

The effect of adding Bambara groundnut (*Vigna subterranea* L.) on the nutritional, functional and sensory quality of a provitamin A-biofortified maize complementary instant porridge

BY

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Abstract

Introduction

Malnutrition has been declared a serious nutritional and health problem globally. The two major forms of malnutrition that are on the rise in the sub-Saharan African (SSA) region are protein-energy malnutrition (PEM) and vitamin A deficiency (VAD). The most recognized period during which these two forms of malnutrition occur is 0 to two years. This is also the period during which complementary foods are introduced. PEM and VAD in SSA mainly affects children living in the poor rural areas whose diets lack diversity. The complementary foods given to these children are mainly composed of starchy-based staple food crops, such as cereals, roots and tubers. These foods are characterised by high viscosity, poor protein content and quality, in terms of the two essential amino acids: lysine and tryptophan, as well as limitations in several micronutrients, including vitamin A. Strategies used to address PEM in SSA include: dietary diversification, amino acid fortification of cereals, biofortification and complementation. Quite similar, the strategies being used to address VAD include biofortification and dietary diversification as well as vitamin A supplementation and fortification. Biofortification is a new strategy that could be used together with cereal-legume complementation to address both PEM and VAD among the vulnerable populations. Bambara groundnut (BGN), an underutilised, but nutrient-rich legume that is adapted to harsh agro-climatic conditions prevalent in SSA could be highly suitable for compositing with provitamin A-biofortified maize (PABM) in complementary foods to address PEM and VAD in countries in the SSA region, including South Africa.

Aim

The aim of this study was to determine the effect of adding BGN on the nutritional, functional and sensory quality of a PABM complementary instant porridge.

Objectives

- (i) To produce composite complementary instant porridges by partially substituting PABM flour with different levels of BGN flour.
- (ii) To determine the effect of BGN on the nutritional composition of a PABM complementary instant porridge.
- (iii) To determine the effect of BGN on the functional properties of a PABM complementary instant porridge.
- (iv) To determine the effect of BGN on the consumer acceptance of a PABM complementary instant porridge.

Methods

The study was conducted using grain, separately, of two BGN (brown and red), one yellow PABM and one white maize (reference) varieties. The PABM flour was substituted with flour of either red or brown BGN variety at 0, 10, 20 and 30% (w/w) levels. The composite flours were used to make an instantised complementary porridge. The grains and the composite complementary instant porridges were analysed for their nutritional composition using standard and referenced methods. The functional properties of the grains and the composite complementary instant porridges including: texture, colour, water absorption capacity (WAC), solubility index (SI) and swelling volume (SV) were also assessed. The consumer acceptability of the composite complementary instant porridges was evaluated on a 9-point hedonic rating scale by a 55-member panel of students and staff of the Agriculture campus of the University of KwaZulu-Natal (UKZN), Pietermaritzburg (PMB), South Africa.

Results

The crude protein fat, fibre and ash content of the composite complementary instant porridges increased with an increase in either of the BGN. The firmness, WAC, SI and SV decreased with

increasing concentration of flour of either of the BGN. The decrease in SV would have a positive effect on the quality of the porridge as nutