NUTRITIONAL, SENSORY AND HEALTH-PROMOTING PROPERTIES OF PROVITAMIN A-BIOFORTIFIED MAIZE STIFF PORRIDGES AND EXTRUDED SNACKS

BY

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PREFACE

The work described in this thesis was carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal from February 2010 to December 2014, under the supervision of Dr Muthulisi Siwela, Dr Eric O Amonsou, Dr Nomusa R Dlamini and Dr Unathi Kolanisi

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DECLARATION

I, Daniso Beswa declare that the thesis hereby submitted by me for the Philosophiae Doctorate degree in Food Security at the University of KwaZulu-Natal is my own original and independent research work. This thesis or any part of it has not been previously submitted by me for any degree or examination to another faculty or University. The research work reported in this thesis does not contain any person's data, pictures, graphs or other information unless specifically acknowledged as being sourced from those persons.

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ABSTRACT

Provitamin A-biofortified maize has been developed to contribute to the alleviation of vitamin A deficiency (VAD), which is prevalent in the sub-Saharan African region where vitamin A- deficient white maize is a leading staple. The biofortified maize is new compared to white maize and as such its grain properties, including milling, nutritional composition (save provitamin A composition) and sensory characteristics, are barely known. There is a challenge in the adoption of the biofortified maize as a food crop due to its low consumer acceptance, which necessitates more consumer studies. Furthermore, there is a need to develop high value commercial food products using provitamin A-biofortified maize to promote its wide spread utilisation and thereby enhance the vitamin A status of the population.

The milling and nutritional properties of grains of 34 varieties of provitamin A-biofortified maize were assessed relative to a white variety (control/reference). The milling properties of the biofortified varieties as indicated by the milling index (69.9-112.1) and hectolitre mass (65.8-82.9 kg/hl) were better compared to 93.5 and 78.5 kg/hl of the white variety, respectively. The ash content of one biofortified maize variety PVAH 48 was comparable to that of the white variety (1.02 g/100 g), whilst five biofortified varieties showed significantly high Fe content (25.67-70.33 mg/kg) compared to the white variety (20.67 mg/kg). The protein (9.8-12.8 g/100 g) and lysine (0.16-0.37 g/100 g) content of the biofortified varieties were significantly high compared to 10.5 g/100 g and 0.21 g/100 g of the white variety, respectively.

The sensory quality of stiff porridges made with provitamin A maize varieties were evaluated using descriptive analysis and the 5-point facial hedonic test by a trained panel and an untrained consumer panel, respectively. The provitamin A maize porridges were described as having a cooked maize flavour and aroma, sticky, fine with low intensity of residual grain and slight bitter aftertaste. Provitamin A carotenoid retention in the porridges was determined. Provitamin A carotenoid retention in the porridges was considerably high (91-123%). Relative to white maize porridge, the biofortified porridges were fairly acceptable, although their acceptability seemed to be reduced by their stickiness and bitter aftertaste.

Leaf powder of Amaranth (*Amaranthus cruentus*), a vegetable widely consumed by rural communities in Southern Africa and reported to have good nutritional and health-promoting properties, was used to partly replace flours of four biofortified maize varieties at 0%, 1% and 3% (w/w) and extruded into snacks. The effects of Amaranth addition on the quality and health-promoting potential of the snacks was assessed, as well as the physical and sensory quality. The physical and sensory qualities of the extruded snacks, in terms of texture and expansion, tended to decrease with increasing Amaranth concentration. However, as Amaranth concentration was increased, the levels of many nutrients (including provitamin A carotenoids and protein) as well as health-promoting potential as indicated by phenolic content (31.0-98.7 mg of GAE/g dry weight) and antioxidant activity (114.3-186.7 µmol TE/g dry weight) also increased.

The less acceptable sensory attributes observed in the biofortified maize should be attenuated through the manipulation of food product formulations. Other plant materials, such as Amaranth, can be used to enhance the nutritional and health-promoting properties of provitamin A-biofortified maize foods. Provitamin A-biofortified maize seems to have a potential for use in the alleviation of VAD and the general enhancement of food and nutrition security, as well as overall wellbeing.

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DEDICATION

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ABBREVIATIONS

PVAH	Provitamin A Hybrid
WAI	Water absorption index
WSI	Water solubility index
VAD	Vitamin A deficiency
UPLC	Ultra-performance liquid chromatography
HPLC	High performance liquid chromatograph
ADF	Acid detergent fibre
NDF	Neutral detergent fibre

CHAPTER 1: INTRODUCTION, THE PROBLEM AND ITS SETTING

1.1. Introduction

Maize is a major staple crop in developing countries of Latin America, Asia and Africa (Sofi et al 2009); where it is primarily produced for human consumption. Globally, 22 countries are leading in maize consumption, and 16 of these countries are in Africa (Nuss and Tanumihardjo 2011). In these countries, maize-based diets are predominantly consumed by low-income populations (Li et al 2010). Maize is the preferred staple crop probably due to its adaptability to diverse ranges of growing conditions and its relatively high yield potential. In sub-Saharan Africa, white maize is preferred over yellow (including the provitamin A-biofortified varieties) (De Groote and Kimenju 2008). However, white maize is devoid of provitamin A (Nuss and Tanumihardjo 2011; Menkir et al 2008). This results in vitamin A deficiency (VAD) among mainly maize consumers in developing countries.

VAD is a major public health problem affecting many children and women (Li et al 2010; Howe and Tanumihardjo 2006). It has a series of adverse functional and clinical consequences (Solomons and Orozco 2003). More research work is underway to alleviate VAD among maize diet consumers particularly in the low-income populations. As a result, maize varieties with high concentration of provitamin A have been developed through bio-fortification (Li et al 2010).

It is proposed that biofortification of maize with provitamin A may have a profound effect on its nutritional quality, milling quality and sensory properties of its food products. Nutritional changes other than increased provitamin A concentration may occur during breeding. Although limited to one study with a fair number of provitamin A maize varieties, Pillay et al (2013) reported a significant increase in protein and starch content of provitamin A-biofortified varieties. The protein and starch content of these varieties was high compared to the reference white maize variety. Maize storage proteins (zeins) are known to have unique functional and biochemical properties which are exploited in the development of a variety of food products (Lawton 2002).

In South Africa, maize is primarily milled into maize meal for cooking porridge (Taylor and Duodu 2009). Zeins are associated with hardness, a most important quality attribute for dry-milling of maize grain (Lee et al 2007). There is more total zein found in hard endosperms than in soft endosperm (Paiva et al 1991). The reduction of protein and amino acid content of maize kernel may affect the ratio of starch to zein and their arrangement in the kernel endosperm. Therefore, any reduction in protein and amino acid content of provitamin A-biofortified yellow maize may affect its milling and functional properties.

Most maize consumers in sub-Saharan Africa prefer white maize over yellow/orange maize due to various reasons (Meenakshi et al 2010; De Groote and Kimenju 2008; Muzhingi et al 2008; Smale and Jayne 2003). However, Pillay et al (2011) found that consumer preference of provitamin A-biofortified maize was influenced by food type and consumer age groups. The sensory characteristics of provitamin A maize, which influence the consumer acceptability of the provitamin A maize seem not to have been subjected to a rigorous scientific investigation. The elucidation of the sensory characteristics of provitamin A maize grain could enable the moderation of the less acceptable characteristics during food product development.

There is a need to develop and evaluate the quality of a variety of food products, including value added commercial foods, containing biofortified maize to promote its utilisation as a grain for food and nutrition security and wellbeing. In sub-Saharan Africa, maize is usually consumed with leafy vegetables. Common high value commercial maize-based food products such as extruded maize snacks are possible candidates for a provitamin A maize-vegetable food product. While extruded maize snacks are popular worldwide, they are mostly, unfortunately, made of white maize which does not contain vitamin A. Extruded snacks made with provitamin A-biofortified maize has the potential to contribute to the alleviation of VAD provided that they are widely accepted and consumed. *Amaranthus cruentus*, a green leafy vegetable naturalised to Southern Africa, is nutrient-rich, and contains substantial levels of Zn, Fe, Ca, and its protein contains high levels of lysine and methionine. The leaves also contain significant levels of phenolic compounds which have antioxidant properties.

Extruding provitamin A-biofortified maize with addition of amaranth could result in nutritive and health-promoting snacks. Addition of amaranth leaf powder may affect the physical and sensory properties of extruded provitamin A-biofortified maize snacks and therefore the quality of the snacks developed should be evaluated.

1.1. Summary of research focus

This study focused on the potential of provitamin A-biofortified maize to alleviate vitamin A deficiency among maize dependent populations. The effects of provitamin A biofortification on maize grain milling properties, nutritional composition and sensory quality of its maize products were investigated. The white maize variety was used as a reference. The sensory characteristics and consumer acceptability of biofortified maize are investigated using provitamin A-biofortified maize porridge; the rationale for choosing the porridge was that it is a popular, widely consumed maize food in sub-Saharan Africa. The effects of adding Amaranth leafy powder on the quality and health-benefitting potential of extruded biofortified maize snacks were investigated. The retention of provitamin A during processing of the studied food products was also investigated. Some results (chapters 4 and 5) of this work have been presented in national and international conferences (Appendix 1).

1.2. Purpose of the study

The purpose of this study was to evaluate the effects of provitamin A biofortification on grain and nutritional properties of maize grain, and the consumer acceptability of provitamin A-biofortified maize as well as the retention of provitamin A during processing provitamin A biofortified maize grain into food products.

1.3. Hypotheses and study objectives

1.3.1. Hypothesis 1

Provitamin A content will be high in provitamin A maize varieties, especially in the varieties with a more intense yellow to orange colour. This is because the concentration of pigment in grains has been found to be significantly associated with yellow to orange colour intensity (Howe and Tunamihlardjo 2006; Pillay et al 2013).

1.3.2. Objective 1

1.3.2.1. To determine the provitamin A carotenoid content of provitamin A-biofortified maize varieties.

1.3.3. Hypothesis 2

The perceived sensory properties of provitamin A-biofortified maize varieties will vary according to the food products prepared and consumer perceptions and expectations. Ethnic background plays a role in the preference (De Groote and Kimenju 2008). For instance, consumers in Zimbabwe regard yellow maize as a “poor man’s grain” and inferior to white maize (Muzhingi et al 2008). A study by Pillay et al (2011) suggested that the acceptability of different types of foods prepared using provitamin A-biofortified maize varied with consumer demographics.

1.3.4. Objective 2

1.3.4.1. To determine the consumer acceptability of provitamin A-biofortified maize stiff porridge to the Venda people of South Africa.

1.3.4.2. To characterise the sensory properties of provitamin A-biofortified maize stiff porridge.

1.3.5. Hypothesis 3

Provitamin A-biofortifed maize grains will be characterised by polyhedral and irregular shapes with some appearing spherical shapes just as reported for white maize. Provitamin A-biofortified maize will also have better milling quality compared to white maize similar to the findings of Pillay et al (2011).

1.3.6. Objective 3

1.3.6.1. To assess the microstructure of grains of provitamin A-biofortified maize varieties compared with reference white maize grain

1.3.6.2. To assess the milling properties of grains of biofortified maize varieties compared with white maize grain.

1.4. Study parameters and general assumptions

The descriptive sensory analysis of provitamin A-biofortified maize stiff porridges was done at the University of KwaZulu-Natal, South Africa. The consumer acceptability tests of stiff porridges and extruded snacks were conducted at Ngulumbi village and University of Venda, in Limpopo province of South Africa. These consumers were selected based on their regular consumption of maize-based staple foods, especially stiff porridge.

1.5. 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: Grain properties and nutritional composition of provitamin A-biofortified maize varieties

Chapter 4: Provitamin A retention and sensory quality of provitamin A-biofortified maize stiff porridges

Chapter 5: Effects of amaranth addition on the quality and health-promoting potential of extruded provitamin A-biofortified maize snacks

Chapter 6: General Discussion

Chapter 7: Conclusions and Recommendations

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CHAPTER 2: LITERATURE REVIEW

Abstract

The extent of vitamin A deficiency (VAD) in developing countries has led to intensive research strategies to improve the vitamin A status of the affected populations. The major challenge in these populations is mainly unaffordable high costs of alternative sources of vitamin A. As a result, these communities subsist on monotonous white maize-based diets with little diversification. Maize varieties which are bred for high concentration of provitaminA carotenoids are currently being evaluated for their potential to alleviate VAD. It is hypothesised that increasing the concentration of provitamin carotenoids A in the grain may have an effect on its end-use properties. This chapter reviews consumer acceptability of provitamin A-biofortified maize, retention of pro-vitamin A carotenoids during food processing, bioavailability of provitamin A carotenoids and the prospects of adding value to provitamin A-biofortified maize snacks by processing it into xtruded snacks with the addition of a green leafy vegetable (Amaranth).

2.1. Introduction

Micronutrient deficiency is still one of the leading causes of many preventable diseases in most Asian and sub-Saharan African countries. The most vulnerable are pre-school age children and women of reproductive age (Menkir et al 2008; SanJoaquin and Molyneux 2009; Li et al 2010; Courraud et al 2013; Phorbee et al 2013). Among the extensively reviewed micronutrient deficiencies, vitamin A deficiency (VAD) is the most cited cause of nutrition-related illnesses in sub-Saharan Africa (Sibeko et al 2004; Aguayo and Baker 2005; Maziya-Dixon et al 2006; Sommer 2008; WHO 2009; Nuss and Tanumihardjo 2010; Pillay et al 2011). VAD is divided into sub-clinical (measles, diarrhoea and other infectious diseases) and clinical (xerophthalmia) categories (Zimmermann and Qaim 2004; Akhtar et al 2013).

VAD is prevalent in resource-poor populations due to their subsistence on monotonous diets of cereal grains (Naqvi et al 2009; Bouis and Welch 2010). Animal-based diets, which are good sources of vitamin A, are unaffordable to poor communities and therefore cereal-based diets remain the only source of pro-vitamin A carotenoids (Helland et al 2002; Tumuhimbise et al 2013). Preformed vitamin A (retinol) can be obtained by consuming animal products like liver (chicken, pork, beef) and cod liver oil. Vitamin A occurs in plants (such as carrots, sweet potatoes and leafy green vegetables). Dietary carotenoids are categorized as pro-vitamin A carotenoids (i.e. α-carotene, β-carotene and β-cryptoxanthin) and non-provitamin A carotenoids that include lutein and zeaxanthin (Zimmermann and Qaim 2004); Aluru et al 2008). The pro-vitamin A carotenoids are broken to vitamin A (retinol), jus before absorption in the intestinal mucosa of the animal/human. White maize is the predominant staple crop in sub-Saharan Africa and the white colour of its kernels demonstrates the lack of carotenoids. Since white maize is the predominant staple crop in sub-Saharan Africa, and generally lacks carotenoids (Naqvi et al 2009; Tumuhimbise et al 2013),the consumers of mainly maize-based diets are at increased risk of VAD (Li et al 2010). Current strategies to alleviate VAD include improving vitamin A availability through a diversified diet, increased consumption of pro-vitamin A carotenoids by biofortification of staple foods and delivery of high-dose vitamin A supplements (Senete et al 2011; Phorbee et al 2013).

Biofortification is regarded as a safe, effective and affordable, sustainable strategy for alleviating VAD (Nestel et al 2006; Bouis and Welch 2010; Azmach et al 2013). It involves increasing the provitamin A carotenoids levels of staple crops which consequently increases the concentration of carotenoid pigments (both provitamin A and non-provitamin A carotenoid pigments) in the grain resulting in grain color changing from white to yellow (or orange) (Tumuhimbise et al 2013). The unfamiliar colour of provitamin A-biofortified staple crops affects their acceptance (Tumuhimbise et al 2013). Carotenoids also impart other sensory properties to the maize making it significantly different from white maize. These properties include unusual flavor and aroma, which also contribute to the low acceptance of the yellow maize (Muzhingi et al 2008; Pillay et al 2011; Tumuhimbise et al 2013). In order to determine and improve the impact of pro-vitamin A-biofortified maize on the alleviation of VAD, it is important that the biofortified maize is acceptable to target consumers and sufficient amount of pro-vitamin A carotenoids are retained when cooked into food and easily absorbed by the consumer when eaten (Bouis and Welch 2010).

2.2. Significance of maize as a staple crop in sub-Saharan Africa

Maize is the most important grain crop in sub-Saharan Africa where it serves as a staple diet for the majority of resource-poor populations (Wurtzel et al 2012). Its central role as a staple food is comparable to that of rice or wheat in Asia (Nuss and Tanumihardjo 2011). Maize colour is the main trait that distinguishes the major varieties and their uses. Yellow and mixed grain coloured maize is primarily used as livestock feed, forage (Vermeulen et al 2005). White maize is strongly preferred for human consumption due the association of yellow maize with food aid for the poor (Stevens & Winter-Nelson, 2008; Pillay et al 2011), thus yellow maize is used as livestock feed. As a human food, maize grain is diversely utilised for consumption (Bolade 2009).

Maize is prepared and consumed into a wide range of food forms for consumption by both children and adults. Its kernels can be consumed off the cob, parched, boiled, fried, roasted, ground, and fermented for use in breads, porridges, gruel, cakes, and alcoholic beverages (Nuss and Tanumihardjo 2010). Porridge is the most dominant food form of maize in sub-Saharan Africa (Haug et al 2010). In African communities, maize plays an important part in food preparations for commemorative and mourning gatherings. Other non-food uses of maize include industrial use as a raw material for plastics, syrups and alcohol for biofuels (Zeppa et al 2012).

Maize is widely grown throughout the world due to its ability to grow in diverse climatic conditions. White maize constitutes more than 90% of Africa's total maize production (Khumalo et al 2011). In sub-Saharan Africa, it is mainly grown for subsistence. However, few countries in this region produce maize on a large scale for both subsistence and commercial purposes. According to Hannon (2012), South Africa has emerged as the largest maize producer and exporter in Africa. Maize production is quite stable in South Africa, Nigeria, Ethiopia and Egypt.

These countries wereranked among the top twenty maize producers in the world (Nuss and Tanumihardjo 2010).In 2013, South Africa was among the top ten maize producing countries in the world (FAOSTAT 2014). Currently, South Africa, Nigeria, Ethiopia, Egypt and United Republic of Tanzaniaare in the top six maize producers in Africa and therefore a food (maize) basket of the continent (Table 2.1).

Table 2.1.

Top ten maize producing countries in sub-Saharan Africa

Country	Maize production (Tonnes)			
	2010	2011	2012	2013
South Africa	12815000	10360000	11830000	12365000
Nigeria	7676850	9180270	9410000*	10400000*
Egypt	7041099	6876473	8093646	6500000*
Ethiopia	4986125	6069413	6158318	6674048
United Republic of Tanzania	4733070	4340823	5104248	4700000*
Malawi	3419409	3699147	3618699	3639866
Kenya	3464541	3376862	3600000	3390941
Zambia	2795483	3020380	2852687	2532800
Uganda	2373501	2551000	2734000	2748000
Ghana	1871695	1683984	1949897	1816000*

*= Unofficial figure

Source: FAOSTAT, 2014

2.3. Vitamin A deficiency and the potential of pro-vitamin A-biofortified maize to alleviate it

2.3.1. Prevalence of VAD in sub-Saharan Africa

The wide consumption of maize and the high prevalence of VAD in developing countries have caught the attention of research programmes, such as HarvestPlus. In an effort to alleviate VAD, HarvestPlus has discovered sustainable ways to reach vulnerable populations. The HarvestPlus strategy is to improve the provitamin A carotenoid content of familiar staple crops that people eat every day and to disseminate them to vitamin A deficient communities (Bouis and Welch 2010). This is achieved through a safe and effective conventional breeding method called biofortification (Nestel et al 2006; Meenakshi et al 2010; Yan et al 2010).

Vitamin A is an essential nutrient which is responsible for normal growth of body tissues and tissue repair (Weingartner 2009). VAD is more prevalent in pre-school age children and women of reproductive age (West 2002). In sub-Saharan Africa, about 42.4% of children 0 to 59 months of age are at risk for vitamin A deficiency (Aguayo and Baker 2005). According to West (2002) VAD may increase morbidity and mortality during pregnancy and the early postpartum period in women of reproductive age (pregnant and breastfeeding). However, there is no published quantified VAD prevalence in sub-Saharan African women. The VAD disorders which can be observed early in life include all active clinical stages of xerophthalmia including corneal xerophthalmia and its potentially blinding sequelae, impaired mechanisms of host resistance, increased severity of infection, anemia, poor growth and mortality (Singh and West 2004).

2.3.2. Current interventions to alleviate VAD

VAD has been declared a global public health problem by the United Nations and goals to achieve the fight against it have been set by World Health Organization (Samba et al 2013). Efforts to alleviate VAD are part of the United Nations Millenium Development Goals (WHO 2014). Nutritional strategies to reduce VAD in developing countries have been devised and tested through research studies around the world. Such interventions include vitamin A supplementation programme, food fortification, dietary diversification and biofortification of staple crops (Akhtar et al 2013; Stevens and Winter-Nelson 2008).

In South Africa, an Intergrated Nutrition Programme (INP) was launched by the the Departmant of Health in 1995. This programme (INP) was aimed at ensuring optimum nutrition for all South Africans by preventing and managing malnutrition (Faber et al 2009). INP combines various strategies such as supplementation, food fortification, promotion of dietary diversification as well as other related public health measures towards achiecving its aim (Labadarios et al 2005).

2.3.2.1 Supplementation programmes

The supplementation programme has played a crucial role in improving the nutritional status of children for decades. In this programme, pre-school children are given capsules containing high doses of vitamin A (twice yearly) (Phorbee et al 2013). These capsules are distributed in high-risk areas. Some of the challenges reported in this programme include inability to reach all the vulnerable communities due to poor road infrastructure, vomiting in children due to high doses of vitamin A (WHO 2011). High doses of vitamin A may be harmful to children, especially those with compromised immunity (Latham 2010). Despite the reported challenges, supplementation is among the intervention programmes that significantly reduce the child morbidity and mortality rate (Sablah et al 2013; Imdad et al 2010).

2.3.2.2 Food fortification

Fortification involves deliberately increasing the content of an essential nutrient in a food, so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health (Kyamuhangire et al 2013). The essential nutrients are usually added to accessible and affordable foods that are regularly consumed by a significant proportion of the population at risk (Faber and Wenhold 2007). Wheat flour and maize meal are some of the crops that have been identified for fortification with vitamin A and iron worldwide. The National Food Fortification Programme was implemented by the South African Department of Health in 2003, with the mandatory fortification of staple maize meal and wheat flour. Vitamin A, together with iron and zinc are some of the micronutrients used to fortify the local staple white maize meal. However, despite this programme, vitamin A deficiency is still reported in children frm ower income communities.

2.3.2.3 Dietary diversification

Dietary diversification is long-term approach that complements the supplementation and food fortification programmes (Faber and Wenhold 2007). In this approach, consumers are encouraged to include animal products or more fruits and vegetables in their diets in order to satisfy their vitamin and mineral requirements. Animal-based food products are good sources of the most bioavailable forms of vitamin A and iron and of absorbable zinc (Horton et al 2008).

Affordability of animal-based foods is a challenge to most poor communities and as a result poor communities subsist on monotonous plant based diets (Tumuhimbise et al 2013).

2.3.2.4 Biofortification of staple crops

Biofortification of staple crops with higher provitamin A carotenoid content is a direct strategy of reaching the target consumers, particularly, rural families that only have limited access to markets and healthcare facilities needed to provide fortified foods and nutritional supplements (Bouis and Welch 2010). Staple crops of priority for biofortification include wheat, rice, maize, sorghum, sweet potato and cassava, which are leading subsistence crops in developing countries. Unlike other VAD interventions, biofortification is the most cost effective and sustainable strategy to alleviate VAD. Biofortification involves breeding staple crops to have high concentration of provitamin A carotenoids (Stevens and Winter-Nelson 2008). Current results on the use of provitamin A-biofortified crops to alleviate VAD hold promise. According to Bouis et al (2009), results of a study conducted in Mozambique, showed a decline from 60% to 38% in vitamin A deficiency among pre-school children in treatment villages, while vitamin A deficiency remained constant in control villages. Testing the effectiveness of consuming pro-vitamin A-rich staple crops on improving the vitamin A status of consumers is crucial. In a study conducted by West et al (1999) in the VAD rife area of southern Nepal it was found that pregnancy-related mortality decreased by 40% among the women who received vitamin A or beta-carotene supplements on a weekly basis before, during, and after pregnancy (West et al 1999).

2.3.3 Potential of provitamin A-biofortified maize to alleviate VAD

Maize is an important staple food in sub-Saharan Africa particularly for resource-poor populations. Its poor nutritional composition, in particular, vitamin A content, protein quality, zinc and iron contents are currently being improved to meet the daily dietary intake of maize diet consumers. In sub-Saharan Africa, the most prevalent micronutrient deficiency is VAD. Biofortification is the current leading technology being used to improve the micronutrient content of maize (Yan et al 2010). The target provitamin A concentration of maize is 15μg g^{-1} (Aluru et al 2008; Yan et al 2010).

Provitamin A-biofortified maize varieties with provitamin A carotenoid concentration (8 μg g^{-1}) close to the target amount of provitamin A have already been identified (Ortiz-Monasterio et al 2007; Khush et al 2012). The concentration of provitamin A retained and its bioavailability may be affected during food preparations (Ortiz-Monasterio et al 2007; Pillay et al 2011).

2.3.3.1 Provitamin A carotenoid retention

Processing of maize into food generally involves the application of heat that has the potential to thermally decompose heat-labile nutrients. It is important that sufficient amount of provitamin A carotenoids are retained after cooking food. Many studies have shown that provitamin A carotenoids (β-carotene and β-cryptoxanthin) are fairly stable to heat processing, however, excessive exposure to heat may result in significant losses (Galaverna and Dall'Asta 2014).

In a study by Mugode et al (2014), a high provitamin A carotenoids retention (>100%) was observed after cooking provitamin A-biofortified maize into thick porridge (*nshima (*Zambia and

Malawi*), sadza (*Zimbabwe*), pap* (South Africa). Pillay et al (2014) reported a substantially high carotenoid retention when provitamin A-biofortied maize was cooked into *phutu* (crumbly maize porridge eaten in South Africa) and samp (dried corn kernels that have been stamped and chopped until broken but not as fine as Mielie-meal or mielie rice. It is commonly eaten among the Xhosa variant in South Africa). The carotenoid retention of phutu ranged from 79.6-128.6% for zeaxanthin, 78-118.2% for β-cryptoxanthin and 80.9-107.9% for β-carotene. The samp retained 89.9-121.6% of zeaxanthin, 78.6-102% of β-cryptoxanthin and 92.9-110% of β-carotene. According to Kong and Singh (2012), heat processing enhances the availability of vitamins and carotenoids by releasing them from the food matrix. A significant increase in lycopene, β-carotene and α-tocopherol content was observed after oven baking of tomato at 160°C (Hwang et al 2012). Cooking of green leafy vegetables resulted in considerably high β-carotene retention, 18-380% after boiling for 8 min and 2-3 times high after stir-frying for 4 and 8 min (Chang et al 2013).

These results demonstrate that cooking enhances the release of carotenoids by disrupting the food matrix.

2.3.3.2 Bioavailabilityof provitamin A carotenoids

The success of provitamin A-biofortified maize can only be measured through bioavailability when it is processed into food and consumed. Bioavalability refers to the proportion of carotenoids from a meal that is absorbed, present in circulation and available for utilisation, metabolism or storage by the organism (Goltz and Ferruzzi 2012). After the food has been ingested, the carotenoids need to be released from the food matrix and subsequently transformed into an absorbable form. The carotenoids release involves mechanical and enzymatic disruption of the food matrix (Alminger et al 2012). The released carotenoids are then transferred to lipid droplets and incorporated into mixed bile salt micelles (Failla et al 2008). Some studies have investigated the bioavailability of provitamin A carotenoids through *in vivo* and *in vitro* experiments.

Howe and Tanumihardjo (2006) investigated the bioavailability of provitamin A carotenoids from maize using vitamin A deficient Mongolian gerbils. The vitamin A in the liver of the gerbils fed on orange maize was significantly higher than that of those fed ordinary yellow maize. Mamatha et al (2012) reported significantly high levels of lutein and zeaxanthin in liver of mice which were fed on processed maize (blanched + dried + milled) compared to the group which was fed on unprocessed maize. The provitamin A status of children in South Africa and Mozambique improved after daily consumption of orange fleshed sweet potato (Tumuhimbise et al 2013). Feeding rats with orange fleshed cassava resulted in a weight gain with appreciable level of bioavailable β-carotene (Phorbee et al 2013). Muzhingi et al (2011) concluded that biofortified yellow maize rich in β-carotene has a potential as an efficient food source to combat VAD in the countries where maize is a predominant staple. In their in vivo bioavailability experiment, these authors reported that all the subjects used converted yellow maize β-carotene to vitamin A and absorbed. The retinyl acetate, a reference dose, was also converted to retinol (Muzhingi et al 2011).

These results demonstrate that encouraging the consumption of provitamin A-biofortified maize instead of white maize could be a sustainable strategy to alleviate VAD in populations where maize is predominantly used as the subsistence food.

2.4 Potential effects of biofortification of maize with provitamin A on its grain milling quality and nutritional composition

2.4.3 *The processing quality of standard white maize grain and the possible effects of provitamin A biofortification on milling quality*

Yellow maize is biologically and genetically very similar to white maize except for the difference in appearance which is mainly due to the presence of carotenoid oil pigments in the yellow maize kernel (FAO 2014). Maize kernels comprise of pericarp (hull or bran), germ and endosperm (Yuan and Flores 1996). The kernel endosperm is the major anatomical part of interest in milling of grain for human food preparations and industrial applications (starch granules). Hence, maize is primarily milled into maize meal or flour which is used as an ingredient in the preparation of various food products (Taylor and Duodu 2009; Li et al 2014). There are various types of milling techniques of which wet and dry milling are the most common methods of maize milling (Li et al 2014; Nuss and Tanumihardjo 2010). In wet milling maize kernels are separated into starch, protein, germ and fibre fractions, while dry milling involves separation of grain anatomical parts and grinding into various particle sizes using roller mills (Arendt and Zannini 2013). The physical quality and chemical composition of grain play a role in selection of the milling method to be used. Maize grain with low test weight has lower percentages of hard endosperm and produces lower yields of prime grits when dry milled (Pan et al 1996). Kernel hardness is the most important quality attribute in milling as it affects functional properties of milled products (Weightman et al 2008; Gaytán-Martínez et al 2006). Hard kernels exhibit high performance in dry-milling, while soft kernels perform well in wet-milling (Lee et al 2007).

The major component of endosperm is starch which comprises two polymers, amylose (linear) and amylopectin (highly branched) which are stored in starch granules. The starch granules are surrounded and embedded in protein (alcohol-soluble prolamin) matrix which is known to be responsible for kernel endosperm hardness (Mestres and Matencio 1996; Lee et al 2006). As mention in Chapter 1, section 1.1., during biofortification of maize nutritional changes other than increased provitamin A concentration may occur during breeding. The protein and starch content of the grain increases (Pillay et al 2013). The changes in protein content of maize as reported by Pillay et al (2013) supported the earlier findings by Raboy et al (1989). Protein (zein) and starch polymer amylose influence grain hardness (Blandino et al 2010; Lee et al 2006). Thus, biofortification may have an effect on the milling properties of the maize grain.

Most nutrients are concentrated in the outer layers of the kernel (Rana et al 2014; Gyori 2010). Therefore, separating the kernels into their anatomical parts has an effect on the nutritional composition of maize-derived products as most of the nutrients remain in the bran-rich fraction which is a by-product of milling (Shepherd et al 2008). In the case of provitamin A-biofortified maize grain, the provitamin A carotenoids are concentrated in the endosperm and would therefore, be minimally affected by milling (Egesel et al 2003; Azmach et al 2013).

2.4.4 Nutritional composition of standard white maize grain and the possible effects of provitamin A biofortification on nutritional composition

White maize is responsible for the high proportion of daily caloric intake of most people in developing countries and the major source of nutrients for the poor (FAO and CIMMYT 1997). The maize nutrients are distributed in different compartments of the kernel. A typical white maize kernel comprises 72% carbohydrate, 87% fibre in seed coat, 10% protein divided between the endosperm and germ, 3.5-6% lipids (Nuss and Tanumihardjo 2010). White maize protein (zein) is deficient in essential amino acids such as lysine and tryptophan and is therefore poor in nutritional quality (Shukla and Cheryan 2001). Zein also plays a crucial role in kernel hardness which is the most important property in grain milling. Other important nutrients in white maize include vitamins, especially the B-vitamins. Vitamin A in the form of beta-carotene (provitamin A) imparts its pigment to its host plant food resulting in an orange to yellow colour. Therefore, the absence of orange to yellow pigmentation in white maize is an indication of the absence of vitamin A. Biofortification of white maize to have high concentration of provitamin A results in maize with orange to yellow kernels. Biofortification may also have an effect on other nutrients in the kernel as shown in Table 2.2. Pillay et al (2013) reported a significant increase in starch, fat, protein and zinc of provitamin A-biofortified maize compared to white maize varieties. Protein and zinc are among the key nutrients which are of food security concern in developing countries, especially in sub-Saharan Africa. An improvement in the composition of these nutrients would be a remarkable achievement in the alleviation of micronutrient deficiency in maize consuming populations.

Table 2.2.

Nutritional composition of white maize and provitamin A-biofortified maize (dry weight)

Nutrient	White maize	ProvA maize*
Moisture (%)	17.1 (0.1)	12.6
Starch (g/100 g)	59.4 (0.3)	66.7
Fat (g/100 g)	3.0 (0.0)	4.7
Protein (g/100 g)	10.7 (0.1)	12.8
Iron(mg/100 g)	5.90 (0.00)	3.21
Zn (mg/100 g)	2.12 (0.08)	2.18
Phosphorus (mg/100 g)	393.97 (17.17)	363.28

Adapted from Pillay et al (2013).

*Grand mean of nutrient composition of 32 varieties evaluated.

2.4.5 Consumer acceptability and sensory properties of provitamin A-biofortified maize

Maize biofortification is considered a feasible and low-cost means of directly reaching the vitamin A-deficient populations who subsist on maize as their major staple crop (Bouis and Welch 2010). These populations generally reside in areas with limited reach by mainstream vitamin A fortification and supplementation programmes (Li et al 2010). Maize is the only staple cereal crop that naturally accumulates carotenoids in the edible seed endosperm (Wurtzel et al 2012; Azmach et al 2013; Pillay et al 2014). As mentioned in the introduction section, biofortification alters the physical and sensory properties of maize. The significant colour changes of maize grain raises acceptability concerns in communities where white maize is a predominant staple crop. In Mozambique, Stevens and Winter-Nelson (2008) conducted a sensory study on provitamin A-biofortified maize which was prepared into *xhima* (thick porridge). This study revealed that consumers liked the *xhima* prepared with orange maize and preferred its aroma compared to that of a familiar white variety. Nuss et al (2012) investigated the intake patterns of traditional foods made with pro-vitamin A-biofortified maize and adaptation by Zambian children aged 3 to 5 years. Quick adaptation by these children to porridge and *nshima* made with orange maize was observed. Furthermore, these children had preference for product from freshly harvested bright orange maize (Nuss et al 2012)

A consumer acceptability study by Pillay et al (2011) in KwaZulu-Natal province, South Africa, involved both children and adult consumers. Provitamin A-biofortified maize was presented to the consumers in the form of *phuthu* (crumbly porridge) and samp. The results from this study suggested that the biofortified maize was more acceptable to secondary school children in the form of samp relative to white maize samp, which was more preferred by adults. The study by De Groote and Kimenju (2008) and De Groote et al (2011) in Kenya, revealed that consumers of a high education level and urban consumers preferred white maize whilst there was a preference for yellow maize in some non-urban parts of the country. Women had stronger preference for both white maize and biofortified maize compared to men. It was found that consumers were interested in commercially fortified maize and would buy yellow maize provided it was offered at a 37% cost savings over white maize (De Groote and Kimenju 2008; De Groote et al 2011).

These findings demonstrate that provitamin A-biofortified maize is fairly acceptable to sub-Saharan African consumers, especially children. There is still a need to intensively educate adult consumers about pro-vitamin A-biofortified maize and its benefit healthwise and to make provitamin A-biofortified maize available and affordable. It has been reported that the attitude of consumers towards biofortified crops changed when they were educated about the nutritional benefits of biofortified (Meenakshi et al 2012).

2.5 Possible effects of using provitamin A-biofortified maize and Amaranth leaf powder on the quality and health-promoting potential of extruded maize snacks

2.5.3 Expected quality of extruded maize snacks and factors affecting their quality

Extruded snacks are expected to be crispier, highly expanded and less bulky with high consumer appeal (eye-catching appearance). Extrusion cooking is a technology with versatility, involves low production cost, produces a better product quality and has no process effluents (Sawant et al 2013). It produces a wide range of products such as breakfast cereals, snacks, pet foods, and texturized vegetable protein from starchy food material (Obadina et al 2013). The extent of the physical and chemical changes that take place during extrusion process depends on the type of starch, presence of other constituents such as sugars, moisture content of the feed material, feed rate, residence time inside the extruder, screw configuration, screw speed and temperature profile (da Silva et al 2009). Processing parameters, conditions and composition of the feed material play a crucial role in the quality characteristics of extruded snacks. Such quality characteristics include physical properties, nutritional quality and sensory properties of snacks which are reviewed in subsequent subsections (2.5.2 - 2.5.4).

2.5.4 Physical properties

Physical properties such appearance, texture, colour and expansion ratio are among the critical quality attributes that have an immediate effect on consumer acceptance of the extruded snacks (Bisharat et al 2013; Oke et al 2013; Anton and Luciano 2007). At the point of purchase, the most important physical properties of extruded snacks include colour, expansion ratio and hardness. Colour is the first property which directly impacts on consumer appeal and therefore a very important physical property for marketing of the snacks (Coutinho et al 2013). Selection of ingredients and mechanical parameters of the extruder have an effect on the physical quality of extruded snacks (Singh et al 2012; Day and Swanson 2013). Colour is influenced by diverse factors including Maillard and caramelization reactions, hydrolysis and degradation of pigments (Santillan-Moreno et al 2009), expansion ratio, extrusion temperature, moisture content, screw speed, screw configuration and die geometry (Fan et al 1996). Colour, expansion ratio, hardness and bulk density are correlated. Expansion ratio is also associated with other properties including crispness and water absorption (Bisharat et al 2013). Extrudates with high expansion ratio tend to have thin and fragile walls between air cells, crispier texture, low bulk density and light appearance as opposed to less expanded. According to Bisharat et al (2013) high expansion ratio and low bulk density are the desirable physical properties of ready-to-eat extruded snacks.

2.5.5 Composite maize extruded snacks and their quality

Maize is the cereal grain in extrusion cooking of snacks (Robutti et al 2002). It is rich in starch which is the main component of interest in extrusion. Like other cereal grains, maize is relatively low in essential amino acid lysine and tryptophan (Świątkiewicz and Bojanowski2004; Johnson 2000), minerals such as zinc and iron (Jin et al 2013; Rai et al 2012) devoid in vitamin A (particularly white maize) (Menkir et al 2008; Nuss and Tanumihardjo 2011). Strategies to improve the nutritional quality of maize-based extruded snacks include compositing maize flour with alternative sources of the limiting nutrients. Onwulata et al (2001) incorporated whey products in an attempt to improve the nutritional content of extruded maize snacks. Increasing fibre content and barrel temperature during extrusion of blends containing fibre from sugar cane bagasse, maize starch and whey protein concentrate resulted in compromised expansion ratio, while improving functional properties of the snack (Martinez-Bustos et al 2011). It appears that blending the maize starch with non-cereal endosperm material had detrimental effects on expansion properties of starch. Kocherla et al (2012) enhanced the taste and nutritional content of snacks incorporating egg albumin powder and cheese powder in the feed material. Contrary to the observations by other researchers, a snack with improved nutritional content and desired physical properties was achieved (Kocherla et al 2012).

2.5.6 Nutritional quality

Nutritionally, snacks are known to be energy-dense with high fat, low in proteins, vitamins and micronutrients (James and Nwabueze 2013). Therefore, the growing trend towards more snacking may increase the severity of micronutrient deficiency of consumers and lead to excessive energy intake. Extrusion processing is known to improve protein quality and protein (and starch) digestibility together with retaining active nutrients (Day and Swanson 2013). In extrusion technology, this involves addition of other ingredients which possess properties of interest such as health-promoting properties, nutritional properties, and functional properties. Incorporation of green leafy vegetables in extruded snacks could potentially improve the nutrient content and health properties of the snacks. Extrusion is a high-temperature short-time operation and therefore minimizes thermal decomposition effect on nutrients (Moscicki and van Zuilichem 2011).

2.5.7 Sensory quality

Sensory quality is an important factor that determines the acceptability of extruded snacks. The success or failure of a new extruded snack food product is directly related to sensory attributes, where texture plays a major role. In such foods, where expansion is desired and puffed products are expected, texture is of major importance, with crispness being one of the most important attributes (Anton and Luciano 2007). Extruded snacks are characterized by air filled foam-like cellular structure with brittle cell walls. During biting of the snacks, texture is the most desirable sensory attribute of extruded products. The bite on extruded snack is expected to produce an audible fracturing due to crispness, crunchiness and crackliness. Thus, a mong the texture attributes of food products, crispness is one of the most important and desirable textural attributes in quality evaluation of extruded products (Duizer 2001; Jakubczyk et al 2013). According to Duizer (2001) if a crisp product does not produce the expected sound upon biting, then it is considered to be stale and of poor quality or has been produced using inappropriate ingredients or processes.

2.5.8 Health-promoting potential of extruded snacks

The consumer awareness about health and nutrition has led to a niche market for nutritious food products with health benefits. Consumers want snacks that taste and smell good, feel good, look good and in addition are nutritionally superior and healthy (Kocherla et al 2012). In extrusion technology, this involves addition of other ingredients which possess properties of interest such as health-promoting properties, nutritional properties, and functional properties among other factors. Extruded snacks are known to be nutrient-dense with low biological value (Ozer et al 2006). Hence, the growing trend towards more snacking may increase the severity of micronutrient deficiency among consumers. Incorporation of green leafy vegetables in extruded snacks could potentially improve the nutrient content and health properties of the snacks.

Green leafy vegetables are known to be good source of protein, possess health-promoting properties such as high phenolic content and antioxidant activity (Kwenin et al 2011; Venskutonis and Kraujalis 2013; Abourashed 2013; Ferrari et al 2014). The inclusion of green leafy vegetables in extruded snacks could benefit children who are known to have a challenge in consuming vegetable-based diets and adult consumers with busy working life which is characterized by irregular dietary habits. Ferrari et al (2014) studied the physical properties of extruded snacks made with cassava leaf flour and cassava starch. Favorable physical properties were achieved at 10% concentration of cassava leaf powder extruded at 100°C and 255 rpm. Nutritional composition of extruded snacks made with moringa leaf powder and oat flour improved with increased concentration of moringa leaf powder, while the physical properties of the snack were negatively affected (Liu et al 2011).

Limsangouan et al (2010) composited rice starch with soy bean and fortified it with a vegetable and herbs. The results demonstrated that functional snacks with sufficient antioxidant properties could be produced from rice starch, soy bean, vegetable and herbs. Jain et al (2012) prepared a mixture of spinach leaf powder, dried carrot, mint, lotus stem, rice flakes and niger seeds. This mixture was subsequently composited with maize and extruded into snack. The results showed that at 10% concentration of the mixture a sensory acceptable snack with better nutritional composition could be produced (Jain et al 2012)

2.6 Conclusion

The ability of maize to accumulate carotenoids and other nutrients makes it the most significant cereal crop in the alleviation of vitamin A deficiency and other nutritional and health problems, thereby contributing to food and nutrition security in sub-Saharan Africa. However, there is either no or scarcity of knowledge of its grain properties (including milling), nutritional composition and sensory characteristics, which would facilitate its adoption as a food crop and utilisation as a major ingredient in traditional and high value commercial food products. Its complementarity with other food materials, especially green leafy vegetables in food preparations could be an additional advantage. Research findings documented in the literature suggest a great potential of compositing provitamin A-biofortified maize with green leafy vegetables in the production of nutritious extruded snacks and health benefits from synergistic combination of bioactive compounds. Currently, there are no published reports on attempts to develop a nutritious snack using provitamin A-biofortified maize and a green leafy vegetablThe magnitude of vitamin A deficiency (VAD) in developing countries has led to intensive research strategies to improve the vitamin A status of the affected populations. The major challenge in these populations is mainly the unaffordable high costs of alternative sources of vitamin A. As a result, these communities subsist on monotonous maize-based diets with little diversification. Maize varieties which are bred for high concentration of pro-vitamin A carotenoids are currently being evaluated for their potential to alleviate VAD. It is hypothesized that increasing the concentration of pro-vitamin A in the grain may affect its end-use properties. This chapter reviews the consumer acceptability of pro-vitamin A-biofortified maize, retention of pro-vitamin A carotenoids during food processing and the prospects of adding value to pro-vitamin A-biofortified maize through processing it into extruded snacks with the addition of a green leafy vegetable (Amaranth).

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CHAPTER 3: GRAIN PROPERTIES AND NUTRITIONAL COMPOSITION OF PROVITAMIN A-BIOFORTIFIED MAIZE VARIETIES

Abstract

Grains of 34 provitamin A-biofortified maize varieties were evaluated for colour, milling properties and nutritional composition compared to grain of a reference white maize variety. The biofortified grains had intense yellow to orange colour (Hunter a*: 11.46-19.79) compared to the white variety (Hunter a*: 6.3). Grain hardness (milling index) of the biofortified varieties was higher (95-112) compared to the white variety (93.5). The hectolitre mass of the biofortified varieties was generally higher (66-83.9 kg/hl) than that of white maize (78.5 kg/hl), but their thousand kernel weight (150.8-305 g) was lower than that of the white variety (317 g). Some biofortified varieties were lower in total mineral content (ash) (0.52-1.09 g/100 g) and Zn (17.33-37.67 mg/kg) relative to the white variety (1.02g/100 g ash and 26.3 mg/kg Zn). The majority (29) of the biofortified varieties had relatively lower Fe content (5.67-19.33 mg/kg) relative to the white variety (20.67 mg/kg). Generally, the fat content of the biofortified varieties was low (2.64-5.91g/100 g) compared to the white variety (5.81 g/100 g). The biofortified varieties were high in fibre, and were generally high in crude protein (9.8-12.8 g/100 g) compared to the white variety (10.5 g/100 g). The lysine content (0.16 to 0.37 g/100 g) of the biofortified varieties was higher than that of the white variety, but their methionine content (0.05 to 0.15 g/100 g) was lower than that of the white variety (0.20 g/100 g). The provitamin A content of the biofortified varieties was high 0.7-7.0 µg g^{-1} compared to the values reported for non-provitamin A-biofortified yellow maize (0.25-2.5 µg g^{-1}). These results indicate that biofortification with provitamin A significantly enhanced the nutritional and physical quality of maize grain.

3.1. Introduction

Maize (*Zea mays*L.) is a key source of essential nutrients for the majority of resource-poor communities of most developing countries, especially in sub-Saharan Africa. The white maize type is most preferred for human consumption (Vermeulen et al 2005). Like other cereals, the maize kernel is comprised of the starchy endosperm, germ and bran as its principal anatomical parts (Yuan and Flores 1996). The maize endosperm is the component of great interest for humans. However, together with the germ, it is protected by the multi-layered bran from damage by sunlight, pests and diseases (Lee et al 2013). Therefore, the extraction of the endosperm involves the removal of the hard multi-layered bran and germ by milling. The endosperm is rich in starch, which is packed in the form of granules that are held together by a protein matrix (Lee et al 2013; Williams et al 2009; Shukla and Cheryan 2001).

A single maize kernel comprises both glassy and floury endosperm in a particular ratio (Williams et al 2009). The ratio of glassy to floury endosperm plays a role in kernel hardness. Grain hardness is an important quality attribute in milling of cereal grains and in the end-use quality of cereal-derived products (Blandino et al 2010; Williams et al 2009). Maize kernels are comprised of mainly the glassy (horny) and floury (starchy) endosperm (Williams et al 2009). Grain hardness is related to the proportions of hard (horny/glassy) and soft (floury/ starchy) endosperm (Zhang et al 2009). Horny or flinty maize varieties refer to maize types with nearly all hard endosperm and floury types refer to maize varietieswith predominantly soft endosperm (Pratt et al 1995). The arrangement of protein and starch in the endosperm is the key factor determining endosperm type and hence grain hardness. The endosperm of hard kernels is tightly compacted with starch granules and protein bodiesembedded in the protein matrix while that of soft kernels comprises loosely packed spherical starch granules embedded in protein matrix (Williams et al 2009). Zein and amylose play a significant role in influencing grain hardness (Blandino et al 2010; Lee et al 2006). The milling involves removal of the bran and germ which are rich in most nutrients. This significantly affects the nutritional composition of cereal-derived products (Rana et al 2014). Most nutrients are concentrated in the bran and germ which are collected as by-products at the end of milling.

The bran is rich in phenolic compounds, vitamins, minerals and fibre while the germ contains vitamins, some protein, minerals and lipids (Lee et al 2013). The endosperm constitutes carbohydrates (starch), protein, vitamins and minerals (Lee et al 2013). Maize, particularly white maize, is poor in essential amino acids (lysine and tryptophan) and deficient in vitamin A (provitamin A carotenoids). Therefore, white maize is nutritionally poor in its refined form. The vitamin A intake of consumers who are heavily dependent on maize-based diets is sub-optimal (Menkir et al 2008). Thus, the dependency on maize-based diets increases the risk of vitamin A deficiency (VAD) (Li et al 2010) which is known to cause several health disorders including night blindness, growth retardation, depressed immune response and increased childhood mortality (Dhliwayo et al 2014). Cost-effective and sustainable strategies such as biofortification are currently dominating in nutritional enhancement of staple crops, including maize (Khush et al 2012; Bouis and Welch 2010; Stevens and Winter-Nelson 2008; Nestel et al 2006).

In this study, biofortification was used to increase the concentration of provitamin A carotenoids in the maize endosperm. It was hypothesized that biofortification may affect the physical and chemical properties of grains of provitamin A-biofortified maize varieties and therefore influence the milling properties of its grain. Few studies (e.g. Pillay et al 2013) have investigated the effect of biofortification with provitamn A on the chemical composition and physical properties (including milling properties) of maize grain as such knowledge on this subject is scanty. Therefore, the aim of this study was to determine selected physical (including milling) properties and nutritional composition of grains of provitamin A-biofortified maize varieties compared to white maize grain.

3.2. Materials and Methods

3.2.1. Materials

Grain of each of 34 provitamin A maize hybrids (PVAH) produced by conventional breeding methods at Cedara Research Station, KwaZulu-Natal, South Africa were used. A reference white maize variety, PAN 67, was also produced under the same conditions and location as the biofortified varieties. The grain ears were harvested manually and then allowed to dry for 21 days at ambient temperature (± 25°C). After manual threshing, the grains were stored at 4°C until used. The grains were analysed for physical properties, milling properties and nutritional composition.

3.2.2. Grain physical properties

3.2.2.1. Colour

The colour of provitamin A-biofortified maize grain and the reference sample (white maize grain) was measured using the pre-calibrated Hunter Lab colorflex spectrophotometer (Hunter Associate Laboratories, Reston, VA). Three replicate samples of each maize variety weremeasured for colour and recorded in the Hunter L* a* b* colour system whereby L* is a measure of lightness (0 = black to 100 = white), a* measures redness to greenness (+*a**= redness and –*a**= greenness), and b* measures yellowness to blueness (+*b**= yellowness; -*b**= blueness).

3.2.2.2. *Milling index*

The milling index of maize grain was measured using a near infrared transmittance (NIT) instrument (Infratec 1241, Grain Analyzer, Foss Tecator, Eden Prairie, MN). About 500 g of sound kernels was placed in a hopper and analysed according to the manufacturer's operating procedure. NIT analysis was done at 860 nm.

3.2.2.3. *Hectolitre mass*

The hectolitre mass of the grains was determined following the American Association of Cereal Chemists International (AACCI) Method 55-10. The hectolitre mass was measured using an apparatus that consisted of a hopper and a 0.5 L receiver (cup). The sample was poured through a funnel into the receiver until the grain overflowed. The grain was then levelled off and weighed on a standard laboratory scale. The grain weight in the 0.5 L cup was converted to kilograms per hectolitre (kg/hL) using the following formula:

$$1b/bu \times 1.287 = kg/hl$$

3.2.2.4. *Thousand kernel weight*

A representative sample of clean, sound kernels of each maize variety was randomly sampled and counted using a numigral seed counter (Chopin SA, Villeneuve-La-Garenne, France). The weight of 1000 kernels was measured and expressed as grams per 1000 kernels. The measurements were replicated three times.

3.2.2.5. Microscopic examination of the maize kernel

Maize kernels were cut into small uniform pieces. The kernel pieces were mounted on the stubs using a double-sided cellophane tape. The kernel pieces and the stubs were gold-coated using Polaron SC500 sputter coater. The kernel pieces were then coated (on the sides) with conductive carbon and examined using a scanning electron microscope (Leo 1450VP, Germany) at 5.00 kV. The samples were also photographed (Figure 3.1)

3.2.3. Nutritional composition of provitamin A-biofortified maize grain

3.2.3.1. Sample preparation

Provitamin A-biofortified maize grain was milled into flour using a laboratory hammer mill (Glen Creston, Stanmore, England) fitted with a 1.0 mm sieve.

3.2.3.2. Moisture content

The moisture content of the whole grain was determined according to the AOAC method (1990). Exactly 2 g of each of the samples was weighed into clean, dry pre-weighed crucibles. The samples were dried in a hot-air drying oven at 105°C and for 3h. The dried samples were transferred into a desiccator and allowed to cool to a constant weight. The dried sample weights were recorded and the difference in weight was calculated as a percentage of the original sample.

$$\%Moisture\ content = \frac{W_2 - W_1}{W_2 - W_3} \times 100$$

Where W_1 = Initial weight of empty dish W_2 = Weight of dish + un-dried sample W_3 = Weight of dish + dried sample.

3.2.3.3. Mineral content

Total ash, Zn and Fe

The total ash was determined according to the Association of Official Analytical Chemists (AOAC) method (1980). Finely milled maize flour samples were weighed into clean, dry porcelain crucibles and placed in a muffle furnace pre-heated to 600°C. The experiment was allowed to run overnight. About 2.5 g of ash sample was transferred into a sample cup. The zinc (Zn) and iron (Fe) were extracted with 25 mL of Ambic-2 extraction solution as described by Hunter (1974). The extract was stirred for 10 min at 400 rpm and filtered through Whatman no.1 filter paper into a clean sample cup. Then, Zn and Fe were determined directly on undiluted extract with an atomic absorption spectrometer. The following formula was used to calculate Zn and Fe content and concentration expressed as mg/kg or ppm:

$$mg/mL\ \ \boldsymbol{A}\ in\ sample = \frac{\boldsymbol{c} \times 25}{2.5}$$

Where **A** = either Zn or Fe, **c =** mg mL **A** in the extract

3.2.3.4. Crude fat

The fat content of dry milled maize flour was determined using Soxhlet extraction method as described by Enyisi et al (2014). About 2 g maize flour was weighed into the Soxhlet cellulose extraction thimbles. The thimbles were plugged with a cotton wool to avoid loss of sample. The thimbles were transferred into the Soxhlet extractor. Sufficient petroleum ether was poured into the Soxhlet extractor beakers. The beakers were clamped into the Soxhlet machine and the electric heating plates were lifted to the base of the beakers. The samples were subjected to extraction for 3 h. The machine was switched off and allowed to cool for 10 minutes. Recovered solvent was transferred into an air oven (100^{o}C) for 1 h and then cooled in desiccators and weighed. The amount of oil extracted was calculated and expressed as percentage of original sample.

$$\% crude\ fat = \frac{weight\ loss\ of\ sample\ (extracted\ fat)}{Weight\ of\ sample}$$

3.2.3.5. Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF)

The neutral detergent fibre and acid detergent fibre were determined according to Robertson and Van Soest (1981).

Neutral Detergent Fibre (NDF)

Preparation of neutral detergent solution: Using a 2 L volumetric flask, 10 L distilled water was added to a 20 L glass carboy. Then, 334.98 g disodium dihydrogen ethylene diamine tetraacetate (EDTA), 82.08 g disodium hydrogen phosphate, 540 g lauryl sulphate, 122.58 g sodium borate decahydrate, and 180 ml ethylene glycol mono-ethyl ether (purified grade) were added. The solution was stirred using a magnetic stirrer plate while adding the remaining 8 L distilled water. The solution was allowed to stir overnight until pH 6.9-7.1 was reached.

Sample analysis: Dry maize grain samples were ground and passed through a 1 mm screen. About 1 g of maize flour was weighed in crucible and 100 mL of neutral detergent solution was added at room temperature with 0.5 g of sodium sulphite and some drops of n-octanol. The mixture was heated to boil and refluxed for 60 min from onset of boiling. The mixture was filtered and washed 3 times with boiling water, then twice with cold acetone. Drying of collected material was done at 105°C for 8 h and the dried material was allowed to cool in a desiccator. The material was weighed and neutral detergent fibre calculated as follows:

$$NDF\ \% = \frac{(weight\ of\ crucible + weight\ of\ residue) - weight\ of\ crucible}{weight\ of\ sample} \times 100$$

Neutral detergent solubles: NDS % = 100 - NDF %.

The material was charred in a muffle at 550 °C for 2 h and cooled in a desiccator. The ash was weighed and the ash insoluble in neutral detergent was calculated:

$$Ash\ insoluble\ in\ neutral\ detergent = \frac{loss\ on\ ashing}{weight\ of\ sample} \times 100$$

Acid Detergent Fibre (ADF):Maize grain samples ground to pass through a 1 mm screen were weighed (1 g) in crucibles. About 100 mL of acid detergent solution (20 g cetyl trimethylammonium bromide, 1 L 1.00 N H_2SO_4 previously standardised) and some drops of n-octanol were added at room temperature. The mixture was boiled and refluxed 60 min from onset of boiling. The mixture was filtered and washed 3 times with boiling water, then twice with cold acetone. The residue was dried at 105 °C for 8 hours and cooled in a desiccator. The dried residue was weighed and the ADF calculated as follows:

$$ADF\ \% = \frac{(weight\ of\ crucible + weight\ of\ residue) - weight\ of\ crucible}{weight\ of\ sample} \times 100$$

The residue was ashed in a muffle at 550°C for 2 h and cooled in a desiccator. The ash was weighed and the ash insoluble in acid detergent was calculated:

$$Ash\ insoluble\ in\ acid\ detergent = \frac{loss\ on\ ashing}{weight\ of\ sample} \times 100$$

3.2.3.6. Crude protein and Essential Amino acids

Crude protein

Crude protein analysis (total N) was conducted according to the AOAC (1990) method as described by Sader et al (2004), using the LECO FP 528 Nitrogen analyser (LECO Corporation, St. Joseph, MI 49085). The LECO Nitrogen analyser uses the Dumas combustion method, where organic nitrogen is converted to nitrogen gas. Samples were weighed (0.1 g) and transferred to aluminium combustion boats, and then placed into the LECO combustion chamber set at 850°C. The mixtures of gases released during combustion are catalytically converted to N_2 quantitatively by passing the gas through a conductivity cell. Nitrogen and crude protein content was calculated as follows:

$$\%N = \frac{(P_c \times V_c \mathrm{X} 0.0449)}{(T \times W)}$$

Where T = final syringe temperature in °K and W = sample weight in milligrams

$$\%Protein = \%N \times 5.70$$

Essential amino acids

Essential amino acids were analysed as described by Yan et al (2014). Finely milled maize flour samples were derivatised using the AccQ- Fluor reagent kit (Waters, Milford, MA, USA) according to the manufacturer's directions. The extracts (5 µL) were mixed with 35 µL of borate buffer and the reaction was initiated by the addition of 10 µL of 6-aminoquinolyl-N-hydroxysuccinimidyl carbonate reagent (AQC), followed by immediate mixing and incubation for 10 min at 55 °C. The derivertized samples (1 µL) were injected into an AccQ Tag C18 column (1.7 µm, 2.1 x 100 mm; Waters) and separated with ultra-performance liquid chromatography (UPLC) (Waters, USA). Solvent A containing 10% AccQ-Tag Eluent A purchased from Waters; solvent B was acetonitrile/water (60:40). The flow rate was 0.7 mL/min. Eluted amino acid derivatives were detected using a Waters PDA eλ detector, with an excitation wavelength of 250 nm and an emission wavelength of 395 nm. Data were recorded and analysed using Waters Empower software.

3.2.3.7. Carbohydrate content

The carbohydrate content of whole grain was calculated as weight by difference between 100 and the summation of other proximate parameters as follows:

$$\text{Carbohydrate content} = 100 - (\text{moisture} + \text{protein} + \text{Fat} + \text{Ash} + \text{Fibre})$$

3.2.3.8. Provitamin A carotenoids

The provitamin A carotenoid contents of four provitamin A-biofortified maize grain varieties as well as the reference white maize variety were determined. Grain samples of each of the biofortified maize varieties and white maize variety were milled, separately, into flour using a laboratory hammer mill (Glen Creston, Stanmore, England) fitted with a 0.5 mm aperture screen. Carotenoids were then extracted from maize flour samples using the method of Kurilich and Juvik (1999) as modified by Howe and Tanumihardjo (2006) and Rodriguez-Amaya and Kimura (2004). The carotenoid extracts were analysed on a C18 column (1.7 µm; 2.1 mm x 100 mm) in a UPLC system consisting of a binary Solvent Manager, Sample Manager and PDA detector. Solvent A consisted of ammonium acetate 10 mM: 2-propanol (90:10), solvent B consisted of acetonitrile: 2-propanol (90:10). The flow rate was set at 0.3 ml/min, injection volume at 2 µl and absorbance measured at 450 nm. Solutions of pure carotenoid pigments that were selected on the basis that they have been found in provitamin A-biofortified maize grain were used as standards, i.e. lutein, zeaxanthin, β-cryptoxanthin, and β-carotene (all-trans and cis isomers). The total provitamin A concentration was calculated as β-carotene using the formula:

Total provitamin A content = (all-trans+9-cis+13-cis β-carotene isomers) + 0.5(β-cryptoxanthin)

3.3. Statistical Analysis

All the analyses were done in triplicates. The data of grain quality and nutritional composition were subjected to analysis of variance (ANOVA). Comparison of multiple means was performed using the Fisher's Least Significant Difference test (LSD) ($p< 0.05$).

3.4. Results and Discussions

3.4.1. Effect of pro-vtamin A biofortification on the colour of maize grain

The effect of biofortification on the colour of maize grain is presented in Table 3.1. Grain colour varied significantly among the biofortified varieties. The lightness (L*) mean values varied from 36.4 to 42.3 with PVAH 81 and PVAH 46 having the lowest and the highest mean values, respectively. As expected, the white variety had significantly high L* mean value compared to the biofortified varieties. The biofortified varieties had significantly high intensity of redness and yellowness as shown by high a* and b* mean values compared to the white variety. The redness values ranged from 11.5 to 19.8 with PVAH41 and PVAH 59 having the lowest and the highest mean values, respectively, while the yellowness ranged from 24.0 to 33.6 with PVAH 41 and PVAH 59 having the lowest and the highest mean values, respectively. The redness and yellowness values for white maize variety were 6.3 and 24.6, respectively. The colour of the grain used in this study was less intense compared to the biofortified maize grain studied by Pillay et al (2011). The colour of grain studied by Pillay et al (2011) ranged from 53.6-57.0 for lightness, 16.5-25.7 for redness and 29.3-37.5 for yellowness. Illustrative images of representative grain samples are shown in Figure 3.1.

Table 3.1.
Effect of provitamin A-biofortification on the colour of maize grain

Maize varieties	Colour (HunterLab values)		
	L*	a*	b*
White maize	$46.33^{g} \pm 1.06$	$6.25^{a} \pm 1.10$	$24.64^{a} \pm 2.62$
PVAH 79-100	$37.19^{a} \pm 1.26$	$16.58^{e} \pm 2.30$	$28.09^{a} \pm 2.53$
PVAH 1-26	$36.60^{a} \pm 0.66$	$18.95^{j} \pm 2.34$	$31.01^{e} \pm 3.63$
PVAH 27-49	$39.01^{a} \pm 3.23$	$17.17^{f} \pm 2.41$	$29.21^{b} \pm 2.46$
PVAH 50-75	$38.98^{a} \pm 1.92$	$17.03^{f} \pm 0.29$	$28.73^{b} \pm 1.14$
PVAH 20	$37.53^{a} \pm 0.64$	$13.89^{a} \pm 0.86$	$25.38^{a} \pm 0.84$
PVAH 28	$38.30^{a} \pm 1.22$	$17.61^{h} \pm 0.84$	$31.36^{f} \pm 2.20$
PVAH 38	$41.41^{e} \pm 1.11$	$11.87^{b} \pm 1.10$	$27.60^{a} \pm 1.15$
PVAH 40	$40.62^{a} \pm 0.30$	$14.29^{a} \pm 2.75$	$30.84^{d} \pm 4.82$
PVAH 41	$41.25^{e} \pm 2.06$	$11.46^{b} \pm 0.18$	$24.01^{a} \pm 1.24$
PVAH 44	$37.63^{a} \pm 1.39$	$17.14^{f} \pm 1.82$	$30.54^{c} \pm 4.33$
PVAH 45	$39.92^{b} \pm 0.34$	$15.80^{d} \pm 0.28$	$31.60^{g} \pm 1.62$
PVAH 46	$42.26^{f} \pm 2.93$	$13.59^{a} \pm 2.18$	$26.30^{a} \pm 3.42$
PVAH 48	$38.83^{a} \pm 0.92$	$13.61^{a} \pm 2.12$	$26.87^{a} \pm 2.82$
PVAH 50	$38.89^{a} \pm 0.69$	$15.79^{f} \pm 0.22$	$28.50^{a} \pm 0.91$
PVAH 51	$40.02^{b} \pm 1.68$	$13.53^{b} \pm 2.24$	$25.49^{a} \pm 2.11$
PVAH 54	$37.10^{a} \pm 1.16$	$14.55^{b} \pm 2.11$	$26.89^{a} \pm 3.96$
PVAH 56	$38.80^{a} \pm 1.92$	$12.66^{a} \pm 1.77$	$25.54^{a} \pm 0.58$
PVAH 59	$37.71^{a} \pm 1.13$	$19.79^{k} \pm 1.32$	$33.62^{i} \pm 1.60$
PVAH 61	$39.29^{a} \pm 1.66$	$14.22^{a} \pm 2.54$	$25.94^{a} \pm 2.78$
PVAH 65	$37.96^{a} \pm 1.45$	$15.84^{a} \pm 0.33$	$27.32^{a} \pm 1.23$
PVAH 67	$38.16^{a} \pm 0.74$	$15.74^{d} \pm 0.91$	$29.13^{b} \pm 0.44$
PVAH 72	$39.26^{a} \pm 1.63$	$17.41^{g} \pm 1.04$	$31.39^{f} \pm 1.58$
PVAH 80	$37.85^{a} \pm 1.97$	$15.50^{d} \pm 1.73$	$29.06^{b} \pm 0.72$
PVAH 81	$36.37^{a} \pm 1.62$	$18.53^{i} \pm 0.45$	$32.58^{h} \pm 1.46$
PVAH 82	$39.04^{a} \pm 1.46$	$15.84^{d} \pm 0.86$	$27.05^{a} \pm 1.85$
PVAH 84	$40.17^{c} \pm 0.84$	$14.79^{c} \pm 1.18$	$26.42^{a} \pm 1.55$
PVAH 86	$39.11^{a} \pm 0.14$	$13.88^{a} \pm 2.15$	$25.36^{a} \pm 3.11$
PVAH 88	$38.60^{a} \pm 1.66$	$15.50^{d} \pm 1.90$	$27.59^{a} \pm 1.84$
PVAH 90	$40.29^{c} \pm 2.90$	$14.80^{c} \pm 1.19$	$29.02^{b} \pm 1.37$
PVAH 91	$36.85^{a} \pm 0.79$	$16.07^{e} \pm 1.77$	$32.28^{h} \pm 3.19$
PVAH 94	$41.33^{e} \pm 1.87$	$13.67^{a} \pm 1.77$	$28.09^{a} \pm 1.35$
PVAH 97	$39.18^{a} \pm 2.71$	$14.53^{a} \pm 0.79$	$26.54^{a} \pm 0.74$
PVAH 101	$38.36^{a} \pm 2.25$	$15.30^{d} \pm 2.03$	$27.47^{a} \pm 2.04$
PVAH 103	$38.31^{a} \pm 1.28$	$15.54^{d} \pm 2.10$	$27.14^{a} \pm 3.03$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different at $p<0.05$, determined by LSD test. PVAH= Provitamin A hybrid. L* = lightness (0 = black to 100 = white), a* = redness to greenness (+*a**= redness and –*a**= greenness), and b* = yellowness to blueness (+*b**= yellowness; -*b**= blueness)

3.4.2. Effect of provitamin A biofortification on the milling properties of maize grain

Maize grain is first converted into various fractions through milling (wet or dry-milling). The milling process involves removal of the outer layers and the germ. Maize grain quality varies among different maize varieties. This variation is due to various factors including the packing and composition of the endosperm components. The milling properties of provitamin A-biofortified maize grain varieties and a reference white maize variety are presented in Table 3.2. The milling indices of provitamin A-biofortified maize grain varied across the varieties. The milling indices of the biofortified maize grains were higher (95.4-112.1) than that of white maize grain (93.5) with the exception of PVAH 50-75, PVAH 79-100, PVAH 1-26 and PVAH 27-49, which had milling indices ranging from 69.9 to 80.7. The milling index is indicative of the extent of grain hardness which is the main physical parameter for selecting the maize end-use (Lee et al 2005; Williams et al 2009). Thus, provitamin A-biofortified maize varieties were relatively harder compared to the white variety. This implies that provitamin A-biofortified maize varieties are suitable for dry-milling as it requires grain with hard endosperm (Pratt et al 1995).

The density of maize grains was evaluated in terms of thousand kernel weight and hectolitre mass. The white maize variety had a higher thousand kernel weight value (317.1 g) compared to the biofortified maize varieties (150.8-305 g). The hectoliter mass values also varied among the biofortified maize grains. These values were generally higher (66.0-82.9 kg/hl) than that of white maize grain (78.5 kg/hl). The hectoliter mass is one of the grain physical traits which can be used to predict the end-use properties of the grain (Ayalew et al 2014). Grain density is related to milling index (grain hardness) (Pomeranz et al 1984). High hectolitre mass values are associated with intense hardness of grain and are an indication of good dry-milling quality. High hectolitre mass values (91.7-96.3 kg/hl) of provitamin A-biofortified maize varieties relative to white maize (88.2 kg/hl) were reported by Pillay et al (2013). The observed hectolitre values in the current study were, in general, lower that those reported by Pillay et al (2013). Chuck-Hernandez et al (2009) also reported a similar hectolitre mass value (76.3 kg/hl) for yellow dent maize. Grain density determines the type of milling suitable for the grain. Grain with low hectolitre mass usually contain lower percentages of hard endosperm and is suitable for wet-milling (Pan et al 1996).

Table 3.2.
Effect of provitamin A biofortification on milling properties of maize grain

Maize variety	Milling index	Thousand kernel weight (g)	Hectolitre mass (kg/hl)
White maize	$93.50^{c} \pm 1.85$	$317.10^{g} \pm 3.11$	78.5
PVAH 79-100	$69.87^{a} \pm 10.57$	$206.5^{d} \pm 0.85$	80.1
PVAH 1-26	$80.67^{b} \pm 1.17$	$267.3^{f} \pm 0.57$	81.6
PVAH 27-49	$79.00^{b} \pm 1.56$	$234.4^{b} \pm 0.21$	81.5
PVAH 50-75	$73.30^{a} \pm 2.10$	$227.7^{b} \pm 00$	78.8
PVAH 20	$105.59^{i} \pm 2.71$	$150.75^{a} \pm 1.77$	76.4
PVAH 28	104.47g ± 2.94	$305.1^{e} \pm 0.57$	65.8
PVAH 38	$96.63^{c} \pm 2.43$	$208.95^{d} \pm 4.74$	75.9
PVAH 40	$97.81^{d} \pm 4.89$	$200.20^{d} \pm 6.79$	77.7
PVAH 41	$102.51^{f} \pm 0.43$	$170.85^{a} \pm 3.32$	77.0
PVAH 44	$105.53^{i} \pm 0.65$	$230.30^{b} \pm 6.22$	82.9
PVAH 45	$112.11^{l} \pm 0.89$	$289.0^{c} \pm 1.34$	66.0
PVAH 46	$98.40^{d} \pm 4.44$	$225.00^{b} \pm 2.26$	79.0
PVAH 48	$104.78^{g} \pm 1.45$	$201.75^{d} \pm 1.63$	77.2
PVAH 50	$105.16^{h} \pm 2.27$	$204.95^{d} \pm 2.76$	77.2
PVAH 51	$107.27^{i} \pm 1.22$	$166.9^{a} \pm 2.97$	66.2
PVAH 54	$107.39^{i} \pm 1.08$	$167.35^{a} \pm 2.90$	75.0
PVAH 56	$98.68^{d} \pm 0.37$	$165.05^{a} \pm 0.92$	79.0
PVAH 59	$110.66^{l} \pm 1.35$	$177.75^{a} \pm 3.75$	79.4
PVAH 61	$105.09^{h} \pm 0.33$	$165.45^{a} \pm 5.45$	77.0
PVAH 65	$104.67^{g} \pm 0.55$	$204.15^{d} \pm 7.71$	78.8
PVAH 67	$105.13^{h} \pm 1.47$	$168.10^{a} \pm 2.55$	75.7
PVAH 72	$102.34^{f} \pm 1.42$	$219.55^{b} \pm 2.19$	80.6
PVAH 80	$109.24^{k} \pm 0.89$	$193.85^{d} \pm 0.64$	80.2
PVAH 81	$104.60^{g} \pm 0.96$	$233.15^{b} \pm 3.75$	80.4
PVAH 82	$107.94^{j} \pm 1.21$	$225.20^{b} \pm 3.25$	81.9
PVAH 84	$109.23^{k} \pm 2.86$	$196.35^{d} \pm 5.16$	80.8
PVAH 86	$104.90^{g} \pm 1.39$	$211.1^{d} \pm 3.11$	67.2
PVAH 88	$99.03^{d} \pm 0.81$	$215.05^{b} \pm 6.29$	79.2
PVAH 90	$99.89^{d} \pm 3.26$	$224.60^{b} \pm 3.96$	79.7
PVAH 91	$104.03^{g} \pm 1.57$	$210.75^{b} \pm 0.07$	80.7
PVAH 94	$100.22^{e} \pm 1.16$	$230.70^{b} \pm 3.54$	80.3
PVAH 97	$95.35^{c} \pm 1.30$	$221.45^{b} \pm 3.47$	77.3
PVAH 101	$95.15^{c} \pm 0.27$	$211.60^{b} \pm 1.41$	77.9
PVAH 103	$98.72^{d} \pm 2.65$	$189.50^{d} \pm 3.39$	78.5

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different at $p<0.05$ (LSD). PVAH= Provitamin A hybrid.

3.4.2.1. Illustrative images of representative provitamin A-biofortified maize grain

The packing of major endosperm components (starch and proteins) has an influence on the milling properties of the grain. To better understand the endosperm quality of the biofortified maize varieties, kernels of representative maize varieties with high, middle and lower milling indices were subjected to scanning electron microscope (SEM) analysis (Figure 3.1). The endosperm of PVAH 50 (high milling index representative) exhibited a more compactly packed microstructure compared to other biofortified varieties and the reference white variety. The starch granules for PVAH 50 were mostly small and uniform in size, oval shaped and intertwined by the protein matrix. The grain with densely packed endosperm structure have high density and therefore exhibit high milling index (hardness) (Gaytan-Martinez et al 2006). The microstructures of the biofortified varieties PVAH 72 and PVAH 40 were similar to that of the white variety. These varieties exhibited a combination of oval and spherical shaped loosely packed starch granules with a wide variation in granule size. The loosely packed grain microstructure is associated with soft grain endosperm and better wet-milling quality (Chandrashekar and Mazhar 1999). A less dense protein matrix was observed in the microstructure of the reference white maize variety. This was also supported by the low protein content of the white variety (10.48 g/100 g) compared to the biofortified varieties (11.10-11.34 g/100 g) (Table 3.3). The high values of grain hardness and hectolitre mass as well as their endosperm microstructure of provitamin A-biofortified maize grain suggest that these varieties may be utilised in food applications in the same manner as the white maize grain.

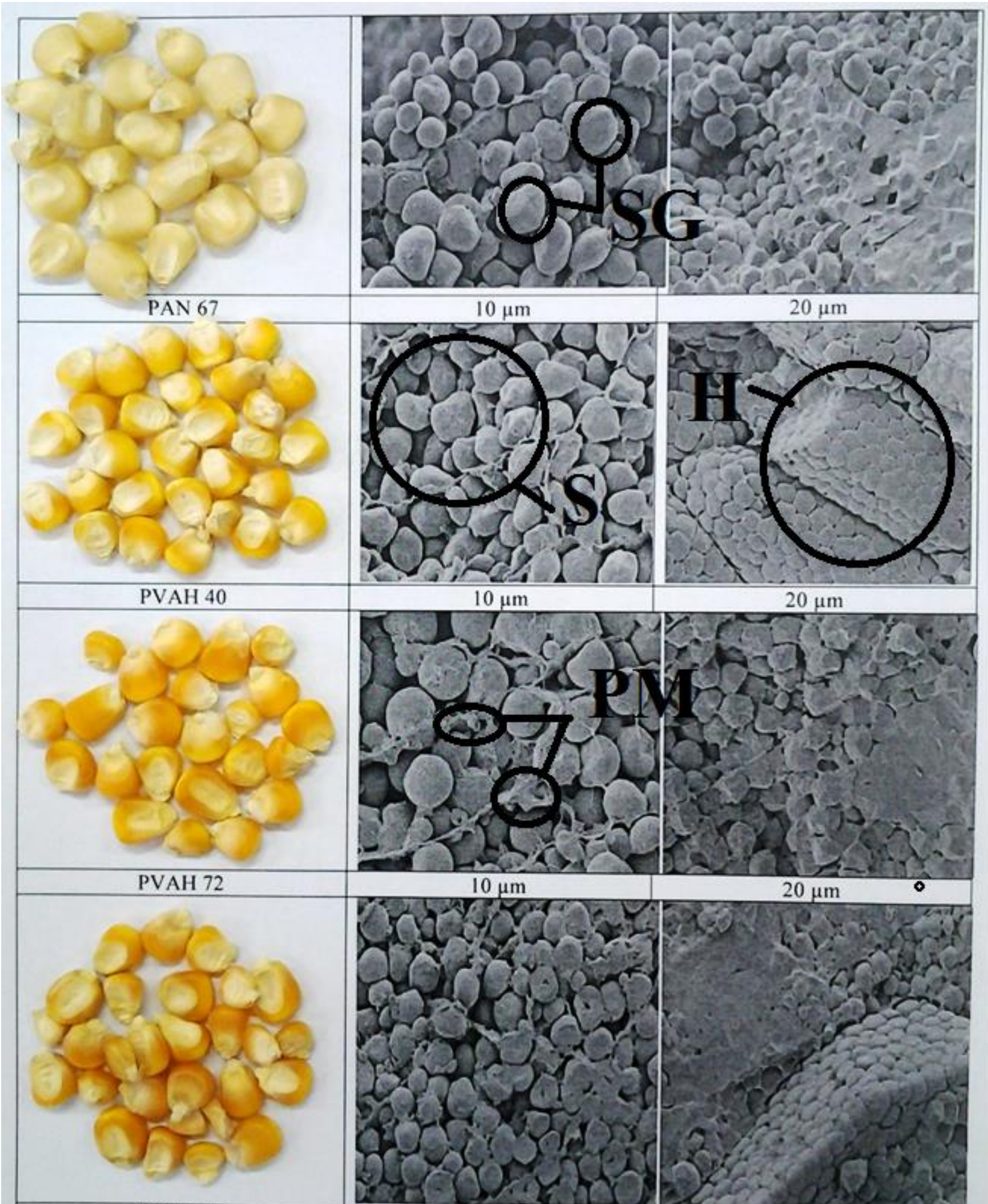

Figure 3.1. Illustrative images and microstructure of provitamin A maize varieties exhibiting variation in packing of starch granules and protein matrix; white maize grain used as a reference. SG = starch granule; PM = protein matrix; S = soft; H = hard; PVAH= Provitamin A hybrid.

3.4.3. Effect of biofortification on the nutritional composition of maize grain

The nutritional composition of the biofortied maize varieties and the reference white variety are presented in Table 3.3. The biofortified varieties were generally lower in ash, fat, Zn and Fe compared to the white variety. However, some biofortified varieties showed significantly higher levels of fat, ash, Zn and Fe than the white variety. The moisture and fibre content of the biofortified varieties were generally high compared to that of the white variety. The biofortified varieties were significantly higher in carbohydrate content than the white maize grain.

3.4.3.1. Moisture content

The moisture content of the biofortified varieties varied from 4.8-9.3 g/100 g whilst the white variety contained 8.1 g/100 g. High moisture content of some of the biofortified varieties does not seem to be a major concern with regard to milling quality since the moisture of the grain is first standardised before milling. However, high moisture could make the grain susceptible to fungal infection.

3.4.3.2. Total mineral (ash), Zn and Fe content

The mineral content varied among the biofortified varieties with their ash and Zn having significantly low mean values compared to the white variety with the exception of PVAH 48 and PVAH 61 (Table 3.3). PVAH 48 had significantly high content of ash (1.09 g/100 g) and Zn (37.7 mg/kg) while PVAH 61 had highest content of Fe (70.3 mg/kg) compared to the white variety which contained 1.02 g/100 g ash, 26.3 mg/kg Zn and 20.7 mg/kg Fe. Maize is generally known to have low levels of Fe and Zn. In the current study, the Zn and Fe content of the biofortified varieties was substantially higher than those reported in a study by Pillay et al

(2013). The Fe content of the biofortified varieties studied by Pillay et al (2013) ranged from 19.0 to 58 mg/kg while Zn ranged from 18.0 to 29.0 mg/kg. On the other hand, the Zn and Fe content of biofortified varieties in this study was significantly higher compared to that of the white maize reported by Banziger and Long (2000). These authors reported a variation in white maize as ranging from 16.4 to 22.9 mg/kg for Fe and 14.7 to 24.0 mg/kg for Zn. The Zn and Fe are among the micronutrients which have been declared limiting in cereal grains (Jin et al 2013; Ortiz-Monasterio et al 2007). Therefore, any improvement in their content may be a remarkable step towards reducing the scourge of micronutrient deficiency.

3.4.3.3. Crude fat

The fat content of most of the biofortified varieties was significantly lower than that of the white variety, whilst one biofortified variety (PVAH 48) had higher fat content (5.91 g/100 g) than all the biofortified varieties (2.64-5.20 g/100 g) and the white variety (5.81 g/100 g) (Table 3.3). Humans need significantly high levels of daily energy (Leonard et al 2010). Dietary fat is a significant source of energy; it contributes double the amount of energy when compared with either carbohydrates or protein on weight basis. Therefore, maize varieties with significantly high fat would also make a significant contribution towards meeting the daily energy requirements of the human body.

3.4.3.4. Fibre content

The fibre content of the maize grain varied among varieties (Table 3.3). The fibre content of the biofortified varieties varied from 8.1-23.1 g/100 g with most of the biofortified varieties containing more fibre content than the white variety (15.4 g/100 g). PVAH 27-49 had the lowest fibre content whilst PVAH 101 contained the highest fibre than all the biofortified varieties and the white variety. Consumption of foods rich in dietary fibre is associated with health benefits such as reduced risk of obesity, coronary heart disease, diabetes, gastro-intestinal disorders, including constipation, inflammatory bowel diseases like diverticulitis and ulcerative colitis, and colon cancer (Tungland and Meyer 2006). Dietary fibre is also known to lower blood pressure, improve blood glucose control in diabetes, aids in weight loss and improve immune function (Anderson et al 2009). From these results, it can be concluded that the biofortified varieties would have better health benefits when compared with the white variety.

3.4.3.5. Carbohydrate content

Cereal grains are known as the major source of carbohydrates (Sarwar et al 2013). In this study, the biofortified maize varieties contained significantly high carbohydrate content than the white variety. The carbohydrate content of the biofortified varieties ranged from 53.4-69.1 g/100 g with PVAH 48 having the lowest value whilst PVAH 27-49 had the highest carbohydrate content than all the biofortified varieties and the white variety (59.3 g/100 g). High carbohydrate content of provitamin A-biofortified maize varieties was also reported by Pillay et al (2013). Carbohydrates are the major source of energy for humans in sub-Saharan Africa. Therefore, high carbohydrate content of provitamin A-biofortified varieties could make these varieties a better source of energy when compared with the white maize.

Table 3.3.
Effects of provitamin A biofortification on proximate composition and selected minerals of maize grain

Maize varieties	Moisture (g/100 g)	Ash (g/100 g)	Fibre (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Carbohydrate (g/100 g)	Zn (mg/kg)	Fe (mg/kg)
White maize	$8.1^{lmn} \pm 0.1$	$1.0^{a} \pm 0.0$	$15.4^{m} \pm 0.0$	$5.8^{a} \pm 0.01$	$10.5^{f} \pm 0.0$	$59.3^{defg} \pm 0.1$	$26.3^{a} \pm 0.6$	$20.7^{a} \pm 0.6$
PVAH79-100	$9.1^{r} \pm 0.0$	$0.7^{f} \pm 0.0$	$9.75^{c} \pm 0.0$	$3.7^{e} \pm 0.01$	$10.5^{e} \pm 0.0$	$66.3^{o} \pm 0.1$	$20.3^{d} \pm 0.6$	$8.7^{e} \pm 0.6$
PVAH 1-26	$9.3^{s} \pm 0.0$	$0.52^{o} \pm 0.0$	$9.9^{d} \pm 0.03$	$3.5^{cc} \pm 0.01$	$10.6^{g} \pm 0.0$	$66.2^{o} \pm 0.1$	$20.3^{d} \pm 0.6$	$8.7^{e} \pm 0.6$
PVAH27-49	$7.3^{h} \pm 0.3$	$0.9^{k} \pm 0.0$	$8.1^{a} \pm 0.3$	$4.3^{dd} \pm 0.01$	$10.4^{d} \pm 0.0$	$69.1^{p} \pm 0.5$	$23.7^{b} \pm 0.6$	$11.3^{b} \pm 0.6$
PVAH 50-75	$8.4^{op} \pm 0.0$	$0.7^{p} \pm 0.0$	$9.1^{b} \pm 0.0$	$3.8^{ee} \pm 0.01$	$10.2^{b} \pm 0.0$	$68.0^{p} \pm 0.0$	$23.7^{b} \pm 0.6$	$8.3^{e} \pm 0.6$
PVAH 20	$8.9^{q} \pm 0.0$	$0.9^{b} \pm 0.0$	$15.0^{k} \pm 0.0$	$3.3^{b} \pm 0.01$	$12.8^{ff} \pm 0.0$	$62.1j^{klm} \pm 5.2$	$22.3^{b} \pm 0.6$	$13.3^{b} \pm 0.6$
PVAH 28	$7.1^{g} \pm 0.0$	$0.7^{n} \pm 0.0$	$16.9^{t} \pm 0.0$	$4.1^{ff} \pm 0.01$	$10.8^{k} \pm 0.0$	$60.4^{fghij} \pm 0.0$	$17.33^{c} \pm 0.6$	$8.3^{e} \pm 0.6$
PVAH 38	$5.7^{b} \pm 0.0$	$0.7^{c} \pm 0.0$	$15.6^{n} \pm 0.0$	$4.2^{c} \pm 0.01$	$11.4^{v} \pm 0.0$	$62.4^{klmn} \pm 0.0$	$18.7^{c} \pm 0.6$	$18.0^{c} \pm 1.0$
PVAH 40	$4.8^{a} \pm 0.0$	$0.8^{d} \pm 0.0$	$14.7^{j} \pm 0.0$	$4.6^{d} \pm 0.01$	$11.2^{o} \pm 0.0$	$63.9^{n} \pm 0.1$	$19.0^{c} \pm 1.0$	$17.3^{d} \pm 0.6$
PVAH 41	$8.0^{lm} \pm 0.1$	$0.8^{e} \pm 0.0$	$13.4^{h} \pm 0.0$	$3.7^{e} \pm 0.01$	$12.4^{dd} \pm 0.0$	$61.7^{ijk} \pm 0.1$	$22.3^{b} \pm 0.6$	$17.3^{d} \pm 0.6$
PVAH 44	$8.5^{p} \pm 0.0$	$0.7^{f} \pm 0.0$	$11.2^{e} \pm 0.0$	$4.3^{f} \pm 0.01$	$11.7^{x} \pm 0.0$	$63.6^{mn} \pm 0.0$	$17.3^{c} \pm 0.6$	$8.7^{e} \pm 0.6$
PVAH 45	$6.2^{de} \pm 0.0$	$0.7^{f} \pm 0.0$	$16.73^{r} \pm 0.0$	$4.9^{g} \pm 0.01$	$11.3^{r} \pm 0.0$	$60.2^{fghi} \pm 0.0$	$18.3^{c} \pm 0.6$	$28.7^{f} \pm 0.6$
PVAH 46	$7.4^{hi} \pm 0.0$	$0.8^{g} \pm 0.0$	$17.6^{v} \pm 0.0$	$3.9^{h} \pm 0.01$	$11.8^{cc} \pm 0.0$	58.5cde ± 0.0	$19.7^{e} \pm 0.6$	$19.3^{g} \pm 0.6$
PVAH 48	$7.3^{h} \pm 0.0$	$1.1^{h} \pm 0.0$	$21.1^{y} \pm 0.0$	$5.9^{i} \pm 0.01$	$11.2^{p} \pm 0.0$	$53.4^{a} \pm 0.0$	$37.7^{f} \pm 0.6$	$25.7^{h} \pm 0.6$
PVAH 50	$7.4^{hi} \pm 0.0$	$0.8^{g} \pm 0.0$	$19.1^{x} \pm 0.0$	$4.4^{j} \pm 0.01$	$11.3^{t} \pm 0.0$	$57.0^{c} \pm 0.02$	$22.7^{b} \pm 0.6$	$16.7^{d} \pm 0.6$
PVAH 51	$7.6^{jk} \pm 0.0$	$0.9^{i} \pm 0.0$	$16.2^{p} \pm 0.0$	$2.6^{k} \pm 0.01$	$11.8^{aa} \pm 0.0$	$60.9^{ghijk} \pm 0.0$	$23.3^{g} \pm 0.6$	$15.3^{i} \pm 0.6$
PVAH 54	$8.1^{lmn} \pm 0.1$	$1.0^{j} \pm 0.0$	$15.4^{m} \pm 0.0$	$3.8^{l} \pm 0.01$	$12.6^{ee} \pm 0.0$	$59.1^{def} \pm 0.1$	$25.7^{a} \pm 0.6$	$12.7^{b} \pm 0.6$
PVAH 56	$8.2^{no} \pm 0.0$	$0.9^{k} \pm 0.0$	$17.1^{u} \pm 0.0$	$2.9^{m} \pm 0.01$	$10.6^{h} \pm 0.0$	$60.4^{fghi} \pm 0.0$	$22.7^{b} \pm 0.6$	$12.3^{b} \pm 0.6$
PVAH 59	$6.3^{e} \pm 0.0$	$0.8^{c} \pm 0.0$	$15.1^{kl} \pm 0.0$	$3.9^{n} \pm 0.01$	$10.7^{j} \pm 0.0$	$63.3^{lmn} \pm 0.1$	$22.3^{b} \pm 0.6$	$17.3^{d} \pm 0.6$
PVAH 61	$8.0^{lmn} \pm 0.2$	$0.8^{k} \pm 0.0$	$12.7^{f} \pm 0.0$	$3.4^{o} \pm 0.01$	$11.8^{y} \pm 0.0$	$63.2^{lmn} \pm 0.2$	$21.7^{h} \pm 0.6$	$70.3^{j} \pm 0.6$
PVAH 65	$6.3^{e} \pm 0.4$	$0.6^{m} \pm 0.0$	16.8 ± 0.0	$3.2^{p} \pm 0.01$	$11.3^{s} \pm 0.0$	$61.8^{ijkl} \pm 0.4$	$22.0^{b} \pm 1.0$	$26.3^{h} \pm 0.6$
PVAH 67	$8.0^{l} \pm 0.0$	$0.9^{i} \pm 0.0$	$16.8^{s} \pm 0.0$	$4.4^{j} \pm 0.01$	$11.6^{w} \pm 0.0$	$58.4^{cde} \pm 0.0$	$21.3^{h} \pm 0.6$	$6.7^{k} \pm 0.6$
PVAH 72	$8.1^{mn} \pm 0.1$	$0.9^{k} \pm 0.0$	$15.1^{l} \pm 0.0$	$4.4^{j} \pm 0.01$	$11.1^{n} \pm 0.0$	$60.4^{fghij} \pm 0.1$	$21.7^{h} \pm 0.6$	$8.3^{e} \pm .0.6$
PVAH 80	$7.3^{hi} \pm 0.0$	$0.8^{k} \pm 0.0$	$18.0^{w} \pm 0.0$	$4.3^{q} \pm 0.01$	$11.8^{bb} \pm 0.0$	$57.7^{cd} \pm 0.0$	$25.7^{a} \pm 0.6$	$18.7^{g} \pm 0.6$
PVAH 81	$6.3^{e} \pm 0.0$	$0.8^{g} \pm 0.0$	$16.7^{r} \pm 0.0$	$4.5^{r} \pm 0.01$	$9.8^{a} \pm 0.0$	$61.8^{ijkl} \pm 0.1$	$21.7^{h} \pm 0.6$	$17.3^{d} \pm .06$
PVAH 82	$6.7^{f} \pm 0.0$	$0.7^{c} \pm 0.0$	$16.2^{p} \pm 0.0$	$4.2^{s} \pm 0.01$	11.3 ± 0.0	$60.9^{ghijk} \pm 0.0$	$24.3^{i} \pm 0.6$	$15.3^{i} \pm 0.6$
PVAH 84	$6.1^{cd} \pm 0.0$	$0.7^{c} \pm 0.0$	$13.7^{i} \pm 0.0$	$5.2^{t} \pm 0.01$	$10.6^{h} \pm 0.0$	$63.7^{mn} \pm 0.0$	$18.7^{c} \pm 0.6$	$13.3^{b} \pm 0.6$
PVAH 86	$7.5^{ij} \pm 0.2$	$0.7^{c} \pm 0.0$	$16.4^{q} \pm 0.0$	$4.6^{u} \pm 0.01$	$10.9^{l} \pm 0.0$	$59.9^{efgh} \pm 0.2$	$21.7^{h} \pm 0.6$	$6.3^{k} \pm 0.6$
PVAH 88	$8.3^{op} \pm 0.0$	$0.8^{k} \pm 0.0$	$13.2^{g} \pm 0.0$	$3.7^{v} \pm 0.01$	$11.8^{bb} \pm 0.0$	$62.1^{jklm} \pm 0.0$	$24.3^{i} \pm 0.6$	$16.3^{d} \pm 0.6$
PVAH 90	$7.7^{k} \pm 0.1$	$0.8^{l} \pm 0.0$	$15.1^{kl} \pm 0.0$	$3.7^{w} \pm 0.01$	$11.4^{u} \pm 0.0$	$61.4^{hijk} \pm 0.1$	$20.7^{d} \pm 0.6$	$14.3^{i} \pm 0.6$
PVAH 91	$7.4^{hi} \pm 0.0$	$0.9^{i} \pm 0.0$	$14.8^{j} \pm 0.0$	$4.0^{x} \pm 0.01$	$10.7^{i} \pm 0.0$	$62.3^{klmn} \pm 0.0$	$22.7^{b} \pm 0.6$	$6.0^{k} \pm 1.0$
PVAH 94	$7.6^{jk} \pm 0.0$	$0.8^{l} \pm 0.0$	$13.3^{h} \pm 0.0$	$3.6^{y} \pm 0.01$	$11.0^{m} \pm 0.0$	$63.8^{mn} \pm 0.0$	$22.3^{b} \pm 0.6$	$5.7^{k} \pm 0.6$
PVAH 97	$6.1^{c} \pm 0.0$	$0.8^{c} \pm 0.0$	$15.9^{o} \pm 0.0$	$4.0^{z} \pm 0.01$	$11.8^{z} \pm 0.0$	$61.5^{hijk} \pm 0.0$	$19.3^{c} \pm 0.6$	$16.7^{d} \pm 0.6$
PVAH 101	$6.7^{f} \pm 0.0$	$0.6^{m} \pm 0.0$	$23.1^{aa} \pm 0.0$	$4.3^{aa} \pm 0.01$	$10.2^{c} \pm 0.0$	$55.1^{b} \pm 0.0$	$21.3^{h} \pm 0.6$	$27.7^{f} \pm 0.6$
PVAH 103	$7.6^{jk} \pm 0.0$	$0.7^{n} \pm 0.0$	22.4 ± 0.0	$4.1^{bb} \pm 0.01$	$11.4^{t} \pm 0.0$	$53.9^{ab} \pm 0.0$	$21.3^{h} \pm 0.6$	$17.0^{d} \pm 1.0$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different at $p<0.05$, determined by LSD test. PVAH = Provitamin A hybrid.

3.4.3.6. Crude protein and essential amino acids

The protein and essential amino acid composition of provitamin A-biofortified maize varieties and reference white maize are presented in Table 3.4. The protein and essential amino acid content varied among the biofortified varieties. The protein content ranged from 9.8 to 12.8 g/100 g with PVAH81 and PVAH 20 containing the lowest and the highest mean values, respectively. The protein content of the biofortified varieties was significantly higher than that of the white variety (10.5 g/100 g). The biofortified varieties varied within a narrow range in essential amino content. In this study, the essential amino acids determined were histidine, threonine, lysine, methionine, isoleucine, leucine, valine and phenylalanine. Lysine and tryptophan are among the known limiting essential amino acids in maize (Świątkiewicz and Bojanowski2004). The lysine content of the biofortified varieties varied from 0.16 to 0.37 g/100 g while the methionine varied from 0.05 to 0.15 g/100 g. The content of lysine was generally higher compared to the white variety (0.21 g/100 g), whilst the methionine content of the biofortified varieties was significantly lower than that of white variety (0.20 g/100 g). PVAH 80 and PVAH 97 had the lowest lysine (0.16 g/100 g) and methionine (0.05 g/100 g) content, respectively. The highest content of lysine was found in PVAH 59 (0.37 g/100 g) while PVAH 45, PVAH 51 and PVAH 50 contained high methionine content (0.15 g/100g). These findings on protein content are supported by the results reported by Pillay et al (2013). These authors found that the biofortified maize varieties had high protein content (12.8 g/100 g) compared to white maize (10.7 g/100 g). Though crude protein values in the present study are relatively low compared to those reported by Pillay et al (2013), but the trend was the same. Contrary to lysine values reported by Pillay et al (2013), in the current study four (4) provitamin A-biofortified maize varieties (PVAH 50-75, PVAH 59, PVAH 84 and PVAH101) had higher lysine values

(0.25-0.37 g/100 g) compared to the reference white maize variety (0.21 g/100 g). From the nutritional point of view, this is a significant observation as the lysine content and its availability are the determinant of quality protein cereal grain (Soria and Villamiel 2012). The influence of lysine on the protein content of provitamin A-biofortified maize was demonstrated by higher protein values (10.62-12.77 g/100 g) compared to the reference white maize variety (10.48 g/100 g). The apparent increase in protein and lysine content of the provitamin A-biofortified maize varieties could be associated with genetic transformation. According to Pillay et al (2013), the hybridisation of maize genotypes to produce maize with high concentration of provitamin A and subsequent selection during breeding may cause a significant change in the nutritional composition of the maize, including nutrients other than provitamin A. As mentioned earlier (above) tryptophan is one of the limiting essential amino acids in cereal grains, therefore, there is a need to analyse the tryptophan content of the biofortified maize varieties. This essential amino acid could not be analysed due to insufficient research funds.

Table 3.4.

Effect of provitamin A biofortification on protein and essential amino acid composition of maize grain

Maize varieties	Crude protein (g/100 g)	Essential amino acids							
		Histidine (g/100 g)	Threonine (g/100 g)	Lysine (g/100 g)	Methionine (g/100 g)	Valine (g/100 g)	Isoleucine (g/100 g)	Leucine (g/100 g)	Phenylalanine (g/100 g)
White maize	10.48^{a} ± 0.01	0.18^{a} ± 0.06	0.21^{a} ± 0.03	0.21^{a} ± 0.05	0.20^{a} ± 0.05	0.35^{a} ± 0.04	0.21^{a} ± 0.03	0.90^{a} ± 0.03	0.34^{a} ± 0.03
PVAH 79-100	10.45^{ee} ± 0.01	0.17^{b} ± 0.02	0.26^{c} ± 0.01	0.20^{a} ± 0.03	0.12^{b} ± 0.02	0.30^{m} ± 0.02	0.22^{a} ± 0.02	0.96^{a} ± 0.02	0.37^{b} ± 0.02
PVAH 1-26	10.59^{aa} ± 0.01	0.20^{d} ± 0.03	0.28^{f} ± 0.05	0.21^{a} ± 0.02	0.14^{b} ± 0.03	0.40^{e} ± 0.04	0.27^{d} ± 0.04	1.22^{i} ± 0.07	0.40^{d} ± 0.04
PVAH 27-49	10.37^{cc} ± 0.01	0.18^{b} ± 0.02	0.26^{c} ± 0.02	0.19^{a} ± 0.04	0.13^{b} ± 0.02	0.34^{n} ± 0.02	0.21^{a} ± 0.03	1.00^{b} ± 0.03	0.33^{a} ± 0.02
PVAH 50-75	10.17^{dd} ± 0.01	0.15^{b} ± 0.02	0.34^{k} ± 0.01	0.26^{c} ± 0.03	0.13^{b} ± 0.02	0.51^{k} ± 0.02	0.27^{e} ± 0.02	1.33^{k} ± 0.02	0.42^{f} ± 0.02
PVAH 20	12.77^{b} ± 0.01	0.20^{d} ± 0.03	0.25^{b} ± 0.03	0.20^{a} ± 0.04	0.12^{b} ± 0.03	0.39^{d} ± 0.04	0.28^{f} ± 0.02	1.16^{h} ± 0.03	0.40^{d} ± 0.02
PVAH 28	10.75^{ff} ± 0.01	0.18^{b} ± 0.02	0.25^{a} ± 0.02	0.19^{a} ± 0.03	0.13^{b} ± 0.03	0.39^{c} ± 0.02	0.27^{e} ± 0.02	1.17^{h} ± 0.02	0.39^{c} ± 0.02
PVAH 38	11.43^{c} ± 0.01	0.19^{c} ± 0.02	0.30^{i} ± 0.03	0.24^{a} ± 0.03	0.14^{b} ± 0.02	0.41^{f} ± 0.03	0.25^{b} ± 0.02	1.02^{c} ± 0.03	0.33^{a} ± 0.04
PVAH 40	11.17^{d} ± 0.01	0.18^{b} ± 0.02	0.21^{a} ± 0.04	0.21^{a} ± 0.04	0.13^{b} ± 0.02	0.00^{o} ± 0.00	0.20^{a} ± 0.03	0.96^{a} ± 0.03	0.31^{a} ± 0.03
PVAH 41	12.44^{e} ± 0.01	0.19^{a} ± 0.03	0.27^{d} ± 0.02	0.21^{a} ± 0.02	0.14^{b} ± 0.02	0.44^{j} ± 0.02	0.30^{h} ± 0.02	1.20^{h} ± 0.20	0.39^{c} ± 0.03
PVAH 44	11.70^{f} ± 0.01	0.16^{b} ± 0.01	0.23^{a} ± 0.02	0.21^{a} ± 0.03	0.14^{b} ± 0.02	0.40^{e} ± 0.03	0.25^{b} ± 0.02	1.13^{g} ± 0.03	0.37^{b} ± 0.02
PVAH 45	11.27^{g} ± 0.01	0.17^{b} ± 0.03	0.26^{c} ± 0.03	0.21^{a} ± 0.03	0.15^{b} ± 0.02	0.43^{h} ± 0.02	0.31^{i} ± 0.02	1.13^{g} ± 0.03	0.43^{f} ± 0.02
PVAH 46	11.82^{h} ± 0.01	0.15^{b} ± 0.02	0.27^{d} ± 0.02	0.22^{a} ± 0.04	0.14^{b} ± 0.03	0.44^{i} ± 0.01	0.28^{f} ± 0.01	1.07^{f} ± 0.02	0.40^{d} ± 0.02
PVAH 48	11.24^{i} ± 0.01	0.15^{b} ± 0.01	0.28^{f} ± 0.01	0.21^{a} ± 0.03	0.13^{b} ± 0.04	0.41^{f} ± 0.01	0.25^{b} ± 0.02	1.03^{c} ± 0.01	0.42^{f} ± 0.01
PVAH 50	11.34^{j} ± 0.01	0.15^{b} ± 0.01	0.31^{j} ± 0.01	0.23^{a} ± 0.03	0.15^{b} ± 0.03	0.42^{g} ± 0.02	0.29^{g} ± 0.01	1.25^{j} ± 0.02	0.47^{g} ± 0.02
PVAH 51	11.78^{k} ± 0.01	0.14^{b} ± 0.01	0.34^{k} ± 0.01	0.22^{a} ± 0.04	0.15^{b} ± 0.03	0.53^{k} ± 0.02	0.36^{j} ± 0.02	1.40^{k} ± 0.02	0.54^{h} ± 0.02
PVAH 54	12.56^{l} ± 0.01	0.21^{f} ± 0.01	0.29^{g} ± 0.02	0.22^{a} ± 0.03	0.13^{b} ± 0.02	0.39^{d} ± 0.02	0.24^{a} ± 0.03	1.17^{h} ± 0.02	0.40^{d} ± 0.02
PVAH 56	10.62^{m} ± 0.01	0.20^{e} ± 0.02	0.23^{a} ± 0.02	0.19^{a} ± 0.03	0.13^{b} ± 0.01	0.36^{b} ± 0.02	0.23^{a} ± 0.02	0.92^{a} ± 0.02	0.35^{a} ± 0.02
PVAH 59	10.68^{n} ± 0.01	0.21^{g} ± 0.02	0.27^{e} ± 0.02	0.37^{b} ± 0.15	0.12^{b} ± 0.01	0.42^{g} ± 0.02	0.30^{h} ± 0.02	1.17^{h} ± 0.02	0.43^{f} ± 0.02
PVAH 61	11.75^{o} ± 0.01	0.21^{f} ± 0.01	0.26^{c} ± 0.02	0.18^{a} ± 0.04	0.14^{b} ± 0.03	0.35^{a} ± 0.03	0.25^{b} ± 0.01	1.13^{g} ± 0.01	0.42^{e} ± 0.01
PVAH 65	11.30^{p} ± 0.01	0.20^{d} ± 0.02	0.25^{a} ± 0.02	0.20^{a} ± 0.03	0.13^{b} ± 0.02	0.35^{a} ± 0.02	0.25^{b} ± 0.02	1.04^{e} ± 0.02	0.43^{f} ± 0.02
PVAH 67	11.56^{bb} ± 0.01	0.21^{g} ± 0.02	0.26^{c} ± 0.01	0.18^{a} ± 0.05	0.12^{b} ± 0.02	0.35^{a} ± 0.02	0.25^{b} ± 0.01	1.05^{f} ± 0.02	0.41^{d} ± 0.01
PVAH 72	11.10^{q} ± 0.01	0.21^{f} ± 0.01	0.25^{a} ± 0.01	0.19^{a} ± 0.03	0.13^{b} ± 0.01	0.32^{m} ± 0.01	0.22^{a} ± 0.02	0.96^{a} ± 0.01	0.41^{d} ± 0.01
PVAH 80	11.81^{r} ± 0.01	0.17^{b} ± 0.02	0.24^{a} ± 0.02	0.16^{a} ± 0.02	0.11^{b} ± 0.02	0.35^{a} ± 0.02	0.22^{a} ± 0.03	1.04^{d} ± 0.02	0.38^{b} ± 0.01
PVAH 81	9.81^{s} ± 0.01	0.16^{b} ± 0.03	0.24^{a} ± 0.02	0.18^{a} ± 0.04	0.14^{b} ± 0.01	0.34^{n} ± 0.01	0.22^{a} ± 0.01	0.96^{a} ± 0.02	0.35^{a} ± 0.03
PVAH 82	11.26^{t} ± 0.01	0.22^{i} ± 0.02	0.25^{b} ± 0.03	0.19^{a} ± 0.03	0.13^{b} ± 0.02	0.42^{g} ± 0.02	0.27^{e} ± 0.02	1.17^{h} ± 0.01	0.45^{i} ± 0.01
PVAH 84	10.63^{m} ± 0.01	0.25^{k} ± 0.02	0.29^{h} ± 0.02	0.27^{c} ± 0.12	0.13^{b} ± 0.02	0.44^{i} ± 0.02	0.29^{g} ± 0.02	1.14^{g} ± 0.01	0.42^{f} ± 0.02
PVAH 86	10.92^{u} ± 0.01	0.22^{h} ± 0.01	0.26^{c} ± 0.02	0.20^{a} ± 0.04	0.13^{b} ± 0.02	0.38^{c} ± 0.02	0.25^{b} ± 0.02	1.01^{c} ± 0.03	0.41^{d} ± 0.02
PVAH 88	11.81^{r} ± 0.01	0.22^{i} ± 0.03	0.26^{c} ± 0.02	0.20^{a} ± 0.03	0.13^{b} ± 0.03	0.38^{c} ± 0.02	0.25^{b} ± 0.03	1.15^{g} ± 0.02	0.47^{g} ± 0.03
PVAH 90	11.38^{v} ± 0.01	0.21^{g} ± 0.03	0.26^{c} ± 0.02	0.18^{a} ± 0.02	0.12^{b} ± 0.02	0.35^{a} ± 0.03	0.23^{a} ± 0.02	1.04^{f} ± 0.03	0.35^{a} ± 0.03
PVAH 91	10.66^{w} ± 0.01	0.21^{f} ± 0.01	0.22^{a} ± 0.02	0.21^{a} ± 0.06	0.12^{b} ± 0.02	0.34^{n} ± 0.02	0.24^{b} ± 0.01	0.93^{a} ± 0.02	0.32^{a} ± 0.03
PVAH 94	10.97^{x} ± 0.01	0.22^{h} ± 0.03	0.23^{a} ± 0.02	0.18^{a} ± 0.03	0.07^{c} ± 0.03	0.37^{b} ± 0.02	0.22^{a} ± 0.02	0.90^{a} ± 0.03	0.33^{a} ± 0.02
PVAH 97	11.77^{y} ± 0.01	0.21^{f} ± 0.03	0.25^{b} ± 0.03	0.18^{a} ± 0.03	0.05^{c} ± 0.03	0.31^{m} ± 0.03	0.22^{a} ± 0.02	0.95^{a} ± 0.02	0.34^{a} ± 0.02
PVAH 101	10.20^{z} ± 0.01	0.24^{j} ± 0.01	0.22^{a} ± 0.03	0.25^{c} ± 0.05	0.11^{b} ± 0.02	0.38^{b} ± 0.01	0.26^{c} ± 0.02	0.96^{a} ± 0.02	0.33^{a} ± 0.03
PVAH 103	11.35^{j} ± 0.01	0.21^{g} ± 0.03	0.26^{c} ± 0.02	0.19^{a} ± 0.04	0.14^{b} ± 0.03	0.35^{a} ± 0.02	0.24^{a} ± 0.02	1.03^{d} ± 0.03	0.37^{b} ± 0.02

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different at $p<0.05$ determined by LSD test.PVAH = Provitamin A hybrid.

3.4.3.7. Carotenoid composition

The target amount (by the HarvestPlus Global Challenge Programme) of provitamin A content that could alleviate vitamin A deficiency is 15.0 µg g^{-1} (Nuss and Tanumihardjo 2011; Yan et al 2010; Ortiz-Monasterio et al 2007). The carotenoid composition of biofortified maize varieties is presented in Table 3.5. In this study, the provitamin A carotenoid content of the biofortified varieties was lower than the target amount. The values varied between 0.7 and 7.0 µg g^{-1}. PVAH 94 had the lowest provitamin A content (0.74µg g^{-1}), whilst a provitamin A content of 7.13µg $g^{-1}$was recorded for PVAH 50. These values were also significantly lower compared to those reported by Pillay et al (2013) for provitamin A-biofortified maize (8.2-10.6 µg g^{-1}), but significantly higher than yellow maize values (0.25-2.5 µg g^{-1})reported by Aluru et al (2008). Zeaxanthin content of 32% of biofortified varieties was within the range (16.8-25.5 µg g^{-1}) reported by Pillay et al (2014) for biofortified maize and those for yellow maize (10-30 µg g^{-1}) which were reported in the review by Nuss and Tanumihardjo (2010). About 44% of biofortified maize varieties had higher β-cryptoxanthin content (4.9-9.7 µg g^{-1}) than those reported by Pillay et al (2014) (4.4-6.2 µg g^{-1}) for biofortified varieties and Nuss and Tanumihardjo (2010) for yellow maize (<0.01 µg g^{-1}). The β-carotene contents is this study were substantially lower (0.02-3 µg g^{-1}) compared to Pillay et al (2014) results (3.9-4.8 µg g^{-1}). The carotenoid content of white maize is too low to be detected by chromatographic analysis. Therefore, in our study the yellow maize carotenoid composition which comprised 0.25-2.5 provitamin A, 10-30 zeaxanthin, <0.01 µg g^{-1} β-cryptoxanthin, and 0.5-2.0 µg g^{-1} β-carotene (Prasanna et al 2014; Nuss and Tanumihardjo 2010; Sefawo et al 2010) was used as the baseline. These results indicate that the biofortification significantly enhanced the provitamin A content of the biofortified maize lines.

Table 3.5.

Carotenoid composition of provitamin A-biofortified maize grain (μg g^{-1} dry weight)

Maize varieties	β-cryptoxanthin	Zeaxanthin	β-carotene	Total provitamin A
White maize	n.d	n.d	n.d	n.d
PVAH 79-100	$1.17^{a} \pm 0.02$	$3.26^{b} \pm 0.05$	$0.83^{c} \pm 0.01$	$2.40^{c} \pm 0.08$
PVAH 1-26	$1.53^{b} \pm 0.05$	$4.48^{c} \pm 0.12$	$0.83^{c} \pm 0.03$	$2.58^{c} \pm 0.06$
PVAH 27-49	$1.15^{a} \pm 0.06$	$2.89^{a} \pm 0.15$	$0.86^{c} \pm 0.03$	$2.47^{c} \pm 0.12$
PVAH 50-75	$1.21^{a} \pm 0.06$	$2.93^{a} \pm 0.03$	$0.88^{c} \pm 0.05$	$2.46^{c} \pm 0.07$
PVAH 20	$3.38^{r} \pm 0.18$	$17.51^{r} \pm 0.15$	$1.26^{e} \pm 0.05$	$3.51^{g} \pm 0.38$
PVAH 28	$7.76^{o} \pm 0.25$	$18.30^{t} \pm 0.25$	$2.39^{m} \pm 0.20$	$6.34^{m} \pm 0.23$
PVAH 38	$6.39^{l} \pm 0.19$	$17.08^{p} \pm 0.18$	$1.00^{c} \pm 0.01$	$4.13^{i} \pm 0.21$
PVAH 40	$8.15^{p} \pm 0.25$	$12.33^{k} \pm 0.35$	$1.22^{e} \pm 0.12$	$5.37^{k} \pm 0.16$
PVAH 41	$3.42^{f} \pm 0.09$	$5.18^{d} \pm 0.16$	$1.23^{e} \pm 0.05$	$2.90^{d} \pm 0.13$
PVAH 44	$2.62^{d} \pm 0.10$	$7.70^{f} \pm 0.18$	$0.95^{c} \pm 0.06$	$2.22^{b} \pm 0.12$
PVAH 45	$3.29^{e} \pm 0.17$	$16.98^{p} \pm 0.08$	$1.44^{g} \pm 0.28$	$2.93^{d} \pm 0.11$
PVAH 46	$4.63^{h} \pm 0.24$	$11.95^{j} \pm 0.17$	$0.80^{c} \pm 0.01$	$3.08^{e} \pm 0.19$
PVAH 48	$5.52^{k} \pm 0.25$	$5.98^{e} \pm 0.07$	$2.49^{o} \pm 0.15$	$5.36^{k} \pm 0.14$
PVAH 50	$9.68^{q} \pm 0.02$	$21.24^{x} \pm 0.33$	$2.26^{m} \pm 0.23$	$7.13^{n} \pm 0.23$
PVAH 51	3.94 ± 0.07	$16.25^{o} \pm 0.10$	$0.92^{c} \pm 0.11$	$2.95^{d} \pm 0.07$
PVAH 54	$6.55^{l} \pm 0.23$	$8.18^{g} \pm 0.11$	$2.21^{m} \pm 0.20$	$5.54^{l} \pm 0.25$
PVAH 56	$6.51^{l} \pm 0.15$	$7.69^{f} \pm 0.21$	$1.16^{d} \pm 0.06$	$4.39^{i} \pm 0.23$
PVAH 59	$4.42^{h} \pm 0.15$	$8.23^{g} \pm 0.05$	$1.63^{i} \pm 0.03$	$3.71^{h} \pm 0.20$
PVAH 61	$4.92^{i} \pm 0.10$	$5.36^{d} \pm 0.33$	$2.02^{l} \pm 0.13$	$4.49^{i} \pm 0.18$
PVAH 65	$6.74^{m} \pm 0.16$	$18.54^{u} \pm 0.18$	$3.01^{p} \pm 0.19$	$6.35^{m} \pm 0.25$
PVAH 67	$5.32^{j} \pm 0.11$	$15.15^{m} \pm 0.14$	$2.23^{m} \pm 0.12$	$4.63^{j} \pm 0.29$
PVAH 72	$3.13^{e} \pm 0.10$	$14.04^{l} \pm 0.15$	$0.81^{c} \pm 0.01$	$2.28^{c} \pm 0.14$
PVAH 80	$4.00^{g} \pm 0.24$	$17.32^{q} \pm 0.12$	$0.98^{c} \pm 0.04$	$2.67^{y} \pm 0.50$
PVAH 81	$5.06^{i} \pm 0.14$	$17.68^{s} \pm 0.21$	$2.44^{n} \pm 0.14$	$5.04^{k} \pm 0.14$
PVAH 82	$4.58^{h} \pm 0.18$	$15.60^{n} \pm 0.24$	$1.47^{h} \pm 0.07$	$3.68^{h} \pm 0.25$
PVAH 84	$5.28^{j} \pm 0.16$	$12.03^{j} \pm 0.21$	$0.84^{c} \pm 0.04$	$3.30^{f} \pm 0.21$
PVAH 86	$3.55^{f} \pm 0.20$	$4.53^{c} \pm 0.12$	$1.88^{k} \pm 0.09$	$3.60^{g} \pm 0.34$
PVAH 88	$2.12^{c} \pm 0.11$	$3.54^{b} \pm 0.12$	$1.40^{f} \pm 0.04$	$2.37^{c} \pm 0.16$
PVAH 90	$3.97^{g} \pm 0.13$	$17.62^{r} \pm 0.20$	$1.34^{e} \pm 0.07$	$3.26^{e} \pm 0.19$
PVAH 91	$3.95^{g} \pm 0.07$	$10.95^{h} \pm 0.17$	$0.60^{b} \pm 0.07$	$2.37^{c} \pm 0.29$
PVAH 94	$1.43^{a} \pm 0.10$	$5.23^{d} \pm 0.11$	$0.02^{a} \pm 0.00$	$0.74^{a} \pm 0.05$
PVAH 97	$5.54^{k} \pm 0.20$	$16.35^{o} \pm 0.07$	$1.74^{j} \pm 0.11$	$4.53^{j} \pm 0.17$
PVAH 101	$9.82^{r} \pm 0.21$	$23.24^{w} \pm 0.42$	$1.33^{e} \pm 0.05$	$6.12^{m} \pm 0.26$
PVAH 103	$7.40^{m} \pm 0.15$	$11.51^{i} \pm 0.13$	$2.44^{n} \pm 0.08$	$6.19^{m} \pm 0.19$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different at $p<0.05$, determined by LSD test. PVAH = Provitamin A hybrid. Nd = not detected

3.5. Conclusions

The biofortified varieties showed significant variations in physical and nutritional properties. The main objective of breeding provitamin A biofortified maize varieties was to produce varieties with a high concentration of provitamin A carotenoids. Therefore, from these results it is clear that more work still needs to be done to achieve the HarvestPlus target. Furthermore, since maize grain usually requires dry milling before preparation into food, the milling quality of grains of biofortified varieties seems better than that of white maize grain as they were harder. In this study, the biofortified varieties PVAH 45, PVAH 48, PVAH 61, PVAH 20, PVAH 59, PVAH 51 and PVAH 50 had appreciable levels of provitamin A carotenoids which was coupled with considerably high milling indices, and high protein, lysine, methionine, Zn and Fe contents compared to the white variety. These varieties may be used as the baseline for further improvement of consolidated physical and nutritional quality.

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CHAPTER 4: PROVITAMIN A RETENTION AND SENSORY QUALITY OF PROVITAMIN A-BIOFORTIFIED MAIZE STIFF PORRIDGES

Abstract

Maize has been selected for biofortification with provitamin A carotenoids to alleviate vitamin A deficiency (VAD), which is prevalent in sub-Saharan Africa. In this study, four varieties of biofortified maize, PVAH 79-100, PVAH 1-26, PVAH 27-49 and PVAH 50-75 were prepared into stiff porridges. Provitamin A carotenoid retention in the porridges was determined. Descriptive sensory analysis and the 5-point facial hedonic test were used to evaluate the sensory quality of the porridges. Provitamin A carotenoid retention in the porridges was very high (91-123%). The biofortified maize porridges were described as sticky, fine with low intensity residual grain, slightly bitter aftertaste with cooked maize flavour and aroma. By Principal Component Analysis (PCA), principal component (PC) 1 and 2 explained 84% of the total variation in the sensory attributes of the porridges. PC 1 (48.9%) separated PVAH 1-26 and PVAH 50-75 porridges from those of PVAH 27-49 and PVAH 79-100. The former were characterised by residual grain, stickiness and fineness. About 33% and 28% of the consumer sample (N= 60) liked and disliked the biofortified maize porridges, respectively, whilst about 38% of the consumers neither liked nor disliked the porridges. The bitter aftertaste and stickiness seemed to negatively affect the sensory acceptability of the biofortified maize porridges when compared with the white maize porridge. The sensory acceptability of the porridges was significantly associated with consumer age ($p<0.05$). An appreciable proportion (55%) of the consumers in the 41-60 years age group liked biofortifed maize porridges, whilst a similar proportion (58.8%) of consumers in the 20-40 years age group disliked the porridges. The

findings suggest that biofortified maize stiff porridge can deliver a significant amount of provitamin A to targeted consumers. However, there is a need to improve the acceptance of the biofortified maize by the targeted consumers.

4.1. Introduction

Maize (*Zea mays* L.) is a dominant subsistence crop in much of Asia, Africa and Latin America (Hulshof et al 2007). In most sub-Saharan African countries, maize is consumed in various food forms, including whole and broken kernels, soft and stiff porridges, gruels and beverages. The white maize is the usual type for food use in sub-Saharan Africa (Menkir and Maziya-Dixon, 2004). Soft and stiff porridges are often consumed with milk or meat and vegetables as a family lunch or supper. However, among low income consumers, the soft and stiff porridges are consumed mainly with indigenous vegetables because these consumers cannot afford the high cost of animal products. Unfortunately, white maize is devoid of vitamin A and as such large populations in sub-Saharan Africa are at a high risk of vitamin A deficiency (VAD), because they depend largely on white maize and other vitamin A deficient starchy staples with little dietary diversity (Menkir and Maziya-Dixon, 2004).

Improving the provitamin A carotenoid levels of staple crops through biofortification is regarded as a sustainable strategy for alleviating VAD (Nestel et al 2006). Due to its wide consumption in the sub-Saharan African region, maize is a leading candidate for biofortification with provitamin A carotenoids. Unfortunately, consumer studies in a number of countries in this region have found low acceptance of the biofortified maize compared to white maize, for example in Kenya (De Groote and Kimenju 2008), Zimbabwe (Muzhingi et al 2008) and South Africa (Pillay et al2011). The biofortification of maize increases the concentration of carotenoid pigments (both provitamin A and non-provitamin A carotenoid pigments) in the grain resulting in grain colour changing from white to yellow (or orange) (Tumuhimbise et al 2013).

The yellow colour has been found to significantly contribe to the low acceptance of yellow maize, which seems to be due to the fact that African consumers are accustomed to white maize, as stated earlier. Carotenoids also impart other sensory properties to the yellow maize making it significantly different from white maize, including unusual flavour and aroma, which also contribute to the low acceptance of the yellow maize (Muzhingi et al 2008; Pillay et al 2011).

Besides the sensory properties, the poor acceptance of yellow maize by African consumers seems to be caused also by other factors, including demographic, psychological and socio-economic factors. Yellow maize is negatively associated with its usual use as animal feed and as a food aid item (Muzhingi et al 2008; De Groote et al 2011). Pillay et al (2011) found that, among the South African consumers surveyed, younger school children preferred yellow maize, whilst older school children and adults preferred white maize over yellow maize.

In Kenya, it was found that consumers with a high education level preferred white maize (De Groote and Kimenju 2008); and urban consumers preferred white maize, whilst there was a preference for yellow maize in some non-urban parts of the country (De Groote and Kimenju 2008; De Groote et al 2011). The Kenyan consumers were found interested in commercially fortified maize and would buy yellow maize only if it was sold at a discounted price. On the other hand, in Mozambique yellow maize was more acceptable than local white maize varieties (Stevens and Winter-Nelson 2008). Further, the attitude of consumers towards biofortified crops has been found to change when they were educated about the nutritional benefits involved (Meenakshi et al2012).

The type of food in which provitamin A-biofortified maize is presented to the consumer was found to have an influence on its acceptance (Pillay et al 2011). The study conducted by Pillay et al (2011) in KwaZulu-Natal province, South Africa, demonstrated that the biofortified maize was more acceptable to secondary school children in the form of samp relative to white maize samp, which was more preferred by adults. This finding suggests that the acceptance of biofortified maize can be significantly high if it is presented to the consumer in a maize food type that is yet to be established. The study of Pillay et al (2011) did not include stiff maize porridge in the consumer acceptance test; yet stiff maize porridge is arguably the most popular maize food in sub-Saharan Africa, especially in Southern Africa. More studies are needed to evaluate consumer acceptance of stiff porridge, particularly among Southern African consumers. Although several studies have been conducted on consumer acceptance of provitamin A-biofortified maize, it appears that the descriptive sensory properties of the biofortified maize products have not been studied. Information about the sensory properties of provitamin A-biofortified maize may be useful in the efforts to breed for improved product acceptability. Therefore, in this study, the sensory properties and consumer acceptability of stiff porridge made with provitamin A-biofortified maize were investigated. The other objective was to determine the retention of provitamin A carotenoids in the stiff porridges.

4.2. Materials and Methods

4.2.1. Maize grain varieties

Four varieties of provitamin A-biofortified maize, PVAH 79-100, PVAH 1-26, PVAH 27-49 and PVAH 50-75 (Figure 4.1), produced by conventional breeding methods at Cedara Research Station, KwaZulu-Natal, South Africa, were used in this study. A reference white maize variety, PAN 67, was also produced under the same conditions and location as the biofortified varieties. The grain ears were harvested manually and then allowed to dry for 21 days at ambient temperature (± 25°C). After manual threshing, the grains were stored at 4°C until used.

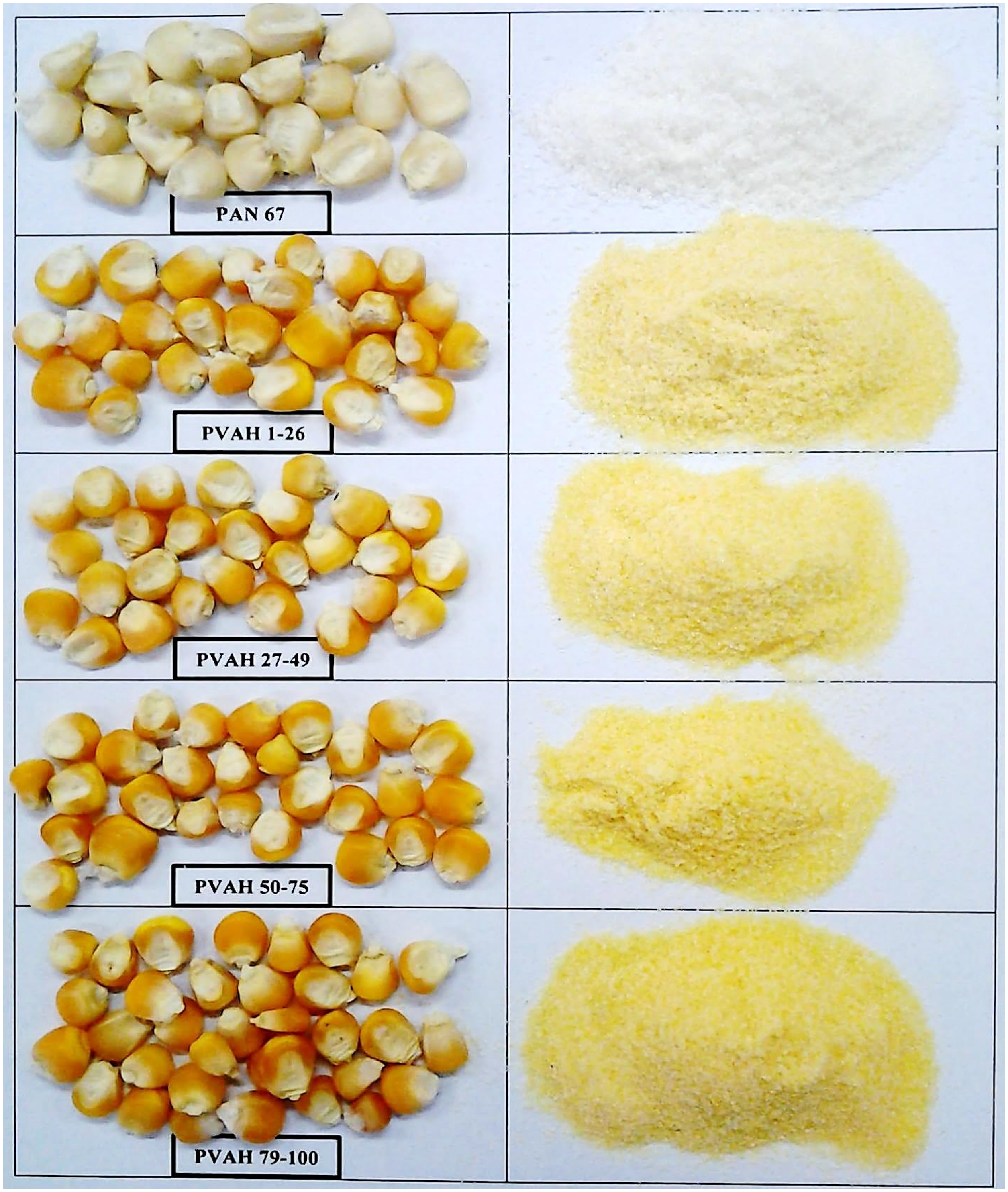

Figure 4.1. Provitamin A-biofortified maize grain and flour used in the preparation of maize stiff porridges.
PVAH = Provitamin A Hybrid; PAN 67= Reference white grain

4.2.2. Grain milling

After the maize grains had been cleaned by a standard method, they were milled with a pilot plant roller mill (Model MK 150, Roff Industries, Kroonstad, South Africa). This type of mill has a three-break system, which yields super meal, maize grits and fine meal. Super meal, the product that passed through the 459 µm aperture screen, was collected from the last two break systems and used for making porridge samples.

4.2.3. Preparation of stiff porridges

Stiff porridges were made with the super meal of each of the four provitamin A-biofortified maize varieties and the reference white variety. The recipe of a typical traditional stiff maize porridge consumed by the *Venda* tribe of South Africa was standardised with the help of three experienced *Venda* women who worked at the University of Venda experimental farm, South Africa (Figure 4.2) (Appendix 2).

Standardization of traditional *Venda* stiff porridge recipe

Cooking of provitamin A-biofortified maize stiff porridges in the presence of CIVIC union delegates

Figure 4.2. Preparation of maize stiff porridges

4.2.4. Provitamin A analysis

The provitamin A contents of grain of each of the four provitamin A-biofortified maize varieties as well as those of their porridges were determined. Samples of stiff porridge prepared as described earlier were cooled, freeze-dried and milled, separately, into flour using a laboratory hammer mill (Glen Creston, Stanmore, England) fitted with a 0.5 mm aperture screen. Grain samples of each of the biofortified maize varieties were milled in the same manner as the porridge samples. The extraction of carotenoids from the flours of the porridges and grains and subsequent analysis was done as described in section 3.2.3.8. The total provitamin A concentration was calculated as β-carotene using the formula:

Total provitamin A = (all-trans+9-*cis*+13-*cis β-carotene* isomers) + 0.5(β-cryptoxanthin).

The apparent retention (AR) of provitamin A in stiff porridge was calculated as follows:

$$\%\ AR = \frac{\textit{provitamin A content per gcooked porridge (dry weight)}}{\textit{provitamin A content per g super meal (dry weight)}}\ X\ 100$$

4.2.5. Sensory evaluation

Stiff porridges were prepared with the four provitamin A-biofortified maize varieties (plus a reference white variety for the consumer test), separately, and subjected to sensory evaluation. The porridges were prepared as described in 4.2.3.

4.2.5.1. Descriptive sensory analysis

A panel of 10 trained panellists, selected from an initial 15 prospective panellists, was used for the sensory profiling of the stiff porridges. The panellists were selected based on interest, availability and sensory acuity, which were determined during their recruitment and training. All panellists were postgraduate students in Agricultural disciplines at the University of KwaZulu-Natal, South Africa. The panellists were regular consumers of maize foods. During the training sessions, the panel generated and defined descriptive terms for each sensory attribute (Table 4.1). At least one reference for each descriptor was identified and an intensity rating scale was developed for each attribute.

The references were sourced and the panellists used them to analyse the sample porridges as part of the training process. The reliability of the panellists was assessed during the training sessions through repeated analyses. The panellists were isolated from each other in booths during the reliability tests to prevent them influencing each other. Five prospective panellists were withdrawn as their reliability was not satisfactory. The porridge samples (100 g) were randomly labelled with three-digit codes obtained from a Table of Random Numbers. The porridge samples (cooled to room temperature) along with references were presented to the 10 panellists for analysis using the protocol of Anyango et al (2011). The porridge samples were served to each panellist in a randomised order, which was determined from a Table of Random Permutations of Nine.

The panellists were isolated from each other in booths to prevent them influencing each other as was done during the reliability tests. The panellists used 13 sensory descriptors to describe and rate the intensity of the sensory attributes of the stiff porridges (Table 4.1). The panellists handled the stiff porridges using their hands, the way stiff porridge is normally consumed. Responses were written directly onto a questionnaire which was provided to each panellist. Analysis was replicated twice.

Table 4.1.
Descriptors developed by the trained sensory evaluation panel for the profiling of the provitamin A-biofortified maize stiff porridges

Attribute	Definition	References	Rating scale
Appearance			
Colour	Degree of colour intensity ranging from yellow to dark yellow.	Rama (rate 2) Deep yellow cheese	0 = light yellow 10 = deep yellow
Glossy	Degree of glossiness (shiny) of porridge ranging from opaque (not glossy) to very shiny.	Rama margarine (South African Brand) Ultramel custard	0 = opaque 10 = shiny
Roughness	The degree of roughness as seen on the surface of the porridge.	Ultramel custard Coarse white maize porridge (25% solid).	0 = not rough 10 = very rough
Aroma			
Overall aroma	The overall aroma intensity of maize porridge		0 = not intense 10 = very intense
Cooked maize aroma	The intensity of cooked maize aroma in the porridge.	Stiff coarse yellow maize porridge (25% solid)	0 = not perceived 10 = strongly perceived aroma
Rama-margarine aroma	The intensity of *Rama*-margarine aroma in the porridge.	Cooked soft yellow maize porridge with Rama margarine (South African Brand).	0 = not perceived 10 = strongly perceived
Texture			
Stickiness	The degree to which the porridge adhere to fingers.	Thin porridge (10% solid). White wheat dough	0 = not sticky 10 = very sticky
Hardness	The force required to compress the porridge	Thin cooked porridge (10% solid). Cooked coarse maize meal (33%)	0 = not hard 10 = very hard
Fineness	The degree of fineness of granules felt in the mouth	Coarse white maize meal (25%) Corn starch (25% solid)	0 = not fine 10 = very fine
Flavour			
Overall flavour	The overall flavour intensity of maize porridge		0 = not intense 10 = very intense
Cooked maize flavour	The intensity of cooked maize flavour in the porridge	25% cooked yellow maize porridge	0 = bland 10 = strong cooked maize flavour
Aftertaste			
Bitter	The bitter sensation after swallowing the porridge	Cold instant coffee solution (30% solid)	0 = Not intense 10 = very intense
Residual grain	The extent to which the particles are felt in the mouth after swallowing	Cooked coarse sorghum flour	0 = none 10 = a lot

4.2.5.2. *Sensory acceptability test*

Sixty (60) regular consumers of stiff maize porridge were recruited (door-to-door) from Ngulumbi village in Sibasa, Limpopo province, South Africa. The consumer panel consisted of 18 males and 42 females with no allergies to maize. The four samples of biofortified maize porridge and a reference porridge sample made with white maize were presented to each panellist in a central location (Figure 4.3). The panellists were seated far apart apart to prevent them from influencing each other. Sample labelling and presentation order were randomized as described in 4.2.5.1.

The consumer panel rated the sensory acceptability of the porridges in terms of colour, texture, taste, aroma and overall acceptability using a 5-point facial Hedonic scale, whereby 5 represented the highest score (like extremely) and 1 the lowest score (dislike extremely). Panellists were also provided with tap water to rinse their palates before and between testing. The 5-point facial Hedonic scale was used instead of the customary 9-point Hedonic scale because the consumers surveyed were semi-illiterate. Longer hedonic scales, e.g. 7 or 9 ratings, tend to confuse subjects with lower literacy levels, while scales that are shorter than the 5-point scale tend to cause end-point avoidance (Stone and Sidel 2004).

	
Representative consumers evaluating the porridges	Consumers leaving after evaluating the porridges

Figure 4.3. Sensory evaluation of stiff porridges

4.2.5.3. Ethical considerations

Ethical approval to conduct this study was obtained from the University of KwaZulu-Natal, Humanities and Social Sciences Ethics Committee (Appendix 3). Approval to perform the consumer acceptance test at Ngulumbi village in Sibasa, Limpopo province, South Africa, was obtained from the local chief who represented the tribal authority (Appendix 4 and 5). Both the descriptive test and consumer panel members signed a consent form (Appendix 6) to indicate their consent to participate in the study and to confirm that they understood the purpose of the study.

The panellists were informed that participation in the study was voluntary and they were free to withdraw from the study at any stage. The consent form for the descriptive test panel members included an agreement about payment for participation and that a panellist could be withdrawn from the study if his/her performance was not satisfactory and in that case they would be paid *pro rata*. In the consent form, it was also stated that personal information of the panellists would be kept confidential.

4.2.6. Statistical Analysis

The data on the grain physical properties, sensory profiling, consumer acceptability and provitamin A retention were evaluated using analysis of variance (ANOVA). Comparison of multiple means was performed using the Fisher's Least Significant Difference test (LSD) ($p < 0.05$). Furthermore, the descriptive sensory data were subjected to Principal Component Analysis (PCA) to evaluate and identify variations between provitamin A-biofortified maize porridges based on their sensory attribute loadings. The Agglomerative Hierarchical Clustering (AHC) method was used for segmentation. Ward's test was used for allocation of panellists to clusters (Ng'ong'ola-Manani et al 2013).

4.3. Results and Discussions

4.3.1. Provitamin A retention

The provitamin A-biofortified maize grains had quite similar content of provitamin A carotenoids which ranged from 2.4 to 2.58 µg g^{-1} (dry weight) (Table 4.2). As expected, there were no detectable provitamin A carotenoids in the reference white maize grain. Cooking the flour from the biofortified maize grains to stiff porridges seems to have influenced the measurable provitamin A carotenoid content of the porridges. The porridges prepared with flours from the biofortified maize grains significantly retained the provitamin A carotenoids (91.4-123.3%) with the porridge from PVAH1-26 having the highest retention while the stiff porridge from PVAH 50-75 had the lowest provitamin A carotenoid retention. The low apparent retention of provitamin A carotenoids in the stiff porridges prepared with PVAH 50-75 was probably due to isomerisation of *trans*-β-carotene isomers to *cis*-β-carotene isomers. In this study, the β-carotene isomers measured in the stiff porridges were mainly *cis*-β-carotene (9-*cis*-β-carotene and 13-*cis*-β-carotene). The *cis*-β-carotene isomers are converted to vitamin A less efficiently than all-*trans*-β-carotene isomers (Nguyen and Schwartz 2000; Vicente et al 2011; Rogers et al 1993).

The substantially high apparent retention of provitamin A carotenoids in the biofortified maize porridges was similarly reported by Pillay et al (2014) when provitamin A-biofortied maize was cooked into *uphutu* (crumbly maize porridge) and samp. It appears that heat processing of materials containing provitamin A carotenoids enhances the availability of provitamin A carotenoids. The increase in apparent retention of provitamin A carotenoinds in the stiff porridges was probably due to cellular disruption of the food matrix by the heat (Boileau and Erdman 2004). This is supported by the results of studies which were conducted by Hwang et al (2012) and Chang et al (2013). Hwang et al (2012) reported a significant increase in lycopene, β-carotene and α-tocopherol content of the tomato which was oven baked at 160°C while Chang et al (2013) observed that cooking of green leafy vegetables resulted in considerably high β-carotene retention, 18-380% after boiling for 8 min and 2-3 times high after stir-frying for 4 and 8 min.

The observations reported in the current study and literature suggest that heat processing of food materials which are high in provitamin A carotenoids enhances their extractability from the food matrix. The differences in the retention of provitamin A in the different biofortified porridge samples of this study may be attributed to differences in provitamin A composition, different provitamin A molecules presumably have different heat sensitivities.

Table 4.2.
Provitamin A retention in provitamin A-biofortified maize stiff porridges

Variety	Provitamin A content (µg g^{-1} dry weight)		Provitamin A retention (%)
	Maize flour	Stiff porridge	
White maize flour	n.d	n.d	n.d
PVAH1-26	$2.58^{a} \pm 0.06$	$3.18^{a} \pm 0.01$	$123.30^{a} \pm 2.93$
PVAH27-49	$2.47^{ab} \pm 0.12$	$2.60^{b} \pm 0.01$	$105.30^{b} \pm 5.27$
PVAH50-75	$2.46^{a} \pm 0.07$	$2.24^{c} \pm 0.06$	$91.39^{c} \pm 4.23$
PVAH79-100	$2.40^{b} \pm 0.08$	$2.60^{b} \pm 0.01$	$108.41^{b} \pm 3.82$

Means ± SD. Mean values followed by different letters in a column are significantly different ($p<0.05$) according to LSD test. PVAH = provitamin A hybrid. n.d = not detected.

4.3.2. Sensory quality of the porridges

The sensory attributes of the biorfortified maize porridges as described by the trained panel are presented in Table 4.3. The porridges were described as sticky, fine with intense cooked maize flavour and aroma. *Rama*-margarine aroma, bitter aftertaste and residual grain were also perceived, but at very lower intensity. Table 4.3 indicates that, generally, the four porridges had similar attribute intensity. However, by principal component analysis (PCA), the porridges were separated based on sensory attributes (Figure 4.4). PC1 and PC2 explained 48.9% and 35.1% of the total variance, respectively. In PC1, the porridge made with PVAH 79-100 was separated from PVAH 1-26 porridge. The PVAH 79-100 porridge was characterised by a cooked maize flavour, roughness, overall aroma, cooked maize aroma and colour, whilst the PVAH 1-26 porridge was described as having an intense residual grain, stickiness, and fineness.

In PC 2, the porridge made with PVAH 27-49 was separated from the PVAH 50-75 porridge. The porridge of the variety PVAH 27-49 was characterised by an intense hardness, whilst the PVAH 50-75 porridge was associated with a glossy appearance and *Rama*-margarine aroma. Hardness was negatively correlated with glossiness and *Rama*-margarine aroma ($p<0.05$). The South African margarine of the brand *Rama* is yellowish in colour; carotenoid pigments may be partly responsible for this colour. Probably, the carotenoid pigments in the provitamin A-biofortified maize porridges contributed to the *Rama*-margarine flavour detected in the porridges of this study. Fineness, stickiness and residual grain may be linked to particle size of the meal. Carotenoid pigments may also have contributed to the stickiness of the porridges. Bitterness was probably caused by phenolic compounds in maize. Cereal grains are known to contain phenolic compounds, which influence the taste of cereal-based foods (Dykes and Rooney 2007).

The descriptive test panellists of this study commented that the two attributes bitter aftertaste and stickiness were not typical sensory properties of stiff white maize porridge. Bitter aftertaste and stickiness are therefore likely to negatively affect the sensory acceptability of provitamin A-biofortified maize stiff porridges compared to white stiff porridge as discussed further below.

Table 4.3.

Sensory attributes of provitamin A-biofortified maize stiff porridges

Attribute	Provitamin A bio-fortified yellow maize porridge samples			
	PVAH 79-100	PVAH 27-49	PVAH 1-26	PVAH 50-75
Colour	$4.9^{b} \pm 1.0$	$4.0^{a} \pm 0.8$	$4.6^{b} \pm 1.0$	$4.8^{b} \pm 1.0$
Glossy	$3.6^{a} \pm 0.9$	$3.6^{a} \pm 0.8$	$4.0^{a} \pm 0.9$	$3.8^{a} \pm 1.1$
Roughness	$4.1^{a} \pm 0.8$	$3.8^{a} \pm 0.7$	$3.9^{a} \pm 0.7$	$4.0^{a} \pm 0.8$
Overall aroma	$4.4^{a} \pm 1.3$	$4.3^{a} \pm 1.3$	$4.4^{a} \pm 1.2$	$4.3^{a} \pm 1.2$
Cooked maize aroma	$5.1^{a} \pm 1.9$	$4.9^{a} \pm 2.0$	$5.0^{a} \pm 1.9$	$5.1^{a} \pm 1.9$
Rama-margarine aroma	$3.3^{a} \pm 1.5$	$3.2^{a} \pm 1.5$	$3.4^{a} \pm 1.7$	$3.4^{a} \pm 1.6$
Stickiness	$5.2^{a} \pm 1.5$	$5.6^{a} \pm 1.4$	$5.7^{a} \pm 1.6$	$5.6^{a} \pm 1.3$
Hardness	$4.1^{a} \pm 1.4$	$4.2^{a} \pm 1.5$	$3.8^{a} \pm 1.3$	$3.9^{a} \pm 1.4$
Fineness	$4.7^{a} \pm 1.3$	$5.0^{a} \pm 1.4$	$5.2^{a} \pm 1.5$	$5.2^{a} \pm 1.4$
Overall flavor	$4.0^{a} \pm 1.9$	$3.7^{a} \pm 1.6$	$3.7^{a} \pm 1.6$	$3.5^{a} \pm 1.6$
Cooked maize flavour	$5.1^{a} \pm 1.9$	$4.9^{a} \pm 1.9$	$5.1^{a} \pm 2.0$	$4.9^{a} \pm 2.0$
Bitter	$1.8^{a} \pm 1.3$	$1.8^{a} \pm 1.4$	$1.8^{a} \pm 1.2$	$1.7^{a} \pm 1.2$
Residual	$2.7^{a} \pm 0.9$	$2.9^{a} \pm 1.0$	$2.8^{a} \pm 1.0$	$2.9^{a} \pm 0.9$

Means ± SD (n =10).

Mean values followed by different superscript letters in the same row are significantly different (p<0.05) according to LSD test.

PVAH = Provitamin A hybrid

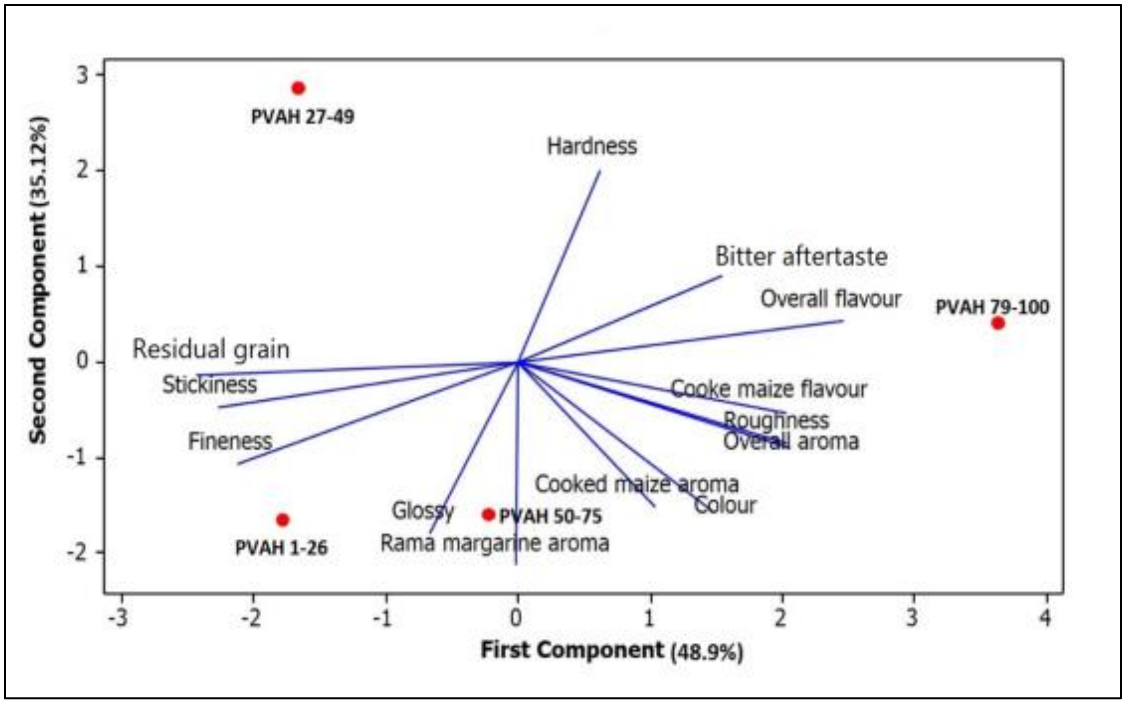

Figure 4.4. Biplot of principal component (PC) 1 versus PC2 loadings for four provitamin A-biofortified maize stiff porridges.

PVAH = Provitamin A hybrid

The acceptability of the porridges to the consumer panel is shown in Table 4.4. The sensory acceptability of the biofortified maize porridges was the same in terms of all the attributes evaluated. There was no significant difference in the sensory acceptability of the biofortified porridges and the white porridge with respect to all the attributes evaluated, including overall acceptability. The numerical values for mean ratings for texture, taste and overall acceptability may have been generally lower fort the biofortified porridges than for the white maize porridge. However, this is of no significance since there was no statistical difference.

The lower ratings for the taste and texture of the biofortified porridges relative to the white maize porridge may be attributed to the bitter aftertaste and stickiness, which were described by the descriptive panel. The bitter aftertaste and stickiness which were described by the descriptive sensory panel (Table 4.4) could have contributed to the lower acceptability of the biofortified maize porridges relative to the white maize porridge. In a study of consumer acceptance of yellow maize meal conducted in South Africa by Khumalo et al (2011), bitterness was also reported and it was suspected to negatively affect the acceptability of the yellow maize meal negatively. Therefore, it appears that there is a need to reduce the bitter aftertaste and stickiness of provitamin A-biofortified maize stiff porridge. This could be achieved through recipe development.

Table 4.4.

Sensory acceptability of provitamin A-biofortified maize stiff porridges

Sensory attribute	Provitamin A-biofortified maize stiff porridges				
	PAN 67	PVAH 27-49	PVAH 1-26	PVAH 50-75	PVAH 79-100
Colour	$4.6^{a} \pm 0.6$	$4.6^{a} \pm 0.7$	$4.6^{a} \pm 0.6$	$4.6^{a} \pm 0.5$	$4.5^{a} \pm 0.9$
Texture	$4.6^{a} \pm 0.7$	$4.5^{a} \pm 0.9$	$4.4^{a} \pm 0.9$	$4.6^{a} \pm 0.6$	$4.3^{a} \pm 1.0$
Taste	$4.7^{a} \pm 0.6$	$4.5^{a} \pm 0.8$	$4.4^{a} \pm 0.9$	$4.5^{a} \pm 0.7$	$4.4^{a} \pm 0.9$
Aroma	$4.6^{ab} \pm 0.7$	$4.6^{ab} \pm 0.7$	$4.4^{a} \pm 1.0$	$4.7^{b} \pm 0.5$	$4.5^{ab} \pm 0.9$
Overall acceptability	$4.7^{a} \pm 0.6$	$4.5^{a} \pm 0.8$	$4.5^{a} \pm 0.8$	$4.6^{a} \pm 0.5$	$4.6^{a} \pm 0.7$

Means ± SD (n =60). Mean values followed by different superscript letters in the same row are significantly different ($p<0.05$) according to LSD test.
PVAH = Provitamin-A hybrid.
PAN 67= Control.
Five-point hedonic scale ranged from 1 to 5 (1= dislike extremely, 2= dislike moderately, 3= neither like nor dislike, 4= like moderately, 5=like extremely).

Consumers were segmented into three clusters based on overall liking of the biofortified porridges (Table 4.5). Cluster 1 comprised 38.3% of the consumer panel; this cluster of consumers neither liked nor disliked the biofortified maize porridges. This cluster was dominated by female consumers (60.9%); almost 50% of the consumers in this cluster had an age range of 41-60 years, whilst the two age groups 20-40 years and 61-80 years had an equal proportion of consumers. Cluster 2 was made up 33.3% of consumer panel and the consumers in this cluster liked the biofortified maize porridges. About 90% of the consumers in this cluster were females; about 55% of the consumers in this cluster were between 41 and 60 years, inclusively, whilst the other two age groups had a similar proportion of consumers. Cluster 3 represented the smallest consumer group (28.3% of the consumer panel). Consumers in this cluster disliked the biofortified maize porridges. Similar to the other two clusters, this cluster was dominated by females (58.8%). Approximately 58.8% of these consumers had an age range of 20-40 years, whilst 5.9% were aged 41-60 years and 35.3% had age ranging from 61-80 years. The Chi-square test showed that, in all the three consumer clusters, consumer's liking of the porridge was not correlated with gender, but was significantly correlated with consumer age ($p<0.05$) (Table 4.5). Although, overall, the consumer panel rated the overall acceptability of the biofortified maize porridges to be as good as the white porridge, segmentation of the consumer panel showed that there were differences in the liking of the biofortified maize porridges across consumer clusters. Actually, only 33.3% of the consumer sample liked the biofortified maize porridges (Table 4.5).

In this study, consumer age seemed to have significantly contributed to the overall acceptance of the porridges.Consumers of middle age range (41- 60 years) either liked the biofortified maize porridges (55% of the consumers in cluster 2) or were neutral in their liking of the porridges (47.8% of the consumers in cluster 1). On the other hand, the highest proportion (58.8% of the consumers in cluster 3) of the consumers who disliked the biofortified maize porridges was in the youngest age group (20-40 years). As reviewed earlier, Pillay et al (2011) also found consumer age influenced the acceptability of provitamin A-biofortified food products. Similarly, these authors also found that consumer gender was not associated with the acceptance of the biofortified maize foods. However, Pillay et al (2011) found that the younger consumers (pre-school and younger school children) liked the biofortified maize products more than older consumers. But, the findings of Pillay et al (2011) cannot be differently compared with the findings of this study, because their study included children whereas the current study did not. Amongst the three age groups, the 20-40 years age group comprised of the most sensorially sensitive, socio-economically active, easily-influenced and fussy individuals. It is likely that their dislike of the biofortified maize porridges was due to a combination of these factors, for example they are likely to have had a higher sensitivity to the undesirable sensory properties of the biofortified maize porridges than consumers of the other age groups. They are also likely to have heard more about the negative stigma attached to yellow maize and influenced each other to dislike it. Although there are limitations to the findings of this study, they suggest that intervention aimed at increasing consumer acceptance of provitamin A-biofortified maize, e.g. nutrition education, should primarily target consumers of the age of about 20-40 years of age. The age group of about 41-60 years, whose 55% of the consumers liked the biofortified maize porridges, could be used to advocate for the consumption of the biofortified maize.

Table 4.5.

Relationship between consumer segments, gender and age

Cluster	Cluster description	Consumer	Gender		*P-values	Age			*P-values
		N, (%)	Male	Female		20-40	41-60	61-80	
1	Neither liked nor disliked	23 (38.3)	39.1	60.9	0.6	26.1	47.8	26.1	0.04
2	Liked	20 (33.3)	10.0	90.0		25.0	55.0	20.0	
3	Disliked	17 (28.3)	41.2	58.8		58.8	5.9	35.3	

N=60. *P-values generated using chi-square test

4.3.3. Conclusions

Except for colour, the physical properties of provitamin A-biofortified maize were found similar to those of white maize and hence it can be processed, e.g. milling, in the same manner as white maize. Provitamin A is substantially retained in the stiff porridges suggesting that stiff porridge is a suitable food type for delivering provitamin A to targeted consumers. The provitamin A-biofortified maize stiff porridge can be described as sticky, fine with low intensity residual grain, slightly bitter aftertaste with cooked maize flavour and aroma. Some of these attributes, e.g. bitter aftertaste and stickiness, may have contributed to the low acceptance of provitamin A-biofortfied maize stiff porridge. The results indicate that consumer age is a contributor to the acceptance of provitamin A-biofortified maize; it seems that when addressing the challenge of the low acceptance of the biofortified maize, it would be necessary to use different strategies on consumers of different age groups. Overall, the findings indicate that there is a need to improve the acceptance of provitamin A-bioforfied maize, which could be achieved through recipe development and nutrition education.

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CHAPTER 5: EFFECTS OF AMARANTH ADDITION ON THE QUALITY AND HEALTH-PROMOTING POTENTIAL OF EXTRUDED PROVITAMIN A-BIOFORTIFIED MAIZE SNACKS

Abstract

Provitamin A-biofortified maize snacks with added leafy vegetable may have potential as nutritious and health promoting products, especially in addressing vitamin A deficiency which is prevalent in Sub-Saharan Africa. The objective of this study was to determine the effect of adding Amaranth leaf powder on the physical properties, nutrient content, including provitamin A content, health-promoting potential and consumer acceptability of extruded provitamin A-biofortified maize snacks. The extruded snacks were prepared using four varieties of provitamin A-biofortified maize that were composited with Amaranth leaf powder at 0, 1 and 3% (w/w). As Amaranth increased, the snacks became darker as shown by decreased L* values while a* values increased. The hardness increased by 93%, water absorption index (WAI) and bulk density (BD) increased, while water solubility index (WSI) and expansion ratio (ER) decreased. The ash content ranged from 0.42-1.26 g/100 g, Zn and Fe ranged from 10.67-26.3 mg/kg, respectively, whilst protein ranged from 8.24-10.74 g/100 g. The content of lysine and methionine ranged from 0.19 to 0.26 g/100 g and 0.12 to 0.14 g/100 g, respectively. The snacks contained fairly high amount of provitamin A which ranged from 1.29 to 1.78 µg g^{-1}dry weight. The phenolic content and antioxidant activity varied from 31.0-98.7 mg Gallic acid equivalents (GAE/g dry weight) and 114.3-186.7 µmol Trolox Equivalents/g (µmol TE/g dry weight). The sensory quality of the snacks was affected by the addition of Amaranth leaf powder as shown by the fact that 32-44% neither disliked nor liked, and 24-32% liked moderately and a very low number (2-

8%) of consumer panel who extremely liked the snacks. Provitamin A-biofortified maize with added Amaranth leaf powder has a potential for use in nutritious and healthy extruded snacks. There are limited studies reporting on how best provitamin A maize can be processed with complementary plant foods which are common in Southern Africa, thus this study serves as a baseline.

5.1. Introduction

Snacks play an important part in the diet of many consumers who have limited free time for main meals and increased number of working hours (Bisharat et al 2013). The changing life styles and eating patterns of consumers have led to an increase in the demand for snack foods (Kocherla et al 2012). However, there are concerns about poor nutritional composition of snacks (Tan and Mattes 2013), and there is a demand for snacks that provide health benefits beyond basic nutrition (Reis and Abu-Ghannam 2014). The most commonly consumed snacks are in the extruded form. Extruded snacks are generally produced from the endosperm of cereal grains (Ozer et al 2006; El-Samahy et al 2007; Sawant et al 2013). Maize, a leading staple in sub-Saharan Africa, is the most dominant cereal in the processing of extruded snacks. Although white maize varieties are used, however these are deficient in provitamin A carotenoids, thus, dependency on maize increases the risk of vitamin A deficiency (Li et al 2010).

Currently, maize varieties which are bred for improved vitamin A content (provitamin A-biofortified maize) are being evaluated for use as a complementary strategy to alleviate vitamin A deficiency (Pillay et al 2013). Provitamin A-biofortified maize can be effectively utilized to improve the vitamin A content of maize-based extruded snacks. On the other hand, there is an interest in the role of phenolic compounds in the diet as antioxidants (Lamuela-Raventos et al 1999); and as a result green leafy vegetables are being incorporated in extruded snacks (Karkle et al 2009).

Green leafy vegetables are known to possess health-promoting properties because of their high phenolic content and antioxidant activity (Kraujalis et al 2013; Kwenin et al 2011). *Amaranthus cruentus*, a green leafy vegetable naturalized to Southern Africa is rich in phenolic antioxidant activity (144.24 µmol Trolox equivalents/g dry weight) compared to other leafy vegetables (Kraujalis et al 2013). Amaranth leaves are considerably high in the provitamin A carotenoid, β-carotene; minerals such as calcium, zinc, potassium, iron; as well as vitamin C (Venskutonis and Kraujalis 2013; Kwenin et al 2011). The health-promoting properties of provitamin A-biofortified maize-based extruded snacks could be enhanced by incorporation of Amaranth leaf powder in the formulation of extruded snacks.

The addition of Amaranth leaf powder could potentially influence the physical quality of extruded provitamin A-biofortified maize snacks. The key physical parameters of extruded snacks are appearance and degree of expansion (expansion ratio) (Obadina et al 2013; Coutinho et al 2013). Amaranth leaf powder may impart its green colour on the snacks while diluting the starch which is a key ingredient in extrusion and expansion. This may result in less expanded non-crispier snacks with high bulk density (Hashimoto and Grossman 2003). Therefore, the objectives of this study were to determine the effect of Amaranth leaf powder addition at different incorporation levels, on the physical and chemical properties of extruded provitamin A-biofortified maize snacks.

5.2. Materials and Methods

5.2.1. Materials

Four provitamin A-biofortified maize varieties (PVAH79-100, PVAH1-26, PVAH27-49 and PVAH50-75) were produced through conventional breeding methods at Cedara Research Station, Pietermaritzburg, South Africa. Fresh leaves of *Amaranthus cruentus* were obtained from the Agricultural Research Council (ARC), Roodeplaat, Pretoria, South Africa.

5.2.2. Sample preparation

Maize cobs of the selected varieties were manually harvested and dried for 21 days at ambient temperature (±25°C). The maize cobs were threshed manually and stored at 4°C until used.Provitamin A-biofortified maize was milled using a hammer mill (Model MK 150, Roff Mills, South Africa). Freshly harvested Amaranth leaves were manually sorted, cleaned and then steam blanched for 4 min, cooled to -5°C and dried at 40°C for 3 days using a forced-air drying oven and stored at -10°C until used. The dried Amaranth leaves were milled using a laboratory hammer mill (Glen Creston, Stanmore, England) fitted with a 1.0 mm sieve.

5.2.3. Extrusion

The maize flour was blended with dried Amaranth leaves powder at 0, 1 and 3% (w/w) Amaranth powder. The snacks were prepared by extrusion cooking using a Werner and Pfleiderer Continua 37 co-rotating twin-screw extruder (Stuttgart, Germany). Extrusion parameters were kept constant during the processing of the snacks. The barrel temperature was set at 140.7°C, feed moisture at 20%, screw speed at 400 rpm and a 5-mm restriction die was used. Samples were collected at the die at a temperature around 127°C. The extrudates were collected into containers, and then allowed to cool to room temperature, and then packaged in polyethylene bags.

5.2.4. Physical quality

5.2.4.1. Colour

The colour of the extrudates was measured using a pre-calibrated Hunter Lab colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) as described by Rhee et al (2004). Approximately 20 g of finely ground extrudates was poured into a dry clean glass sample cup, placed in a sample port, then covered and readings taken. The readings were recorded as Hunter Lab values where L^* (100 = white; 0 = black) is an indication of lightness; a^* measures chromaticity, with positive values indicating redness and negative values indicating greenness; while b^* measures chromaticity, with positive values indicating yellowness and negative values indicating blueness. Each sample was analyzed in triplicate.

5.2.4.2. Expansion ratio

The expansion ratio of each of the 10 randomly selected extrudates was determined by dividing the diameter of each extrudate by the extruder die diameter (5 mm) and results recorded as an average of 10 readings for each sample (Conway and Anderson 1973; Fan et al 1996).The extrudates expansion was calculated as:

$$Expansion\ ratio = \frac{diameter\ of\ extrudate}{diameter\ of\ die}$$

5.2.4.3. Bulk density (BD)

Bulk density (BD) was measured by filling a 400 mL beaker with extruded samples and the weight (g) recorded. The BD was calculated by dividing the weight of the sample (extrudates) by its volume (Pan et al 1998) as follows:

$$Bulk\ density = \frac{Extrudates\ mass(g)}{Extrudates\ volume\ of\ (mL)}$$

5.2.4.4. Hardness

The hardness of extruded provitamin A-biofortified maize snacks was determined using a texture analyzer TA-XTplus (Stable Micro Systems, Surrey, England) according to the method described by Li et al (2005). A probe HDP/WBV (Warner Bratzler set with a “V” slot blade) was attached to the texture analyser and the machine was calibrated. Ten randomly selected extrudates from each variety were analysed using the following parameters: pre-test speed 1.5 mm/s, test speed 2.0 mm/s, post-test speed 10.0 mm/s and a distance 30 mm. The results were recorded as an average of 10 readings per variety.

5.2.4.5. Water absorption index (WAI) and water solubility index (WSI)

WAI and WSI were determined as described by Anderson et al (1969) and Damardjati and Luh (1987). Approximately 2.5 g of ground dry extrudates sample was suspended in 30 mL distilled water in a 50 mL tarred centrifuge tube and mixed in a temperature-controlled water bath at 30°C for 30 min. The test tubes were centrifuged at 3000 rpm for 10 min. The supernatant was carefully decanted into a tared evaporating dish. The weights of the supernatant and the gel were recorded. The supernatant was dried overnight at 105°C using a hot-air oven drier. The dried solids recovered from evaporation were weighed to determine dissolved solids.

WAI and WSI were calculated as:

$$WAI=\frac{weight\ of\ gel\text{-}weight\ of\ ground\ dry\ sample}{weight\ of\ ground\ dry\ sample}$$

$$WSI=\frac{weight\ of\ dissolved\ solids}{weight\ of\ dry\ solids}\times 100$$

5.2.5. Nutritional content of extruded snacks

The mineral content, protein content, carbohydrate composition, essential amino acids and provitamin A carotenoid composition were determined as described in Chaper 3, section 3.2.3. Then, the apparent nutrient retention was calculated using the formula below:

$$\%apparent\ retention=\frac{nutrient\ content\ per\ g\ snack\ sample\ (dry\ weight\ basis)}{nutrient\ content\ per\ g\ raw\ maize\ meal\ (dry\ weight\ basis)}\times 100$$

5.2.6. Chemical properties

5.2.6.1. Total phenolic content

Total phenolic content of the extrudates was determined using the Folin-Ciocalteu method of Singleton and Rossi (1965) as described by Siwela et al (2007) with modifications. Finely ground dry extruded samples were weighed (0.3 g) and extracted with 30 mL acidified methanol (1% conc HCl in methanol) for 2 h at room temperature (approx. 25°C), with vortex mixing at 10 min intervals. The samples were centrifuged for 10 min at 3500 rpm using a temperature-controlled centrifuge set at 25°C. An aliquot of 0.5 mL acidified methanolic extract was transferred into a 50 mL volumetric flask and mixed with 2.5 mL Folin-Ciocalteu phenol reagent and 7.5 mL 20% (w/v) sodium carbonate was added within 8 min after addition of the Folin-Ciocalteu phenol reagent. The contents were mixed in the flask and made up to volume with distilled water and left to stand for 2 h at room temperature (approx. 25ºC), after which absorbance was read at 725 nm using a UV-VIS spectrophotometer. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram dry sample.

5.2.6.2. Antioxidant activity

Antioxidant activity of methanolic extracts was measured using the trolox equivalent antioxidant capacity (TEAC) as described by Siwela et al (2007). Extruded snacks powder (0.3 g) samples were weighed into 250 mL flat bottom flask. The samples were extracted with 30 mL acidified methanol (1% conc HCl in methanol) with shaking for 2 h at room temperature (approx. 25°C).The samples were centrifuged for 10 min at 3500 rpm using a temperature-controlled centrifuge set at 25°C. Aliquots of 8 m*M* 2,2´-azinobis [3-ethyl-benzothiazoline-6-sulfonic acid] (ABTS) and 3 m*M* potassium persulphate were mixed and allowed to react for 12 h at room temperature (approx. 25°C) in the dark to form radical cations $ABTS^{\cdot+}$. A working solution was prepared by adding 5 mL of the Mother solution to 145 mL of phosphate buffer solution in a 250 mL beaker. The solution was mixed by swirling the beaker. Standards or extracts from the extrudates (0.1 mL) were mixed with the working solution (2.9 mL) in the test tubes. The test tubes were vortex mixed for 30 sec, stored in the dark for 30 min and absorbance read at 734 nm using a UV-Vis spectrophotometer.

5.2.7. Sensory quality

The sensory quality of extruded snacks was evaluated using 50 regular consumers of extruded snacks who were recruited from the Department of Food Science and Technology, University of Venda, Limpopo province, South Africa. The consumer panel consisted of 22 males and 28 females with no reported allergies to maize-based diets. Labelled small containing about 20 extrudates were randomly presented to the consumer panel. The consumer panel rated the sensory acceptability of the snacks in terms of colour, texture, taste, aroma and overall acceptability using a 5-point facial Hedonic scale as described in Chapter 4, section 4.2.5.2.

5.2.7.1. Ethical considerations

Ethical approval to conduct this study was obtained from the University of KwaZulu-Natal, Humanities and Social Sciences Ethics Committee (Appendix 3). Consumer panel members signed a consent form (Appendix 6) to indicate their consent to participate in the study and to confirm that they understood the purpose of the study. The panellists were informed that participation in the study was voluntary and they were free to withdraw from the study at any stage. The consent form included an agreement about payment for participation and that a panellist could be withdrawn from the study if his/her performance was not satisfactory and in that case they would be paid *pro rata*. In the consent form, it was also stated that personal information of the panellists would be kept confidential.

5.2.8. Statistical Analysis

The snacks were produced at three levels of Amaranth leaf powder concentration (i.e. 0%, 1%, and 3% w/w, respectively) per variety. All the experiments were conducted in triplicates and the results expressed as mean ± SD. The data on the grain physical properties, nutritional content, consumer acceptability and provitamin A retention were evaluated using analysis of variance (ANOVA). Comparison of multiple means was performed using the Fisher's Least Significant Difference test (LSD) ($p < 0.05$).

5.3. Results and Discussions

5.3.1. Proximate composition of provitamin A-biofortified maize flour and Amaranth leaf powder

The proximate composition of the biofortified maize flours and Amaranth leaf powder is shown in Table 5.1. The biofortified maize flours had higher content of moisture, fat and carbohydrates compared to Amaranth leaf powder. However, the Amaranth leaf powder was significantly higher in protein, fibre and ash compared to the biofortified maize flours. Therefore, Amaranth would make a good complementary ingredient to increase the nutritional composition of the snacks with respect to these nutrients.

Table 5.1.

Proximate composition of provitamin A-biofortified maize flour and Amaranth leaf powder (dry weight)

Maize variety	Moisture (g/100 g)	Ash (g/100 g)	Fibre (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Carbohydrate (g/100 g)
PVAH79-100	$9.09^{c} \pm 0.03$	$0.66^{b} \pm 0.01$	$9.75^{c} \pm 0.03$	$3.71^{c} \pm 0.01$	$10.45^{c} \pm 0.01$	$66.34^{b} \pm 0.05$
PVAH1-26	$9.34^{c} \pm 0.03$	$0.52^{a} \pm 0.01$	$9.86^{c} \pm 0.03$	$3.50^{b} \pm 0.01$	$10.59^{d} \pm 0.01$	$66.18^{b} \pm 0.05$
PVAH27-49	$7.28^{a} \pm 0.27$	$0.85^{d} \pm 0.01$	$8.12^{a} \pm 0.27$	$4.33^{e} \pm 0.01$	$10.37^{b} \pm 0.01$	$69.05^{d} \pm 0.54$
PVAH50-75	$8.35^{b} \pm 0.03$	$0.71^{c} \pm 0.01$	$9.06^{b} \pm 0.02$	$3.75^{d} \pm 0.01$	$10.17^{a} \pm 0.01$	$67.95^{c} \pm 0.03$
Amaranth leaf powder	$7.50^{a} \pm 0.20$	$10.61^{e} \pm 0.01$	$18.11^{d} \pm 0.20$	$3.02^{a} \pm 0.01$	$32.51^{e} \pm 0.01$	$28.24^{a} \pm 0.39$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different ($p<0.05$) according to LSD test.PVAH = Provitamin A hybrid

5.3.2. Physical properties of extruded Amaranth-enriched provitamin A-biofortified maize snacks

In this study, the colour of extrudates was affected by the addition of Amaranth leaf powder (Figure 5.1). The snacks changed from yellow to dark yellowish green colour as the concentration of Amaranth leaf powder increased in the formulation. This effect was also confirmed by the Hunter Lab colorimeter results (Table 5.2). The L* values (lightness, 0 = black, 100 = Light) decreased with increasing concentration of Amaranth leaf powder while b* (yellowness) values increased. The L* values ranged from 66.7-78.2 while b* values varied from 29.7-33.7. A similar trend was reported by Ilo et al (1999) for rice-Amaranth extrudates.

The low L* values are an indication of the darkening colourof the snacks which was due to high concentration of Amaranth leaf powder, and according to Leonel et al (2009), an increase in dark colour can also indicate the extent of browning reaction such as caramelization, Maillard reaction, degree of cooking and also be due to pigment degradation during extrusion. Amaranth leaf contains a significant amount of lysine which could have reacted with reducing sugars formed during shear of starch and sucrose (Camire 2001). The yellowish colour as indicated by high b* values of snacks may be attributed to carotenoid pigments in the provitamin A-biofortified maize.

Figure 5.1. Extruded Amaranth-enriched provitamin A-biofortified maize snacks.

PVAH = Provitamin A hybrid.

Table 5.2.

Effect of Amaranth leaf powder on the colour of extruded provitamin A-biofortified maize snacks

Provitamin A maize variety	Amaranth (% w/w)	Colour of extruded snacks		
		L*	a*	b*
PVAH79-100	0	$78.2^{e} \pm 0.1$	$3.6^{d} \pm 0.1$	$31.7^{abcd} \pm 0.2$
	1	$69.6^{bc} \pm 0.2$	$2.1^{a} \pm 0.1$	$31.7^{abcd} \pm 0.3$
	3	$69.4^{c} \pm 0.8$	$3.6^{d} \pm 0.4$	$33.7^{d} \pm 1.0$
PVAH1-26	0	$76.9^{e} \pm 2.3$	$3.3^{cd} \pm 0.3$	$32.3^{bcd} \pm 2.0$
	1	$73.5^{d} \pm 0.8$	$2.3^{ab} \pm 0.1$	$29.7^{a} \pm 0.3$
	3	$69.4^{bc} \pm 0.5$	$4.1^{e} \pm 0.3$	$32.4^{bcd} \pm 1.1$
PVAH27-49	0	$77.4^{e} \pm 2.2$	$3.0^{c} \pm 0.1$	$32.7^{cd} \pm 2.0$
	1	$70.5^{c} \pm 0.8$	$2.3^{ab} \pm 0.5$	$30.3^{ab} \pm 1.2$
	3	$70.5^{c} \pm 0.3$	$2.3^{ab} \pm 0.0$	$30.8^{abc} \pm 0.0$
PVAH50-75	0	$76.8^{e} \pm 1.6$	$3.6^{d} \pm 0.2$	$31.6^{abcd} \pm 0.4$
	1	$67.4^{ab} \pm 0.4$	$2.0^{a} \pm 0.1$	$31.8^{abcd} \pm 2.0$
	3	$66.7^{a} \pm 1.1$	$2.6^{b} \pm 0.1$	$31.2^{abc} \pm 0.5$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different (p<0.05) according to LSD test.PVAH = Provitamin A hybrid.

The incorporation of Amaranth leaf powder affected other physical properties of the extruded snacks, in addition to colour change. The expansion ratio (ER) and water solubility index (WSI) decreased as the Amaranth leaf powder concentration increased, while the water absorption index (WAI), bulk density (BD) and hardness increased (Table 5.3). Expansion ratio ranged from 2.1-3.3, with the snacks made with PVAH 1-26 at 0% Amaranth leaf powder concentration having the highest expansion (3.3) compared to other varieties at the same Amaranth leaf powder concentration. The snacks made with PVAH 79-100, PVAH 1-26 and PVAH 27-49 at 3% Amaranth leaf powder concentration had the lowest expansion ratio (2.1). The extruded snacks made with PVAH 27-49 at 0% concentration of Amaranth leaf powder had the lowest bulk density (0.4 g/cm^3) while all the snacks at 3% Amaranth leaf powder concentration had the highest bulk density (0.7 g/cm^3). The hardness of extruded snacks as measured by texture analyser varied from 2.4-7.3 N.

The low expansion ratio of snacks may be attributed to dilution of starch by fibre (from Amaranth leaf powder) which seems to have interrupted the expanding capacity of starch and resulted in poor bubble growth during extrusion. Fibre is known to rupture the cell walls of extrudates before they reach their maximum expansion (Ferreira et al 2012). The fibre also tends to bind water more tightly during extrusion compared to starch and protein (Seth and Rajamanickam 2012). The negative correlation between expansion ratio and bulk density has been reported by several researchers (Omwamba and Mahungu 2014; Jozinovic et al 2012; Deshpande and Poshadri 2011). These researchers observed a reduction in expansion ratio with corresponding increase in bulk density when high fibre flours were incorporated in the feed material. According to Hashimoto and Grossman (2003) less expanded snacks are usually tough, non-crisp, with compact microstructure and undesirable texture. Such snacks tend to have a hard texture. This relationship between expansion ratio, bulk density and hardness is in agreement with the findings in the current study (Table 5.3). The increase in hardness of extrudates at 3% Amaranth leaf powder concentration is similar to a study reported by Ilo et al (1999) where the texture of rice-Amaranth extrudates increased at high Amaranth leaf powder concentration.

As mentioned earlier, the water absorption index (WAI) of snacks increased as Amaranth leaf powder concentration increased in the feed material. PVAH 27-49 snack at 0% Amaranth concentration had the lowest WAI (3.8 g g^{-1}) while the snack made with PVAH 79-100 at 3% Amaranth leaf powder concentration had the highest WAI (5.9 g g^{-1}) compared to other varieties at same levels of Amaranth addition. The WAI is used as an index of starch gelatinisation since native starches do not absorb water at room temperature (Seth and Rajamanickam, 2012). Contrary to high WAI values, the water solubility index (WSI) decreased at high concentration of Amaranth leaf powder. The WSI values of snacks ranged from 7.0-8.0% at 0% concentration of Amaranth leaf powder, and decreased to 6.0-7.0% at 3%. The snacks made with PVAH 50-75 at 0% Amaranth leaf powder concentration had the highest WSI (8.0%) compared to other varieties at all the concentrations of Amaranth leaf powder. In the current study, it appears that the high water absorption properties of water-soluble fibres interfered with the degradation of starch granules probably resulting in higher WAI values due to fibre absorbing water and lower WSI values, due to reduced starch gelatinisation.

Table 5.3.

Effect of Amaranth leaf powder on other physical properties of extruded provitamin A-biofortified maize snacks (dry weight)

Maize variety	Amaranth (% w/w)	Hardness (N)	Bulk density ($g\ m^{-3}$)	Expansion ratio	Water absorption index ($g\ g^{-1}$)	Water solubility index (%)
PVAH 79-100	0	$3.8^{bc} \pm 0.6$	$0.5^{b} \pm 0.0$	$3.2^{f} \pm 0.2$	$4.3^{c} \pm 0.1$	$7.6^{c} \pm 0.0$
	1	$2.6^{a} \pm 0.7$	$0.5^{b} \pm 0.0$	$2.6^{cd} \pm 0.2$	$5.0^{c} \pm 0.3$	$6.8^{b} \pm 0.4$
	3	$7.3^{e} \pm 2.5$	$0.7^{d} \pm 0.0$	$2.1^{a} \pm 0.3$	$5.9^{d} \pm 0.1$	$6.0^{a} \pm 0.2$
PVAH 1-26	0	$3.3^{abc} \pm 0.3$	$0.5^{b} \pm 0.0$	$3.3^{f} \pm 0.5$	$4.0^{ab} \pm 0.3$	$7.0^{b} \pm 0.2$
	1	$3.1^{ab} \pm 0.6$	$0.5^{b} \pm 0.0$	$2.5^{bc} \pm 0.2$	$5.0^{c} \pm 0.2$	$6.8^{b} \pm 0.6$
	3	$4.1^{cd} \pm 0.6$	$0.7^{d} \pm 0.0$	$2.1^{a} \pm 0.1$	$5.8^{d} \pm 0.1$	$6.0^{a} \pm 0.1$
PVAH 27-49	0	$3.2^{abc} \pm 0.3$	$0.4^{a} \pm 0.0$	$3.1^{f} \pm 0.3$	$3.8^{a} \pm 0.1$	$7.9^{c} \pm 0.2$
	1	$2.4^{a} \pm 0.8$	$0.6^{c} \pm 0.0$	$2.3^{ab} \pm 0.1$	$4.0^{ab} \pm 0.1$	$7.0^{b} \pm 0.2$
	3	$4.7^{d} \pm 1.1$	$0.7^{d} \pm 0.1$	$2.1^{a} \pm 0.2$	$5.3^{c} \pm 0.3$	$6.6^{b} \pm 0.2$
PVAH 50-75	0	$3.6^{bc} \pm 0.3$	$0.5^{b} \pm 0.0$	$3.2^{f} \pm 0.4$	$3.9^{a} \pm 0.1$	$8.0^{c} \pm 0.2$
	1	$3.1^{ab} \pm 0.5$	$0.6^{c} \pm 0.0$	$3.0^{ef} \pm 0.5$	$3.9^{a} \pm 0.1$	$7.6^{c} \pm 0.2$
	3	$4.7^{d} \pm 0.4$	$0.7^{d} \pm 0.0$	$2.8^{de} \pm 0.3$	$4.3^{c} \pm 0.2$	$7.0^{b} \pm 0.2$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different $p<0.05$ according to LSD test.PVAH = Provitamin A hybrid

5.3.3. Effect of Amaranth leaf powder addition on nutritional quality of extruded provitamin A-biofortified maize snacks

Snacks play an important role in curbing hunger between meals. Therefore, adequate amounts of nutrients in snacks could be beneficial in the alleviation of micronutrient deficiencies and associated health conditions. The nutritional content of the biofortified grain used to produce the extruded snacks was reported and discussed in Chapter 3. As mentioned in section 5.1, Amaranth leaf is nutritious and possesses health-promoting properties. The effect of Amaranth addition on nutrient composition is reported in sub-sections 5.3.3.1 to 5.3.3.2.

5.3.3.1. Effect of Amaranth addition on mineral content of provitamin A-biofortified maize snacks

The effect of Amaranth leaf powderaddition on the mineral content of extruded provitamin A-biofortified maize snacks is presented in Table 5.4. Extrusion cooking is known to produce significant retention of nutrients due to the short residence time of food material in the barrel and degradation of anti-nutritional components (Singh et al 2007; Razzaq et al 2012). However, in the current study a decrease in ash content of extrudates (at 0% Amaranth leaf concentration) relative to the ash content of the grain was observed except for PVAH 1-26.Addition of Amaranth leaf powder to the feed material seems to have significantly influenced the nutritional composition of the extruded snacks. The ash content of the snacks made with the biofortified varieties PVAH 79-100 increased as the concentration of Amaranth leaf powder increased while the ash content of snacks made with other biofortified varieties had a tendency to decrease at 1% Amaranth leaf powder concentration (PVAH 1-26) and 3% Amaranth leaf powder concentration (PVAH 27-49 and PVAH 50-75)(Table 5.4). The Zn decreased with increased Amaranth leaf powder concentration whilst the Fe generally increased. The biofortified varieties had higher ash and Fe content than the white variety. The snacks made with 1-26 at 1% and 3% Amaranth leaf powderconcentrations had the lowest ash content (0.42 and 0.65 g/100 g, respectively) whilst PVAH 50-75 had the highest values at the same Amaranth leaf powder concentrations (1.26 and 0.89 g/100 g, respectively). The snacks made with PVAH 1-26 had the lowest Fe content (67.3 mg/kg) at 1% Amaranth leaf powder concentration whilst PVAH 27-49 had the lowest Fe content (112.7 mg/kg) at 3% Amaranth leaf powder concentration. At 3% Amaranth leaf powder concentration, PVAH 79-100 snacks had the highest Fe content (259 mg/kg) compared to all other varieties at the same concentration. These results indicate that Amaranth leaf powder made a significant contribution towards improved mineral content of the extruded snacks. As reported in extensive reviews on the nutritional composition of vegetable Amaranth and its grain, Amaranth is nutritionally superior to cereal grains (Venskutonis and Kraujalis 2013; Escudero et al 1999; Makobo et al 2010). The increase in ash and Fe content of snacks would make a significant improvement in their nutritional quality. Fe is one of the leading deficient micronutrients in maize dependent populations. Therefore, the marked increase of Fe content in extruded snacks would strengthen the efforts to alleviate Fe deficiency in snacking vulnerable populations.

Table 5.4.

Mineral composition of provitamin A-biofortified maize grain, extruded snacks and Amaranth leaf powder (dry weight)

Maize variety and Amaranth	Amaranth concentration	Ash g/100 g	Fat g/100 g	ADF g/100 g	NDF g/100 g	Zn mg/kg	Fe mg/kg
White maize	Grain	1.02^{j} ± 0.01	5.81^{k} ± 0.01	4.90^{de} ± 0.01	10.46^{f} ± 0.01	26.33^{e} ± 0.58	20.67^{b} ± 0.58
	0%	0.40^{a} ± 0.04	0.04^{a} ± 0.02	2.36^{a} ± 0.04	4.53^{c} ± 0.15	25.00^{e} ± 2.65	120.00^{e} ± 2.65
PVAH79-100	Grain	0.66^{def} ± 0.01	3.71^{i} ± 0.01	5.30^{e} ± 0.00	10.60^{f} ± 0.00	20.33^{bcd} ± 0.58	8.67^{a} ± 0.58
	0	0.55^{cd} ± 0.05	0.44^{d} ± 0.06	2.96^{b} ± 0.04	17.18^{h} ± 0.58	25.00^{e} ± 1.73	115.67^{de} ± 4.93
	1	0.70^{efg} ± 0.03	0.06^{a} ± 0.03	1.89^{a} ± 0.12	4.51^{c} ± 0.18	21.00^{cd} ± 2.65	155.33^{f} ± 5.51
	3	0.81^{ghi} ± 0.03	0.12^{b} ± 0.02	6.46^{f} ± 0.28	5.06^{c} ± 0.46	18.67^{bc} ± 1.53	259.00^{j} ± 2.65
PVAH1-26	Grain	0.52^{abc} ± 0.01	3.50^{h} ± 0.02	6.30^{f} ± 0.00	9.90^{e} ± 0.00	20.33^{bcd} ± 0.58	8.67^{a} ± 0.58
	0	0.58^{cde} ± 0.02	0.46^{d} ± 0.01	3.36^{b} ± 0.23	17.12^{h} ± 0.57	26.33^{e} ± 1.53	118.33^{de} ± 1.53
	1	0.42^{ab} ± 0.04	0.13^{b} ± 0.03	2.16^{a} ± 0.52	1.56^{a} ± 0.12	17.33^{b} ± 1.53	67.33^{c} ± 3.79
	3	0.65^{def} ± 0.08	0.45^{d} ± 0.07	3.88^{c} ± 0.34	6.75^{d} ± 0.26	10.67^{a} ± 1.53	117.67^{de} ± 3.21
PVAH27-49	Grain	0.85^{hi} ± 0.01	4.33^{j} ± 0.01	6.20^{f} ± 0.00	11.40^{g} ± 0.00	23.67^{de} ± 0.58	11.33^{a} ± 0.58
	0	0.58^{cde} ± 0.03	0.44^{d} ± 0.03	3.08^{b} ± 0.21	17.12^{h} ± 0.34	24.67^{e} ± 1.53	115.00^{de} ± 2.65
	1	0.80^{ghi} ± 0.03	0.57^{e} ± 0.04	4.65^{d} ± 0.17	5.04^{c} ± 0.29	20.33^{bcd} ± 2.89	188.00^{h} ± 2.65
	3	0.75^{fgh} ± 0.05	0.63^{f} ± 0.04	4.42^{d} ± 0.50	3.30^{b} ± 0.61	12.00^{a} ± 1.73	112.67^{d} ± 5.51
PVAH50-75	Grain	0.71^{fg} ± 0.01	3.75^{i} ± 0.01	6.00^{f} ± 0.00	11.50^{g} ± 0.00	23.67^{de} ± 0.58	8.33^{a} ± 0.58
	0	0.53^{bcd} ± 0.04	0.44^{d} ± 0.04	3.02^{b} ± 0.28	17.45^{h} ± 0.43	25.33^{e} ± 2.08	115.67^{de} ± 4.93
	1	1.26^{k} ± 0.03	0.53^{e} ± 0.04	4.58^{d} ± 0.76	3.52^{b} ± 0.22	23.00^{de} ± 2.65	240.00^{i} ± 4.36
	3	0.89^{i} ± 0.11	0.29^{c} ± 0.03	5.29^{e} ± 0.15	6.52^{d} ± 0.31	21.33^{cd} ± 2.08	179.33^{g} ± 4.62
Amaranth leaf powder		10.57^{l} ± 0.05	3.03^{g} ± 0.02	16.73^{g} ± 0.02	19.88^{i} ± 0.08	31.33^{f} ± 2.08	971.67^{k} ± 2.31

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different ($p<0.05$) according to LSD test.

PVAH = Provitamin A hybrid

5.3.3.2. *Effect of Amaranth leaf powderaddition on protein and essential amino acid content of provitamin A-biofortified maize snacks*

The addition of Amaranth leaf powder in the feed material significantly influenced the protein and amino acid content of the extruded snacks (Table 5.5). Comparing the protein content of the biofortified whole grain with that of the snacks without Amaranth leaf powder(0% Amaranth leaf powder), a significant decrease was recorded in protein content of snacks. This decrease in protein content was probably due to low lysine content of the extruded snacks. The available lysine is an indicator of the extent protein degradation during thermal processing (Singh et al 2007). The lysine content of the whole grain ranged from 0.19 to 0.26 g/100 g (dry weight) which was significantly higher than that of the extruded snacks (0.10 to 0.13 g/100 g dry weight) at 0% Amaranth leaf powder concentration.Probably, the reaction between Ɛ-amine group of lysine and the amide group of asparagines or glutamine which results in the emission of ammonia (Sobota and Rzedzicki 2009). The protein content of the biofortified varieties at 0% Amaranth leaf powder concentration was significantly higher (8.2-10.7 g/100 g) than that of the reference white variety (7.5 g/100 g). The addition of Amaranth leaf powder seems to have positively influenced the protein content of the extruded snacks. However, there was no well-defined trend observed on the influence of Amaranth leaf powder concentration on the protein content of the snacks. The protein content of snacks made with PVAH 1-26 was 9.12 g/100 g at 0% Amaranth leaf powder concentration. The addition of Amaranth leaf powder (1%) resulted in a decrease in the protein content of these snacks (8.24 g/100 g).As the Amaranth leaf powder concentration increasedto 3%, the proteincontent of snacks made with PVAH 1-26 increased significantly (9.13 g/100 g). An opposite trend was observed for snacks made with PVAH 27-49 where a decrease in protein content of the snacks was recorded at 3% Amaranth leaf powder concentration while snacks made with PVAH 50-75and PVAH 79-100 increased with increased concentration of Amaranth leaf powder. The same scenario of protein content of the snacks made with PVAH 1-26 and PVAH 27-49 was observed for methionine and phenylalanine contents across all the biofortified varieties. The essential amino acid content of the biofortified varieties ranged from 0.15-0.20 g/100 g for histidine compared to 0.18 g/100 g for white variety, 0.26-0.34 g/100 g for threonine compared to 0.22 g/100 g for white variety, 0.19-0.26 g/100 g for lysine compared to 0.21 g/100 g for white variety, 0.12-0.14 g/100 g for methionine compared to 0.20 g/100 g for white variety, 0.30-0.51 g/100 g for valine compared to 0.35 for white, 0.21-0.27 g/100 g for isoleucine compared to 0.21 g/100 g for white variety, 0.96-1.33 g/100 g for leucine compared to 0.90 g/100 g for white variety and 0.33-0.42 g/100 g for phenylalanine compared to 0.34 g/100 g for white variety. The Amaranth had significantly high protein and essential amino acid content than both the biofortified varieties and the reference white variety. As a rich source of nutrients, Amaranth leaf powder was expected to improve the nutritional content of the snacks. According to Venskutonis and Kraujalis (2013) and Alegbejo (2013), Amaranth leaf is rich in protein and contains twice the content of essential amino acid lysine compared to cereal grains. The claim by these authors was supported by high protein content of Amaranth (32.5 g/100 g) reported in this study.

Table 5.5.

Essential amino acid composition of extruded provitamin A-biofortified maize snacks (g/100 g dry weight)

Maize variety and Amaranth	Amaranth concentration	Protein	Histidine	Threonine	Lysine	Methionine	Valine	Isoleucine	Leucine	Phenylalanine
Reference white maize	Maize flour	$10.48^{ef} \pm 0.01$	$0.18^{abcd} \pm 0.06$	$0.21^{bcd} \pm 0.03$	$0.21^{e} \pm 0.05$	$0.20^{h} \pm 0.05$	$0.35^{cdef} \pm 0.04$	$0.21^{cdef} \pm 0.03$	$0.90^{cd} \pm 0.03$	$0.34^{a} \pm 0.03$
	Snack (0% Amaranth)	$7.49^{a} \pm 0.04$	$0.17^{b} \pm 0.02$	$0.14^{a} \pm 0.02$	$0.06^{a} \pm 0.01$	$0.10^{b} \pm 0.01$	$0.23^{a} \pm 0.02$	$0.16^{a} \pm 0.02$	$0.78^{a} \pm 0.02$	$0.30^{cde} \pm 0.03$
PVAH79-100	Maize flour	$10.45^{ef} \pm 0.01$	$0.17^{e} \pm 0.02$	$0.26^{ef} \pm 0.01$	$0.20^{cde} \pm 0.03$	$0.12^{bcde} \pm 0.02$	$0.30^{ef} \pm 0.02$	$0.22^{b} \pm 0.02$	$0.96^{de} \pm 0.02$	$0.37^{efg} \pm 0.02$
	Snack (0% Amaranth)	$9.24^{c} \pm 0.35$	$0.15^{a} \pm 0.02$	$0.23^{cde} \pm 0.02$	$0.13^{bcd} \pm 0.02$	$0.15^{cdef} \pm 0.02$	$0.28^{def} \pm 0.03$	$0.21^{ef} \pm 0.02$	$0.90^{cd} \pm 0.03$	$0.30^{bc} \pm 0.04$
	Snack 1% (Amaranth)	$9.72^{cd} \pm 0.40$	$0.20^{bcd} \pm 0.02$	$0.22^{cde} \pm 0.03$	$0.16^{cde} \pm 0.02$	$0.18^{fg} \pm 0.02$	$0.29^{ab} \pm 0.03$	$0.17^{ab} \pm 0.02$	$0.91^{cd} \pm 0.03$	$0.33^{bcde} \pm 0.02$
	Snack 3% (Amaranth)	$10.20^{def} \pm 0.55$	$0.20^{bcd} \pm 0.02$	$0.23^{cde} \pm 0.02$	$0.17^{de} \pm 0.01$	$0.19^{g} \pm 0.01$	$0.36^{bcdef} \pm 0.03$	$0.20^{bcdef} \pm 0.02$	$0.92^{cd} \pm 0.03$	$0.32^{bcd} \pm 0.03$
PVAH1-26	Maize flour	$10.59^{f} \pm 0.01$	$0.20^{bcd} \pm 0.03$	$0.28^{f} \pm 0.05$	$0.21^{e} \pm 0.02$	$0.14^{bcde} \pm 0.03$	$0.40^{g} \pm 0.04$	$0.27^{g} \pm 0.04$	$1.22^{f} \pm 0.07$	$0.40^{fg} \pm 0.04$
	Snack (0% Amaranth)	$9.12^{c} \pm 0.56$	$0.15^{a} \pm 0.02$	$0.18^{ab} \pm 0.03$	$0.10^{ab} \pm 0.02$	$0.14^{cdef} \pm 0.02$	$0.29^{abcd} \pm 0.02$	$0.18^{abcd} \pm 0.01$	$0.90^{cd} \pm 0.02$	$0.33^{bcde} \pm 0.02$
	Snack 1% (Amaranth)	$8.24^{b} \pm 0.58$	$0.17^{abc} \pm 0.02$	$0.20^{bc} \pm 0.02$	$0.13^{bcd} \pm 0.02$	$0.05^{a} \pm 0.02$	$0.28^{abc} \pm 0.01$	$0.17^{abc} \pm 0.02$	$0.79^{a} \pm 0.02$	$0.20^{a} \pm 0.01$
	Snack 3% (Amaranth)	$9.13^{c} \pm 0.43$	$0.20^{bcd} \pm 0.02$	$0.25^{def} \pm 0.01$	$0.13^{bcd} \pm 0.02$	$0.15^{def} \pm 0.02$	$0.33^{f} \pm 0.03$	$0.23^{f} \pm 0.02$	$0.92^{cd} \pm 0.01$	$0.37^{def} \pm 0.02$
PVAH27-49	Maize flour	$10.37^{def} \pm 0.01$	$0.18^{e} \pm 0.02$	$0.26^{ef} \pm 0.02$	$0.19^{de} \pm 0.04$	$0.13^{b} \pm 0.02$	$0.34^{cdef} \pm 0.02$	$0.21^{cdef} \pm 0.03$	$1.00^{e} \pm 0.03$	$0.33^{bcde} \pm 0.02$
	Snack (0% Amaranth)	$9.16^{c} \pm 0.52$	$0.17^{abcd} \pm 0.02$	$0.17^{ab} \pm 0.02$	$0.11^{bc} \pm 0.02$	$0.15^{def} \pm 0.02$	$0.31^{bcdef} \pm 0.02$	$0.20^{bcdef} \pm 0.01$	$0.93^{cd} \pm 0.02$	$0.35^{cdef} \pm 0.02$
	Snack 1% (Amaranth)	$9.79^{cde} \pm 0.34$	$0.18^{abcd} \pm 0.01$	$0.24^{cde} \pm 0.02$	$0.13^{bcd} \pm 0.02$	$0.12^{bcde} \pm 0.01$	$0.32^{ef} \pm 0.02$	$0.22^{ef} \pm 0.01$	$0.87^{bc} \pm 0.02$	$0.30^{bc} \pm 0.03$
	Snack 3% (Amaranth)	$9.10^{c} \pm 0.55$	$0.21^{d} \pm 0.02$	$0.23^{cde} \pm 0.02$	$0.13^{bcd} \pm 0.02$	$0.16^{ef} \pm 0.02$	$0.38^{f} \pm 0.02$	$0.23^{f} \pm 0.02$	$0.96^{de} \pm 0.02$	$0.42^{g} \pm 0.02$
PVAH50-75	Maize flour	$10.17^{def} \pm 0.01$	$0.15^{a} \pm 0.02$	$0.34^{g} \pm 0.01$	$0.26^{f} \pm 0.03$	$0.13^{cde} \pm 0.02$	$0.51^{g} \pm 0.02$	$0.27^{g} \pm 0.02$	$1.33^{g} \pm 0.02$	$0.42^{g} \pm 0.02$
	Snack (0% Amaranth)	$9.29^{c} \pm 0.42$	$0.15^{ab} \pm 0.01$	$0.23^{cde} \pm 0.01$	$0.11^{bc} \pm 0.02$	$0.14^{cdef} \pm 0.01$	$0.29^{abcde} \pm 0.02$	$0.18^{abcde} \pm 0.01$	$0.90^{cd} \pm 0.02$	$0.34^{cde} \pm 0.01$
	Snack 1% (Amaranth)	$10.06^{def} \pm 0.49$	$0.18^{abcd} \pm 0.01$	$0.24^{cde} \pm 0.01$	$0.14^{bcd} \pm 0.01$	$0.11^{bc} \pm 0.02$	$0.33^{abc} \pm 0.02$	$0.17^{abc} \pm 0.02$	$0.82^{ab} \pm 0.09$	$0.28^{b} \pm 0.07$
	Snack 3% (Amaranth)	$10.74^{f} \pm 0.57$	$0.21^{d} \pm 0.02$	$0.25^{def} \pm 0.02$	$0.17^{de} \pm 0.02$	$0.12^{bcde} \pm 0.01$	$0.37^{cdef} \pm 0.02$	$0.21^{cdef} \pm 0.03$	$0.96^{de} \pm 0.02$	$0.32^{bcde} \pm 0.03$
Amaranth	Amaranth leaf powder	$32.50^{g} \pm 0.03$	$0.31^{f} \pm 0.04$	$0.77^{h} \pm 0.04$	$1.13^{g} \pm 0.10$	$0.25^{h} \pm 0.03$	$1.01^{h} \pm 0.06$	$0.75^{h} \pm 0.03$	$1.34^{g} \pm 0.09$	$0.89^{h} \pm 0.02$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different ($p<0.05$) according to LSD test. PVAH = provitamin A hybrid

5.3.3.3. *Effect of Amaranth leaf powderaddition on measurable provitamin A of extruded provitamin A-biofortified maize snacks*

The provitamin A carotenoid content of biofortified whole grain, reference white maize, extruded maize snacks and Amaranth leaf powder is presented in Table 5.6. The carotenoid content of extruded with 0% Amaranth leaf powderwas lower compared to that of the whole grain. This implies that extrusion had an adverse effect on the carotenoid content of snacks. The addition of Amaranth leaf powdershowed a significant improvement in carotenoid content of the snacks. The carotenoid content of the snacks increased as the concentration of Amaranth leaf powderincreased. The β-carotene increased by 61, 66.7, 34.7, and 63.3% for PVAH 1-26, PVAH 27-49, PVAH 50-75 and PVAH 79-100, respectively, with reference to 0% Amaranth leaf powder addition. The β-carotene isomers 9-*cis*-β-carotene increased by 5.3, 7.7, 5.6, and 13.9% for PVAH 1-26, PVAH 27-49, PVAH 50-75 and PVAH 79-100, respectively while 13-*cis*-β-carotene increased by 19.4, 18.8, 12.1, and 12.1% for PVAH 1-26, PVAH 27-49, PVAH 50-75 and PVAH 79-100, respectively. The β-cryptoxanthin content of extruded snacks increased by 48.7, 25.6, 9.8, and 10.3% for PVAH 1-26, PVAH 27-49, PVAH 50-75 and PVAH 79-100, respectively while the total provitamin A content increased by 32.6, 29.3, 18.1, and 30.9% for PVAH 1-26, PVAH 27-49, PVAH 50-75 and PVAH 79-100, respectively.

The Amaranth leaf powder had substantially higher provitamin A carotenoid content when compared to the whole grain of the biofortified varieties. Amaranth leaf powderwas high in all carotenoids and provitamin A contents except for β-cryptoxanthin. The provitamin A carotenoids were not detected in the whole grain of white maize variety. The β-carotene of Amaranth leaf powder was more than 82% higher than that of biofortified maize varieties. The trend shown by the increase in carotenoid content of these snacks indicates that Amaranth leaf powder significantly influenced the final content of provitamin A carotenoids in the extruded snacks. High β-carotene content (28.5 mg/100 g) in Amaranth leaves was also reported by Dlamini et al (2010). A significant amount of provitamin A-carotenoids in the snacks was imparted by Amaranth leaf powder as mentioned, and as observed in Table 5.6, extrudates with higher levels of Amaranth leaf powder, also showed increased total provitamin A carotenoid content.

Table 5.6.

Provitamin A carotenoids composition of maize grain and Amaranth leaf powder

(µg g^{-1} dry weight)

Maize variety and Amaranth	Flour and Snacks	β-carotene	9-*cis*- β-carotene	13-*cis*- β-carotene	β-cryptoxanthin	Tot ProvA*
White maize flour	Whole grain	n.d	n.d	n.d	n.d	n.d
	0% Amaranth	n.d	n.d	n.d	n.d	n.d
PVAH79-100	Whole grain	$0.83^{c} \pm 0.01$	$0.53^{d} \pm 0.03$	$0.47^{bc} \pm 0.05$	$1.17^{fg} \pm 0.02$	$2.41^{g} \pm 0.09$
	0% Amaranth	$0.49^{a} \pm 0.02$	$0.36^{a} \pm 0.01$	$0.33^{a} \pm 0.01$	$0.39^{a} \pm 0.01$	$1.36^{a} \pm 0.02$
	1% Amaranth	$0.70^{b} \pm 0.01$	$0.41^{bc} \pm 0.01$	$0.36^{abc} \pm 0.01$	$0.43^{ab} \pm 0.01$	$1.69^{def} \pm 0.03$
	3% Amaranth	$0.80^{c} \pm 0.02$	$0.40^{abc} \pm 0.01$	$0.36^{abc} \pm 0.01$	$0.43^{ab} \pm 0.02$	$1.78^{ef} \pm 0.04$
PVAH1-26	Whole grain	$0.83^{c} \pm 0.03$	$0.53^{d} \pm 0.01$	$0.47^{bc} \pm 0.02$	$1.53^{i} \pm 0.04$	$2.59^{h} \pm 0.05$
	0% Amaranth	$0.41^{a} \pm 0.01$	$0.38^{abc} \pm 0.02$	$0.31^{a} \pm 0.01$	$0.39^{a} \pm 0.01$	$1.29^{a} \pm 0.01$
	1% Amaranth	$0.66^{b} \pm 0.00$	$0.40^{abc} \pm 0.01$	$0.37^{abc} \pm 0.01$	$0.58^{d} \pm 0.02$	$1.71^{def} \pm 0.02$
	3% Amaranth	$0.62^{b} \pm 0.02$	$0.38^{abc} \pm 0.01$	$0.36^{abc} \pm 0.01$	$0.41^{ab} \pm 0.03$	$1.56^{bc} \pm 0.02$
PVAH27-49	Whole grain	$0.86^{c} \pm 0.02$	$0.57^{d} \pm 0.05$	$0.47^{bc} \pm 0.02$	$1.15^{f} \pm 0.06$	$2.47^{gh} \pm 0.07$
	0% Amaranth	$0.47^{a} \pm 0.01$	$0.41^{abc} \pm 0.02$	$0.32^{a} \pm 0.01$	$0.39^{a} \pm 0.02$	$1.40^{a} \pm 0.02$
	1% Amaranth	$0.80^{c} \pm 0.01$	$0.42^{c} \pm 0.01$	$0.38^{abc} \pm 0.01$	$0.42^{ab} \pm 0.01$	$1.81^{f} \pm 0.02$
	3% Amaranth	$0.68^{b} \pm 0.02$	$0.39^{abc} \pm 0.01$	$0.37^{abc} \pm 0.01$	$0.49^{c} \pm 0.01$	$1.68^{cde} \pm 0.03$
PVAH50-75	Whole grain	$0.88^{c} \pm 0.05$	$0.57^{d} \pm 0.08$	$0.47^{bc} \pm 0.01$	$1.21^{g} \pm 0.06$	$2.52^{gh} \pm 0.13$
	0% Amaranth	$0.49^{a} \pm 0.02$	$0.36^{ab} \pm 0.03$	$0.33^{ab} \pm 0.03$	$1.38^{h} \pm 0.02$	$1.34^{a} \pm 0.02$
	1% Amaranth	$0.66^{b} \pm 0.04$	$0.38^{abc} \pm 0.01$	$0.37^{abc} \pm 0.01$	$0.45^{bc} \pm 0.01$	$1.63^{bcd} \pm 0.05$
	3% Amaranth	$0.63^{b} \pm 0.06$	$0.37^{abc} \pm 0.01$	$0.35^{abc} \pm 0.02$	$0.38^{a} \pm 0.01$	$1.54^{b} \pm 0.07$
Amaranth leaf	Powder	$68.77^{d} \pm 0.20$	$13.57^{e} \pm 0.03$	$14.00^{d} \pm 0.28$	$0.70^{e} \pm 0.03$	$96.68^{i} \pm 0.20$

*Total provitamin A = (all-trans+9-*cis*+13-*cis β-carotene* isomers) + 0.5(β-cryptoxanthin).

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different (p<0.05) according to LSD test.n.d = not detected, PVAH = provitamin A hybrid

5.3.4. *Effect of extrusion on the retention of selected nutrients in extruded provitamin A-biofortified maize snacks*

Some food nutrients undergo thermal degradation during cooking. According to Peluola-Adeyemi et al (2014) extrusion cooking may have desirable and undesirable effects on nutritional value of extruded snacks. Therefore, it is crucial to ensure that nutrients are retained in amounts that are sufficient to meet the recommended daily dietary requirements. Nutrient retention refers to a measure of the proportion of nutrient remaining in the cooked food in relation to the amount of that nutrient originally in a given weight of the food or raw material before cooking. In our study, the selection of the nutrients studied for retention properties was based on their recognition as limiting nutrients that are associated with rife adverse health conditions in most maize dependent populations (Galili and Amir 2013; White and Broadley 2005; Pathak et al 2004; Black 2003; Diaz et al 2003; Allen 2003). The deficiency of these nutrients is a public health concern and their bioavailability is a fundamental aspect of human diet. Provitamin A was significantly retained in the extruded snacks containing Amaranth powder compared to those without (Table 5.7). Extruded snacks made with PVAH 1-26 and PVAH 50-75 without Amaranth leaf powder had the lowest provitamin A carotenoid retention (53.6%), whilst PVAH 27-49 snacks with 1% Amaranth leaf powder retained 136.7%. The lower retention levels of provitamin A carotenoids could be due to heat destruction since provitamin A is known to be unstable at high temperatures (Singh et al 2007). Heat induces isomerisation of *trans*-carotenoids from their usual configuration in nature to the *cis*-form which is known to have low provitamin A activity (Vicente et al 2011; Rogers et al 1993). In contrast the higher provitamin A retention levels observed for the PVAH 27-49 could be due to enhanced extractability caused by extrusion cooking. The provitamin A carotenoids in the raw material are bound in the food matrix which makes them less measurable. During extrusion, the food matrix is disrupted resulting in the provitamin A carotenoids becoming more measurable. This would explain the apparent increase of provitamin A carotenoids in the snack and their significant retention. Nutritionally, this is significant because of the release of provitamin A carotenoids from the food matrix for utilisation by the human body. This is supported by some studies which have reported that cooking of carotenoid-rich food material increases the measurable carotenoid content of food. For instance, it was observed that cooking provitamin A-biofortified maize into *uphutu* (crumbled maize porridge) and samp resulted in significantly high provitamin A retention (Pillay et al 2014). Hwang et al (2012) reported a significant increase in lycopene, β-carotene and α-tocopherol content after oven baking of tomato at 160°C. Cooking of green leafy vegetables resulted in considerably high β-carotene retention, 18-380% after boiling for 8 min and 2-3 times high after stir-frying for 4 and 8 min (Chang et al 2013). The reported increases in the carotenoid contents have been attributed to the enhanced release of carotenoids through the disruption of the plant/food matrix by heat. Heat processing generally enhances the availability of vitamins and carotenoids by releasing them from the food matrix. The protein and essential amino acid retention of snacks made with the biofortified varieties ranged from 77.8-100.4% for protein. The extruded snacks made with PVAH 50-75 with 1% Amaranth leaf powder had significantly higher protein content compared to other snacks at all concentrations of Amaranth leaf powder. The lowest protein retention was observed in the extruded snacks made with PVAH 1-26 at 1% Amaranth leaf powder concentration. The snacks made with the biofortified varieties retained significantly high content of protein compared to the snacks made with white maize variety (71.5%). The lysine retention ranged from 47.5 to 143.3% with PVAH 1-26 at 0% Amaranth leaf powder concentration having the lowest and PVAH 27-49 at 0% Amaranth leaf powder concentration having the highest amount of retained lysine. The retention of methionine ranged from 42.4 to 267.3% with PVAH 1-26 at 1% Amaranth leaf powder concentration having the lowest and PVAH 50-75 at 1% Amaranth leaf powder concentration having the highest methionine. These results demonstrate that the amount of lysine and methionine retained in the extruded snacks made with the biofortified varieties was generally higher than that of the white maize snacks (77.3 and 157.8%, respectively). Significant amounts of ash, Zn and Fe were also retained. The extruded snacks of PVAH 27-49 at 0% Amaranth leaf powder concentration retained 69.7% ash while PVAH 50-75 at 1% Amaranth leaf powder concentration snacks retained 197.7% ash. PVAH 1-26 snacks at 0% Amaranth leaf powder concentrationretained 40.5% Zn whilst at 1% Amaranth leaf powder concentration the same variety (PVAH 1-26) retained 129.4% of Zn. The Fe content retained in the snacks made with biofortified varieties ranged from 56.9% to 224.2%with PVAH 1-26 at 1% Amaranth leaf powder concentration having the lowest content whilst PVAH 79-100 at 3% Amaranth leaf powder concentration retained the highest content. The snacks made with the white variety retained markedly high concentration of Fe (534.5%) compared to the biofortified varieties (56.9-224.2%).

Table 5.7.

Retention of nutrients in extruded provitamin A-biofortified maize snacks

Maize variety	Extruded snacks	Provitamin A (%)	Ash (%)	Zinc (%)	Iron (%)	Protein (%)	Lysine (%)	Methionine (%)
Reference white	Snack (0% Amaranth)	n.d	$42.6^{a} \pm 0.5$	$100.5^{de} \pm 20.6$	$534.5^{g} \pm 68.6$	$71.5^{a} \pm 0.3$	$77.3^{abc} \pm 90.8$	$157.8^{ab} \pm 104.1$
PVAH1-26	Snack (0% Amaranth)	$53.6^{a} \pm 2.3$	$111.0^{g} \pm 1.2$	$129.4^{g} \pm 4.2$	$175.0^{de} \pm 5.2$	$86.1^{bc} \pm 5.4$	$55.6^{ab} \pm 5.4$	$109.4^{a} \pm 15.0$
	Snack (1% Amaranth)	$66.0^{ef} \pm 2.0$	$85.8^{d} \pm 1.0$	$40.5^{a} \pm 5.1$	$56.9^{a} \pm 2.6$	$77.8^{ab} \pm 5.5$	$108.4^{abc} \pm 27.8$	$42.4^{a} \pm 19.0$
	Snack (3% Amaranth)	$60.2^{bcd} \pm 2.0$	$134.2^{i} \pm 1.4$	$65.9^{b} \pm 6.4$	$99.4^{b} \pm 1.8$	$86.2^{bc} \pm 4.1$	$118.8^{abc} \pm 15.7$	$96.9^{a} \pm 30.2$
PVAH27-49	Snack (0% Amaranth)	$56.6^{abc} \pm 2.2$	$69.7^{b} \pm 0.8$	$104.3^{e} \pm 7.3$	$167.2^{cd} \pm 9.2$	$88.3^{cd} \pm 5.0$	$143.3^{c} \pm 83.6$	$102.0^{a} \pm 29.7$
	Snack (1% Amaranth)	$136.7^{h} \pm 0.9$	$97.3^{f} \pm 0.7$	$48.5^{a} \pm 4.5$	$98.1^{b} \pm 7.1$	$87.8^{cd} \pm 5.3$	$67.8^{abc} \pm 53.8$	$94.0^{a} \pm 32.0$
	Snack (3% Amaranth)	$67.7^{f} \pm 3.1$	$85.4^{d} \pm 1.3$	$82.2^{bc} \pm 7.4$	$163.5^{cd} \pm 3.3$	$94.3^{cde} \pm 3.3$	$72.0^{abc} \pm 58.9$	$114.3^{a} \pm 14.3$
PVAH50-75	Snack (0% Amaranth)	$54.9^{ab} \pm 3.2$	$80.2^{c} \pm 1.3$	$107.2^{ef} \pm 10.8$	$133.9^{bc} \pm 5.3$	$98.7^{e} \pm 8.8$	$47.5^{a} \pm 3.8$	$92.1^{a} \pm 32.8$
	Snack (1% Amaranth)	$65.0^{def} \pm 5.1$	$197.7^{j} \pm 2.2$	$84.8^{cd} \pm 13.0$	$155.3^{cd} \pm 9.7$	$100.4^{e} \pm 7.8$	$105.1^{abc} \pm 14.5$	$267.3^{b} \pm 218.4$
	Snack (3% Amaranth)	$61.1^{cde} \pm 5.3$	$134.9^{i} \pm 1.0$	$90.6^{cde} \pm 3.8$	$207.8^{ef} \pm 10.9$	$98.9^{e} \pm 4.8$	$120.1^{abc} \pm 22.9$	$108.3^{a} \pm 36.1$
PVAH79-100	Snack (0% Amaranth)	$56.4^{abc} \pm 1.4$	$88.3^{e} \pm 1.7$	$122.9^{fg} \pm 7.5$	$166.9^{cd} \pm 7.4$	$88.4^{cd} \pm 3.4$	$90.1^{abc} \pm 23.0$	$100.9^{a} \pm 35.1$
	Snack (1% Amaranth)	$70.3^{fg} \pm 2.9$	$112.7^{g} \pm 1.8$	$75.0^{bc} \pm 8.8$	$134.5^{bc} \pm 9.2$	$93.0^{cde} \pm 3.9$	$134.6^{bc} \pm 8.6$	$152.8^{ab} \pm 56.7$
	Snack (3% Amaranth)	$73.8^{g} \pm 3.6$	$125.4^{h} \pm 1.0$	$84.5^{cd} \pm 13.7$	$224.2^{f} \pm 10.6$	$97.6^{de} \pm 5.4$	$127.2^{abc} \pm 21.8$	$181.3^{ab} \pm 66.4$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different ($p<0.05$) according to LSD test.

n.d, not detected. PVAH = provitamin A hybrid

5.3.5. *Effect of Amaranth addition on phenolic content and antioxidant activity of extruded provitamin A-biofortified maize snacks*

The effect of Amaranth leaf powder addition on phenolic content and antioxidant activity of extruded provitamin A biofortified maize snacks is presented in Table 5.8. The phenolic content and antioxidant activity of snacks increased as the concentration of Amaranth leaf powder increased in the feed material. The phenolic content of snacks ranged from 31.0 to 98.7 mg Gallic acid equivalents (GAE)/g dry weight, whilst antioxidant activity was in the range of 114.3 to 186.7 µmol Trolox Equivalent/g (µmol TE/g dry weight) corresponding to snacks containing 0% to 3% Amaranth leaf powder, respectively.

The snacks made with PVAH 27-49 with 3% Amaranth leaf powder had significantly higher phenolic content (98.7 mg GAE/g dry weight) compared to the snacks of other maize varieties at the same concentration of Amaranth leaf powder. However, PVAH 1-26 snacks at 3% Amaranth leaf powder concentration had the highest antioxidant activity (186.7 µmol Trolox Eq./g dry weight) compared to and the snacks of other provitamin A maize varieties at all Amaranth leaf powder concentrations. The lower antioxidant activity of PVAH 27-49 with 3% Amaranth leaf powder (174.0 µmol Trolox Eq./g dry weight), despite having highest phenolic content, can probably be explained by the fact that antioxidant activity is not only influenced by phenolics, but by other molecules present in the extrudates like carotenoids which also have antioxidant activity. As observed from the general trend, the addition of Amaranth leaf powder contributed to a significant increase in the phenolic content and antioxidant activity of the extrudates. Amaranth leaves, like most plants have high levels of phenolics and antioxidants. The correlation between phenolic content and antioxidant activity has been reported by Velioglu et al (1998) in a study on selected fruits, vegetables and grain products. Velioglu et al (1998) observed a significant correlation between phenolic content and antioxidant activity of methanolic extracts from selected fruits, vegetables and grain products. Similar observations were also reported by Bunea et al (2011) in a study on phenolic content and antioxidant activity of some wild and cultivated berries from Romania. In that study, wild berries were high in phenolic content and antioxidant activity compared to cultivated berries. An increase in phenolic content and antioxidant activity of extruded Amaranth-enriched provitamin A-biofortified maize snacks of the present study supports the claims that Amaranth leaves are the substantial source of phenolic compounds that possess high antioxidant activity (Khandaker et al 2008; Kahkonen et al 1999). It has been observed that heat-treatment of plant sources of phenolic compounds, either by boiling, steaming, or microwaving; may result in increased measurable phenolic content and antioxidant activity (Turkmen et al 2005; Dewanto et al 2002). The contribution of heat treatment of material to its elevated antioxidant activity cannot be overlooked. High temperatures induce Maillard reactions and formation of brown compounds. The Maillard reaction compounds are known to significantly contribute to antioxidant activity (Jozinovic et al 2012; Camire et al 2005).

Table 5.8.

Effect of Amaranth on phenolic and antioxidant activity of extruded provitamin A-biofortified maize snacks

Maize variety	Amaranth (%)	Phenolic content (mg of GAE/g dry weight)	Antioxidant activity (μmol TE/g dry weight)
PVAH79-100	0	$34.4^{b} \pm 4.5$	$123.7^{d} \pm 4.4$
	1	$50.9^{ac} \pm 0.3$	$137.0^{ad} \pm 11.2$
	3	$52.4^{ac} \pm 7.5$	$151.6^{a} \pm 12.6$
PVAH1-26	0	$31.0^{b} \pm 9.4$	$149.5^{a} \pm 12.6$
	1	$49.9^{bc} \pm 1.2$	$173.8^{bc} \pm 14.1$
	3	$52.4^{ac} \pm 2.1$	$186.7^{c} \pm 15.4$
PVAH27-49	0	$51.0^{bc} \pm 0.9$	$152.5^{ab} \pm 5.2$
	1	$60.6^{ac} \pm 8.0$	$168.1^{ac} \pm 15.3$
	3	$98.7^{b} \pm 2.0$	$174.0^{bc} \pm 23.5$
PVAH50-75	0	$45.1^{a} \pm 2.1$	$114.3^{d} \pm 6.8$
	1	$50.7^{bc} \pm 1.5$	$123.1^{d} \pm 3.9$
	3	$55.0^{c} \pm 4.7$	$150.7^{a} \pm 17.3$

Means ± SD. Mean values followed by different superscript letters in the same column are significantly different ($p<0.05$) according to LSD test.
PVAH = provitamin A hybrid

5.3.6. Effect of Amaranth leaf powder addition on sensory quality of extruded provitamin A-biofortified maize snacks

The sensory quality of food is of prime importance as it affects its consumer acceptability. The effect of Amaranth leaf powder addition on the sensory quality of the snacks is shown in Table 5.9. As Amaranth leaf powder concentration increased the colour and appearance of the snacks was extremely disliked by 4-12% and 0-20% of the consumer panel, respectively, whilst 2-24% and 6-18% of the consumer panel extremely liked their colour and appearance, respectively. The texture and taste of the snacks were extremely disliked by 2-18% and 4-22% of consumer panel, respectively, whilst 2-14% and 6-12% of the panel extremely liked their texture and taste, respectively. Overall, the snacks were disliked extremely by 0-8% of consumers, disliked moderately by 10-28%, whilst 32-44% neither disliked nor liked them. About 24-32% of the consumer panel moderately liked the snacks, whilst 2-8% extremely liked the snacks.

Sensory attributes such as colour, taste, texture and appearance are some of the key sensory qualities that contribute to consumer acceptance of snacks. The notable proportions of the consumer panel which extremely disliked these sensory attributes indicate that there is a need to improve these sensory attributes to increase the acceptance of the snacks. Consumers are becoming more health-conscious and as more consumers are continuously searching for diets which can positively impact on their health, there is more interest in foods with documented health benefits (Ying and Gantenbein-Demarchi 2013). Therefore, education about the nutritional and health benefits of the snacks made with provitamin A-biofortified maize varieties and Amaranth leaf powder could contribute to an increase in the overall acceptability of the snacks, especially to the consumers falling in the undecided cluster.

Table 5.9.

Percentage of panellists who gave the different ratings for the evaluated sensory attributes (n=50)

Maize variety		Rating	Appearance	Colour	Aroma	Texture	Taste	Overall Acceptability
ProvA snack	0% Amaranth	Dislike extremely	2[a] (4)[b]	2 (4)	4 (8)	1 (2)	4 (8)	1 (2)
		Dislike moderately	4 (8)	4 (8)	6 (12)	16 (32)	20 (40)	5 (10)
		Neither dislike nor like	12 (24)	8 (16)	28 (56)	11 (22)	12 (24)	22 (44)
		Like moderately	26 (52)	27 (54)	10 (20)	16 (32)	10 (20)	21 (42)
		Like extremely	6 (12)	9 (18)	2 (4)	6 (12)	4 (8)	1 (2)
PVAH 79-100	1% Amaranth	Dislike extremely	5 (10)	5 (10)	5 (10)	6 (12)	5 (10)	3 (6)
		Dislike moderately	8 (16)	9 (18)	10 (20)	6 (12)	11 (22)	5 (10)
		Neither dislike nor like	14 (28)	8 (16)	18 (36)	10 (20)	15 (30)	22 (44)
		Like moderately	20 (40)	20 (40)	12 (24)	26 (52)	16 (32)	16 (32)
		Like extremely	3 (6)	8 (16)	5 (10)	2 (2)	3 (6)	4 (8)
	3% Amaranth	Dislike extremely	0 (0)	0 (0)	1 (2)	5 (10)	6 (12)	2 (4)
		Dislike moderately	10 (20)	8 (16)	15 (30)	13 (26)	18 (36)	7 (14)
		Neither dislike nor like	5 (10)	5 (10)	21 (42)	15 (30)	11 (22)	20 (40)
		Like moderately	26 (52)	25 (50)	12 (24)	14 (28)	13 (26)	17 (34)
		Like extremely	9 (18)	12 (24)	1 (2)	3 (6)	2 (4)	4 (8)
PVAH1-26	1% Amaranth	Dislike extremely	5 (10)	5 (10)	5 (10)	2 (4)	6 (12)	4 (8)
		Dislike moderately	20 (40)	14 (28)	14 (28)	17 (34)	13 (26)	14 (28)
		Neither dislike nor like	12 (24)	8 (16)	14 (28)	14 (28)	19 (38)	17 (34)
		Like moderately	10 (20)	15 (30)	13 (26)	15 (30)	8 (16)	14 (28)
		Like extremely	3 (6)	8 (16)	4 (8)	2 (4)	4 (8)	1 (2)
	3% Amaranth	Dislike extremely	9 (18)	5 (10)	11 (22)	8 (16)	7 (14)	0 (0)
		Dislike moderately	10 (20)	11 (22)	9 (18)	15 (30)	15 (30)	14 (28)
		Neither dislike nor like	13 (26)	12 (24)	19 (38)	7 (14)	18 (36)	22 (44)
		Like moderately	14 (28)	14 (28)	8 (16)	17 (34)	8 (16)	12 (24)
		Like extremely	4 (8)	8 (16)	3 (6)	3 (6)	2 (4)	2 (4)
PVAH27-49	1% Amaranth	Dislike extremely	4 (8)	4 (8)	3 (6)	9 (18)	9 (18)	1 (2)
		Dislike moderately	9 (18)	13 (26)	18 (36)	16 (32)	15 (30)	14 (28)
		Neither dislike nor like	18 (36)	14 (28)	13 (26)	5 (10)	15 (30)	16 (32)
		Like moderately	16 (32)	18 (36)	8 (16)	15 (30)	4 (8)	15 (30)
		Like extremely	3 (6)	1 (2)	8 (16)	5 (10)	7 (14)	4 (8)
	3% Amaranth	Dislike extremely	5 (10)	6 (12)	5 (10)	8 (16)	6 (12)	2 (4)
		Dislike moderately	9 (18)	14 (28)	12 (24)	13 (26)	17 (34)	10 (20)
		Neither dislike nor like	12 (24)	11 (22)	16 (32)	8 (16)	13 (26)	21 (42)
		Like moderately	17 (34)	12 (24)	12 (24)	16 (32)	9 (18)	16 (32)
		Like extremely	7 (14)	7 (14)	5 (10)	5 (10)	5 (10)	1 (2)
PVAH50-75	1% Amaranth	Dislike extremely	4 (8)	2 (4)	8 (16)	5 (10)	11 (22)	1 (2)
		Dislike moderately	9 (18)	15 (30)	10 (20)	11 (22)	14 (28)	11 (22)
		Neither dislike nor like	17 (34)	12 (24)	19 (38)	16 (32)	17 (34)	18 (36)
		Like moderately	17 (34)	17 (34)	9 (18)	11 (22)	3 (6)	17 (34)
		Like extremely	3 (6)	4 (8)	4 (8)	7 (14)	5 (10)	3 (6)
	3% Amaranth	Dislike extremely	10 (20)	6 (12)	5 (10)	4 (8)	2 (4)	1 (2)
		Dislike moderately	8 (16)	7 (14)	10 (20)	10 (20)	14 (28)	10 (20)
		Neither dislike nor like	7 (14)	12 (24)	17 (34)	14 (28)	14 (28)	22 (44)
		Like moderately	18 (36)	19 (38)	16 (32)	18 (36)	14 (28)	13 (26)
		Like extremely	7 (14)	6 (6)	2 (4)	4 (8)	6 (12)	4 (8)

[a] Number of subjects; [b] Percentage of total number of participants; PVAH = provitamin A hybrid

5.3.7. Conclusions

The addition of Amaranth leaf powder to the extruded provitamin A-biofortified maize snacks had a significant effect on their quality attributes. The physical properties which play an important role in consumer acceptance of snacks were generally adversely affected as the concentration of Amaranth leaf powder was increased in the snack formulation. On the positive side, the nutrient content of the snacks was significantly improved by the addition of Amaranth leaf powder. The snacks made with the biofortified varieties had high protein, mineral and phenolic content as well as antioxidant activity. As mentioned above, the physical properties are key to the acceptance of the extruded snacks. Therefore, the physical quality of the snack produced with PVAH 27-49 at 1% Amaranth leaf powder concentration was fairly acceptable. This snack was the most tender (2.4 N), with relatively light (L* = 70.5) colour, high WSI (7.0%) and low WAI (4.0 g g^{-1}). The physical quality of PVAH 27-49 snack was complemented by appreciable phenolic content (60.6 mg GAE/g dry weight) and antioxidant activity of (168.1 μM TE/g dry weight). Besides having low crispness/crunchiness, this snack was extremely liked and moderately accepted by 8 and 30% of the consumer panel, respectively.

These results suggest that there is a need to improve the physical properties of extruded provitamin A-biofortified maize snacks, especially the expansion ratio, since high expanded snacks, including the extruded types, should be light and crispy with low bulk density.

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CHAPTER 6: GENERAL DISCUSSION

The purpose of the present study was to evaluate the effects of provtamin A-biofortification on the physical (including milling) and nutritional properties of maize grain, and the consumer acceptability of provitamin A-biofortified maize. Provitamin A Retention of provitamin A carotenoid during the processing of biofortified maize into stiff porridges and extruded snacks was also assessed. In this chapter, an overall interpretation of the findings of the study is made.

The findings of the evaluation of grain physical and nutritional properties of the provitamin A-biofortified maize varieties suggested that the majority of the provitamin A-biofortified maize varieties were superior compared to the reference white maize variety in terms of dry milling quality and levels Zn, Fe, protein and lysine. As stated earlier, in sub-Saharan Africa, dry milling is usually a key step in the processing of maize into several traditional dishes, e.g. porridges, gruels and beverages. The better dry milling quality of grains of the biofortified varieties relative to the white variety indicates that it would be easier to mill biofortified grain using the same equipment used for milling white maize grain. Therefore, there would not be technological and economic hurdles in dry milling of provitamin A-biofortified maize and this could promote its adoption as a food source.

The high levels of Zn and Fe, protein and lysine observed in the biofortified maize varieties compared to the white variety suggest another significant advantage of biofortified maize. Based on FAO/WHO standard, the concentrations of essential amino acids such as lysine in provitamin

A-biofortified is good when compared to the pattern of amino acid requirements for adults. The predominantly poor communities of the sub-Saharan African region are not only vulnerable to vitamin deficiency, but also to other essential nutrients, including minerals and amino acids. Therefore, besides alleviating vitamin A deficiency, provitamin A-biofortified maize would have the added advantage of contributing to addressing other nutrient deficiencies. Similar conclusions have been reported by Pillay et al (2013).

On the other hand, the current study findings echoed the findings of previous studies (e.g. Pillay et al 2011; De Groote and Kimenju 2008; Muzhingi et al 2008) that provitamin A-biofortified maize was less acceptable to regular consumers than white maize. In the present study, biofortified maize stiff porridges were liked by only 33% of the consumer panel; 38% of the panel neither liked nor disliked the porridges; whilst 29% of the consumer panel disliked the porridges. Unlike in previous studies where colour, flavour and aroma seemed to largely contribute to the low acceptability of provitamin A biofortified maize (Pillay et al 2011; De Groote and Kimenju 2008; Muzhingi et al 2008), in this study, the lower acceptability of the biofortified maize porridges appeared associated with their stickiness and bitter after taste. Porridges are a leading maize food product type in sub-Saharan Africa. The low acceptability of the biofortified maize porridges suggests that it would be a challenge to achieve the wide consumption of provitamin A maize by the target communities in sub-Saharan Africa. However, Pillay et al (2011) demonstrated that the acceptability of provitamin A-biofortified maize varied with food type. Also, these authors suggested that consumer education about the nutritional benefits of the biofortified maize could increase its acceptance by the targeted consumers. Therefore, there seems to be possibilities of increasing the acceptance and adoption of biofortified maize by the targeted consumers. This could be achieved by food development to moderate the less acceptable sensory attributes of the biorfortified maize, including stickiness and bitter after taste. Food product development would be coupled with promotion of the biofortifed maize through consumer education in combination with other strategies like providing incentives for growing and consuming the biofortified maize.

Evaluation of the nutritional and health-promoting complementarity of the biofortified maize varieties with *Amaranthus cruentus* in extruded snacks showed encouraging results as both properties increased with increasing Amaranth concentration. These findings highlight a potential to increase the nutritional and health-promoting potential of pro-vitamn A-biofortified maize food products by adding locally available and affordable leafy vegetables. However, the tendency of the leafy vegetable Amaranth to decrease the physical and sensory quality attributes of the snacks indicate that further research would be required to improve these quality attributes and thereby increase the overall acceptability of the snacks to regular consumers of the conventional white maize snacks. Different approaches could be used to improve the quality of the snacks, for example a more acceptable leafy vegetable could be sought or other ingredients could be added to moderate the less acceptable effects of Amaranth on the quality of the snacks.

6.2. The significance of study findings to food and nutrition security

Food and nutrition insecurity is a major challenge at household level in the South African context. Most households lack affordable, secure sufficient and healthy food for all, as a result they purchase and consume poor quality foods *(energy dense)* that compromise their active and productive life. South Africa compared to other Africa countries has taken a prominent stand in realising the right to food mandate through the fortification, supplementation and diet diversification interventions, however malnutrition still persist to be a threat. The South African National Health and Nutrition Examination Survey (SANHANES-1) conducted in 2012 reports that nutritional deficiencies are still prevalent in children in rural and urban areas (Shisana et al, 2013). The SANHANES study reports that an average of 43.6% children is vitamin A deficient, while 11% have iron deficiency. Although these figures show slight improvement from the previous surveys (2005 and 2008), they still point to a food security and health challenge that requires a long term sustainable solution. This situation therefore requires a proactive intervention and a swift transition from just focusing on under nutrition since micro-nutrient malnutrition is posing to be a threat aggravating the health and well-being of the vulnerable groups and communities. In this study the four pillars of food security were considered in order to develop food products using the most popular South African food (maize). Maize foods are a staple to most of households and consumed by both children and adults. Figure 6.1 shows how the four pillars of food security were integrated in the study in attempt to achieve nutrition security.

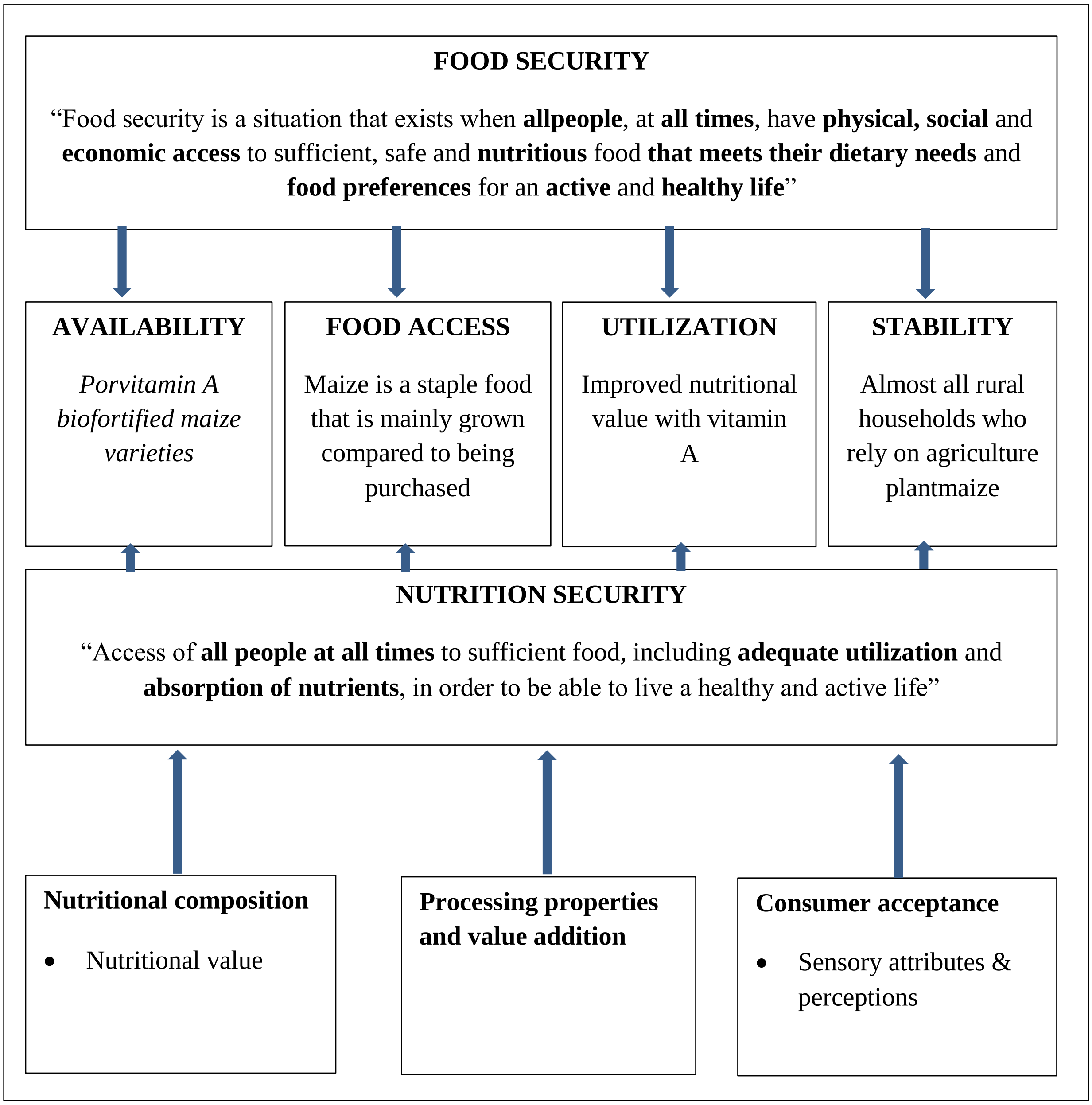

Figure 6.1: Significance of study findings to food and nutrition security

6.2. Reference

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CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

In the current study, it was found that provitaimin A-biofortified maize grain had superior dry milling quality compared to white grain. The better drying milling quality should be a significant attraction for the adoption of the biofortified maize by target communities in sub-Saharan Africa because drying milling is an essential step in the processing of maize in several popular food products.

The findings of this study indicated high retention of provitamin A in biofortified maize products, stiff porridges and extruded snacks. Thus, maize seems a suitable crop for provitamin A biofortification as it would deliver substantial amounts of the vitamin to the targeted consumers when processed into widely consumed maize food products. The addition of Amaranth enhanced the nutritional and health-promoting properties of the biofortified maize.

This study, like previous studies, found that the consumer acceptability ratings of provitamin A maize food products (stiff porridges and extrudated snacks) were not satisfactory. For the first time, a comprehensive characterisation of the sensory properties of provitamin-A-biofortified maize has been done. The study findings indicated that apart from the widely reported less desirable colour, aroma and flavour of the biofortified maize, there are other sensory attributes that also contribute to the low acceptable of the biorfotified maize, they include stickiness and bitter aftertaste. Therefore, a more comprehensive approach to the moderation of the less desirable sensory properties of the biofortified maize should be adopted during food product development.

Overall, it appears that provitamin A-biofortified maize has a potential to contribute to food and nutrition security and the well-being of communities in sub-Saharan Africa, who are predominantly food and nutrition insecure and are highly depended on white maize, which is devoid of vitamin A and as well being limited in other essential nutrients. However, further work, as stated in the next section, is required to exploit this potential.

7.2. Recommendations

There is a need for further improvement of the nutritional properties of the provitamin A-biofortified varieties in order to alleviate interlinked micronutrient deficiencies. Further, the consumer acceptance of the biofortified should be improved; this could be done through further food development, which could encompass trying a wide variety of maize food across the targeted consumers who have varied demographic and socio-cultural profiles and attenuating the intensities of the less desirable sensory attributes of the biorfortified maize. Consumer education and product promotions are recommended as complementary strategies for increasing the acceptance of provitamin A-biofortified maize.

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APPENDICES

Appendix 1. Publications from this work

1. Daniso Beswa, Muthulisi Siwela, Nomusa R. Dlamini and Eric O. Amonsou. Effect of extrusion on retention of provitamin A and other selected nutrients in Amaranth/provitamin A-biofortified maize snacks (Poster presentation). 2nd International Congress Hidden Hunger: Hidden Hunger, Childhood Development and Long-term Prospects for the future. Stuttgart, Germany, 03-06 March 2015.
2. Daniso Beswa, Nomusa R. Dlamini, Eric O. Amonsou, Muthulisi Siwela and John Derera. Effects of Amaranth Addition on the Provitamin A Content, and Physical and Antioxidant Properties of Extruded Provitamin A-Biofortified Maize Snacks. (Accepted). Journal of the Science of Food and agriculture.
3. Siwela M, D BESWA, E.O. Amonsou, N.R. Dlamini and J Derera. Effect of amaranth and provitamin A-biofortified maize on the physical quality and antioxidant activity of a maize extruded snack. AACCI Poster Abstract (http://www.aaccnet.org/meetings/Documents/2013Abstracts/2013Pab137.htm). Meeting of the AACCI, add city, New Mexico, USA, 29 September-2 October 2013.
4. Daniso Beswa, Muthulisi Siwela, Eric O. Amonsou, John Derera and Nomusa R. Dlamini. Effect of *Amaranth cruentus* addition on physical quality and antioxidant activity of extruded provitamin A-biofortified maize snacks (oral presentation). Proceedings of South African Association for Food Science and Technology Biennial Congress and Exhibition, CSIR, Pretoria, 7-9 October 2013.
5. Daniso Beswa, Unathi Kolanisi, Eric O. Amonsou, Nomusa R. Dlamini, John Derera and Muthulisi Siwela. Sensory properties and consumer acceptability of provitamin A-

biofortified maize stiff porridge (Poster presentation). Proceedings of South African Association for Food Science and Technology Biennial Congress and Exhibition, CSIR, Pretoria, 7-9 October 2013.

Appendix 2	Standardized recipe for preparation of stiff porridges

There are only two ingredients for making Venda stiff porridge, 1 part of maize meal and 5 parts of water. Making Venda stiff porridge involves brining water to the boil (96°C) and slowly adding half of maize meal just before the water starts boiling while stirring with a whisk. The stirring continues at low heat for 19 min or until a smooth soft porridge without lumps is achieved. The next step is slowly adding the remaining maize meal while pounding with a wooden spoon. Pounding continues for 46 min or until smooth with no lumps. At the end of cooking, the stiff porridge is immediately poured (while hot) on plate in a form of layers resembling thick pancakes, cooled and eaten with hands.

Appendix 3	Ethical clearance letter from Humanities and Social Sciences Research Ethics committee

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

Research Office, Govan Mbeki Centre
Westville Campus
Private Bag x54001
DURBAN, 4000
Tel No: +27 31 260 3587
Fax No: +27 31 260 4609
mohunp@ukzn.ac.za

25 August 2011

Mr D Beswa (210553813)
School of Agricultural Sciences and Agribusiness
Faculty of Science and Agriculture
Pietermaritzburg Campus

Dear Mr Beswa

PROTOCOL REFERENCE NUMBER: HSS/0748/011D
PROJECT TITLE: Evaluation of grain quality and food properties of provitamin A biofortified yellow/orange maize grain

In response to your application dated 12 August 2011, the Humanities & Social Sciences Research Ethics Committee has considered the abovementioned application and the protocol has been granted **FULL APPROVAL.**

Any alteration/s to the approved research protocol i.e. Questionnaire/Interview Schedule, Informed Consent Form, Title of the Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment /modification prior to its implementation. In case you have further queries, please quote the above reference number.
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 RESEARCH ETHICS COMMITTEE

cc. Supervisor: Dr M Siwela, Dr J Derera & Dr P Tongoona
cc: Mrs M Francis, Faculty of Science & Agriculture, PMB Campus

1910 - 2010
100 YEARS OF ACADEMIC EXCELLENCE

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 4.	Request for permission to conduct research
Appendix 4.1.	**Request letter by the researcher**

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

Tribal authority
Ngulumbi Village, Sibasa
Limpopo Province

Date: 6 June 2012

Dear Sir

Request for permission to conduct research in Ngulumbi Village

My name is Daniso Beswa, and I am a Food Security student at the University of KwaZulu-Natal (UKZN). The research I wish to conduct for my Doctoral thesis involves consumer acceptability of *Vhuswa* prepared from provitamin A-biofortified (produced by conventional breeding) yellow/orange maize grain. This project will be conducted under the supervision of Dr Siwela (UKZN, South Africa), Dr Derera (UKZN, South Africa) and Prof Tongoona (UKZN, South Africa).

I hereby seek your consent to conduct a consumer acceptability research at Ngulumbi Village. The proposal had been presented and approved by Humanities and Social Science Ethics Committee of UKZN. I have provided you with a copy of my one-page proposal which includes consent form and questionnaire to be used in the research process, as well as a copy of the approval letter from Humanities and Social Science Ethics Committee of UKZN.

Thank you for your time and consideration in this matter.

Yours sincerely,

DanisoBeswa (Researcher)

School of Agricultural, Earth and Environmental Sciences
Postal Address: SAEES Pietermaritzburg, Private Bag X01, Scottsville, 3209 South Africa
Telephone: +27 (0) 33 260 5515 Facsimile: +27 (0) 33 260 6094 Email: saees@ukzn.ac.za
1910 - 2010
100 YEARS OF ACADEMIC EXCELLENCE
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 4.2	Supporting request letter from the supervisor

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

Tribal authority
Ngulumbi Village, Sibasa
Limpopo Province

Date: 29 May 2012

Dear Sir

Request for permission to conduct research in Ngulumbi Village

On behalf of the University of KwaZulu-Natal (UKZN), African Centre for Food Security I hereby request Tribal Authority to grant Mr Daniso Beswa to conduct his PhD research at Ngulumbi Village. Mr Beswa is a PhD student at the UKZN and also attached to the Detpartment of Food Science at The University of Venda. His research involves consumer acceptability of *vhuswa* prepared from provitamin A-biofortified (produced by conventional breeding) yellow/orange maize grain. This project is under my supervision (Dr Siwela); the co-supervisors are Dr Derera (UKZN, South Africa) and Prof Tongoona (UKZN, South Africa).

The proposal had been presented and approved by Humanities and Social Science Ethics Committee of UKZN.

Yours sincerely,

Muthulisi Siwela, PhD (Pretoria)

School of Agricultural, Earth and Environmental Sciences
Postal Address: SAEES Pietermaritzburg, Private Bag X01, Scottsville, 3209 South Africa
Telephone: +27 (0) 33 260 5515 Facsimile: +27 (0) 33 260 6094 Email: saees@ukzn.ac.za
1910 - 2010
100 YEARS OF ACADEMIC EXCELLENCE
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 5	Permission letter from Ngulumbi Tribal Authority

Enq.: Vhamusanda Vho-NT Ratshitanga @ 072 289 2942

P. O. Box 39
Sibasa
0970
22 August 2012

Mr Daniso Beswa
Student number 210553813
University of KwaZulu-Natal
P/Bag X01
Scottsville, 3209

Dear Sir!

Re: **Request for permission to conduct research in Ngulumbi Village**

I, Nkhumbuleni Tendani Ratshitanga, Id. No.: **720619 5272 089,** Vhamusanda of **Ngulumbi village** hereby wish to inform you that the Ngulumbi Village community have agreed to grant you permission to conduct your research on **Consumer acceptability of *vhuswa* prepared from provitamin A-biofortified (produced by conventional breeding) yellow/orange maize grain** at Ngulumbi Village.

We wish you well in your research.

VHAMUSANDA VHO-RATSHITANGA N T
NGULUMBI VILLAGE
P O BOX 39
SIBASA, 0970
DATE: [illegible]
SIGNATURE: [illegible]

Regards,

Vhamusanda Vho-NT Ratshitanga

Ngulumbi Village

Cell.: 072 289 2942

Appendix 6.	**Consent forms for participating in sensory analysis of food products made with provitamin A-biofortified maize**
Appendix 6.1.	Consent form for participating in descriptive sensory analysis of provitamin A-biofortified maize stiff porridges

<u>Consent Form</u>

I am **Daniso Beswa**, a students at the University of KwaZulu-Natal doing PhD in Food Security. I am conducting a study (as the requirement for the course) on evaluation of grain quality and food properties of provitamin a biofortified yellow/orange maize grain. All data collected from this study will be confidential and will only be used as part of this research project. Rate each food sample provided using the 5-point hedonic scale and indicate how you feel about the selected sensory attributes on the product evaluation sheet. This information will be used to determine the consumer acceptability of *vhuswa* made from provitamin a biofortified yellow/orange maize grain.

I, …………………………................... (name) hereby confirm that the questionnaire has been clearly explained to me and I understand the purpose of this study and how this information is going to be tested.

I therefore agree to voluntarily participate in this research study.

……………………. …………………………

Signature Date

Appendix 6.2.	***TshiVenda*** **version of consent form for participating in consumer acceptability of provitamin A-biofortified maize stiff porridges and extruded provitamin A-biofortified maize snacks.**

Fomo ya Thendelo

Ndi nṋe vho **Daniso Beswa**, mutshudeni ane a khou ita Digirii ya Vhudokotela ha Vhutsireledzi ha Zwiḽiwa Yunivesithi ya KwaZulu-Natal. Ndi khou ita ngudo (sa ṱhoḓea ya khoso) nga ha u ṱhaṱhuvha vhuḓi ha goroi kana dzithoro na zwiṱaluli zwa zwiḽiwa zwa mavhele matswuku o biofothifaiwaho ane a vha na provithamini. Data yoṱhe yo kuvhanganyiwaho kha ngudo iyi i ḓo vha ya tshiphiri nahone i ḓo shumiswa fhedzi sa tshipiḓa tsha hei thandela ya ṱhoḓisiso. Elani kana kalani tsumbo dza zwiḽiwa zwo ṋetshedzwaho ni tshi khou shumisa tshikalo tsha hedoniki tsha ṱhodzi ṱhanu, ni sumbedze uri ni pfisa hani vhunzani ha zwipfi zwo topolowaho kha bammbiri ḽa u ṱhaṱhuvha tshibveledzwa. Mafhungo haya a ḓo shumiswa u kona u wanulusa u ṱanganedzea nga vharengi ha vhuswa ho itwaho vhu tshi bva kha mavhele matswuku o biofothifaiwaho ane a vha na provithamini.

Arali vha this khou tenda u dzhenelela kha ṱhoḓisiso idzi ri humbela uri vha ḓadzise fomo iyo ire nga fhasi.

Vha nga nkwama nga liṱongo arali huna zwinwe zwinzhi zwine vha ṱoḓa u ḓivha.

Vho-Beswa Daniso

015 162 8081 (dza mushumo)

083 705 5156 (ṱhingo thendeleki)

Nṋe,…………………………………………………………(dzina) ndi khwaṱhisedza uri mbudzisavhathu hei yo ṱalutshedzwa zwi pfalaho kha nṋe nahone ndi a pfesesa ndivho ya ngudo hei na uri mafhungo haya a ḓo lingwa hani.

Ndi dovha hafhu nda tenda u dzhenelela kha ngudo ya ṱhoḓisiso hei nga u tou funa nga nṋe muṋe hu si na u wana malamba.

.... ………………………………

Tsaino Datumu

Appendix 7	**Questionnaires for descriptive sensory analysis of stiff porridges and consumer acceptability of stiff porridges and extruded snacks made with provitamin A-biofortified maize**
Appendix 7.1	**Questionnaires for descriptive sensory analysis of stiff porridges**

School of Agricultural, Earth and Environmental Sciences

University of KwaZulu-Natal

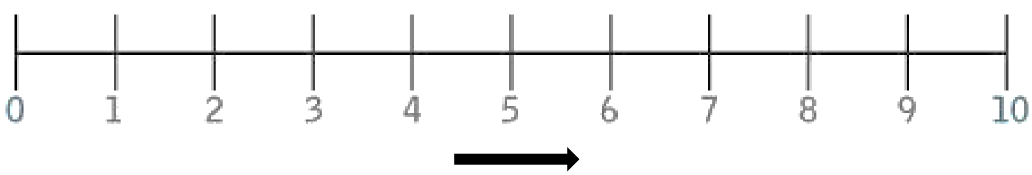
Name……………………………………………..

Set No…………………………………………….

Presented to you are 4 samples to evaluate. Starting from left to right, rate the sensory attributes of each of these samples in the following order: appearance, aroma, texture, flavour and aftertaste. Take a sip of water before and in-between samples.

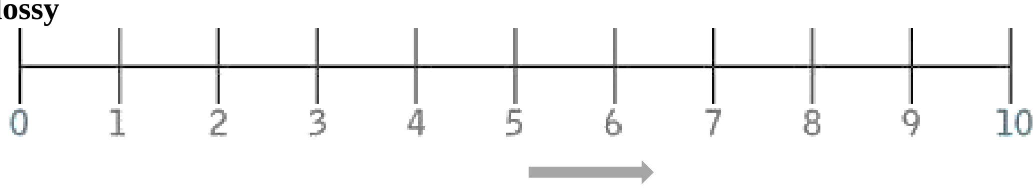
Appearance attribute
Sample…………………..

Colour



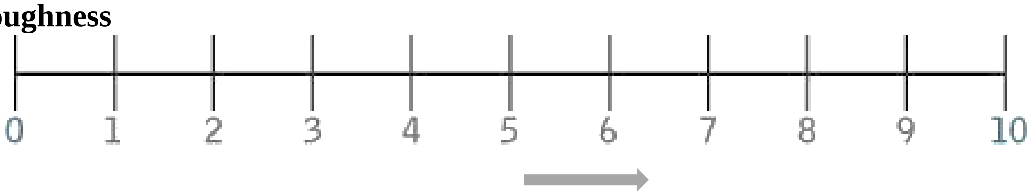
Light yellow Dark yellow

Glossy



Opaque (not glossy) Very shiny

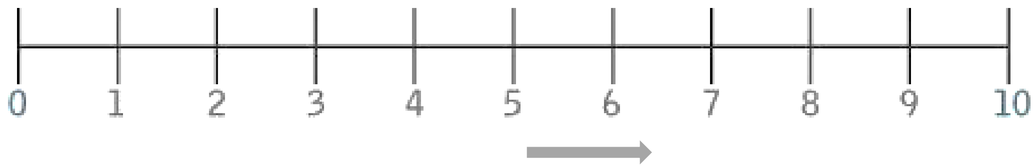
Roughness



Not rough	Very rough

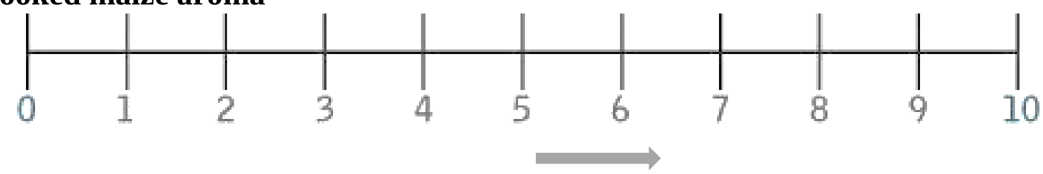
Aroma

Sample…………………..



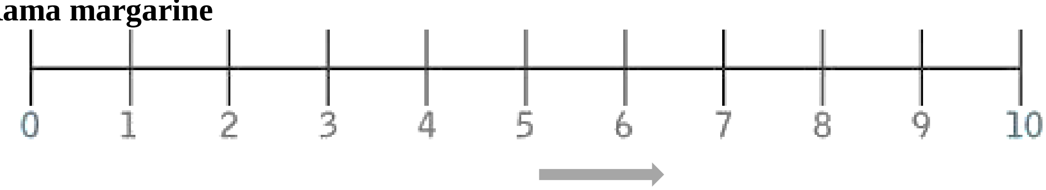
Not intense — Very intense

Cooked maize aroma



Not perceived — Strongly perceived

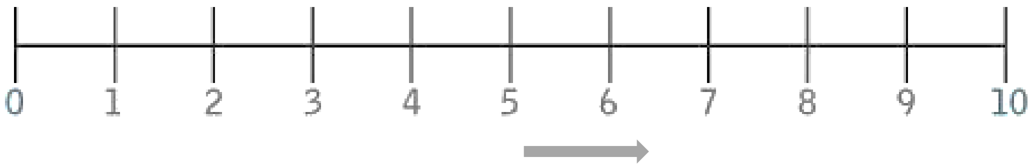
Rama margarine



Not perceived — Strongly perceived

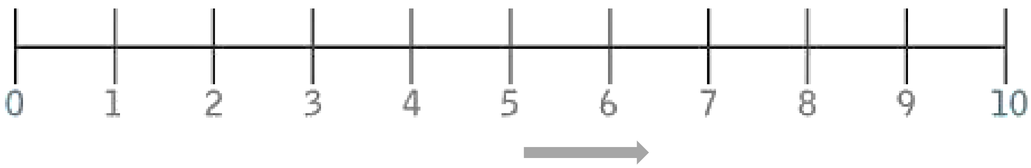
Texture
Sample…………………..

Stickiness



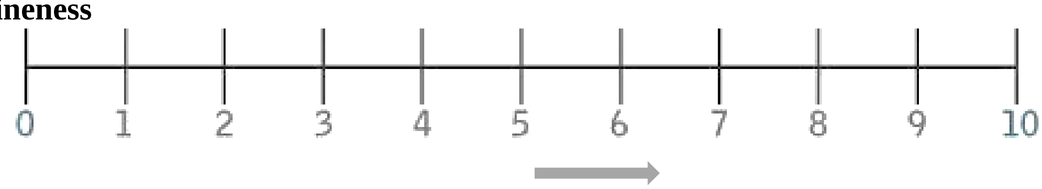
Not sticky Very sticky

Hardness



Not hard Very hard

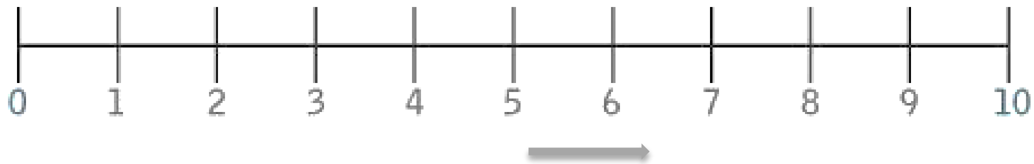
Fineness



Not fine Very fine

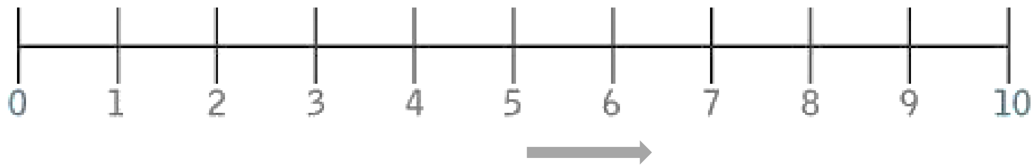
Flavour
Sample…………………..

Overall flavor



Not intense — Very intense

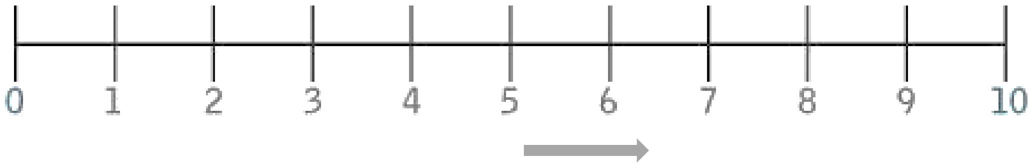
Cooked maize flour



Bland — Strong maize flavour

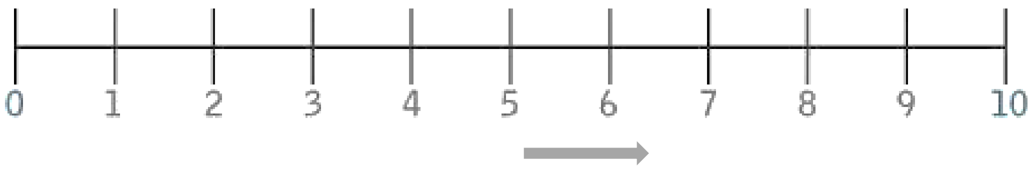
Aftertaste
Sample…………………..

Bitter



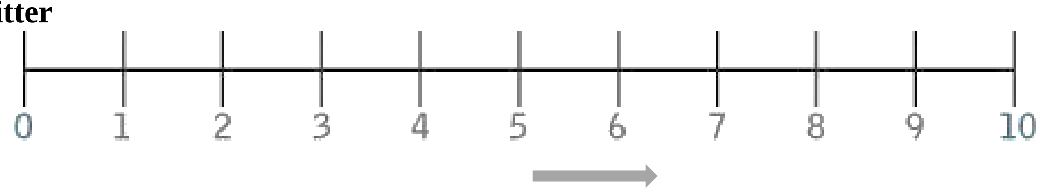
Not intense Very intense

Sample…………………..
Bitter



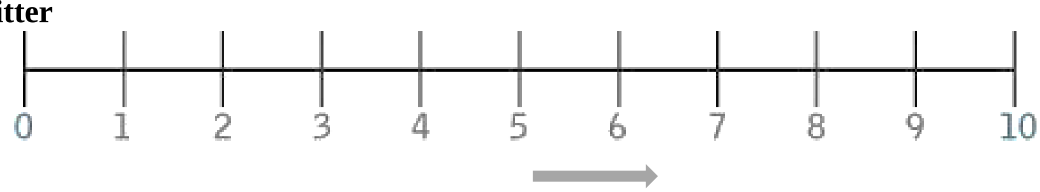
Not intense Very intense

Sample…………………..
Bitter



Not intense Very intense

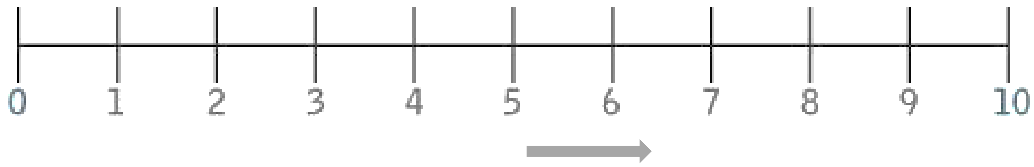
Sample…………………..
Bitter



Not intense Very intense

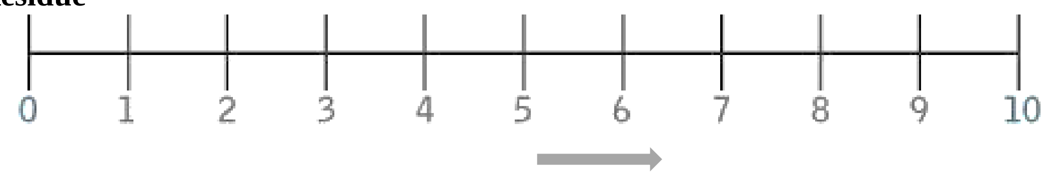
Residual grain

Sample…………………..
Residue



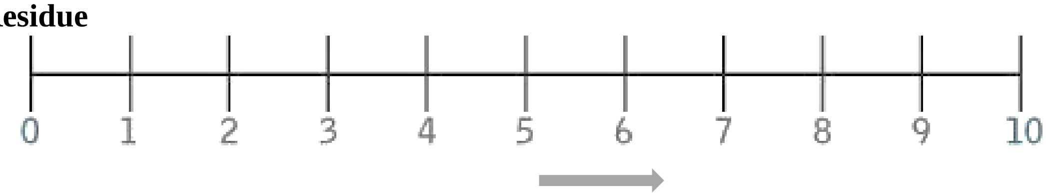
None A lot

Sample…………………..
Residue



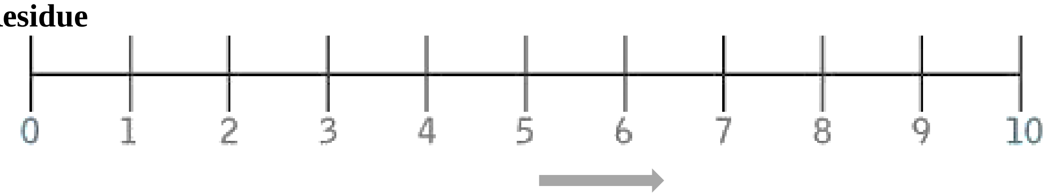
None A lot

Sample…………………..
Residue



None A lot

Sample…………………..
Residue



None A lot

Appendix 7.2.	***TshiVenda* version of questionnaire for consumer acceptability of stiff porridges and extruded snacks**

BAMBIRI ḼA U ṰHAṰHUVHA TSHIBVELEDZWA: MBUNO ṰHANU DZA TSHIKALO TSHA ZWIFANYISO ZWA U ḒIPHINA KANA U ḒITAKADZA

Nomboro ya Phanele: ___________________________ **Vhukale:** ____________________

Mbeu: ____ **Datumu :** _____________________

Khoudu ya Tshibveledzwa: ____________________________

Vha khou humbelwa uri vha thetshele zwiḽiwa zwo vhewaho phanḓa havho. Musi vho no thetshela kha vha sumbedze zwine vha pfisa zwone nga muvhala, u hwasa kana u suvhelela, muthetshelo, munukhelelo na u ṱanganedzwa nga vhoṱhe, nga u vhea tshifhambano kha zwifhaṱuwo zwo teaho afho fhasi.

MUVHALA	 a si wavhuḓi tshoṱhe / a si wavhuḓi / U nga vha wavhuḓi kana u si vhe wavhuḓi / wavhuḓi / wavhuḓisa
U HWASA KANA U SUVHELELA (Ha zwiliwa mulomoni)	 a si zwavhuḓi tshoṱhe / a si zwavhuḓi / zwi nga vha zwavhuḓi kana zwi si vhe zwavhuḓi / zwavhuḓi / zwavhuḓisa

MUTHETSHELO	 a si wavhuḓi tshoṱhe / a si wavhuḓi / U nga vha wavhuḓi kana u si vhe wavhuḓi / wavhuḓi / wavhuḓisa
MUNUKHELELO	 a si wavhuḓi tshoṱhe / a si wavhuḓi / U nga vha wavhuḓi kana u si vhe wavhuḓi / wavhuḓi / wavhuḓisa
U TANGANEDZWA NGA VHOṰHE	 a si zwavhuḓi tshoṱhe / a si zwavhuḓi / zwi nga vha zwavhuḓi kana zwi si vhe zwavhuḓi / zwavhuḓi / zwavhuḓisa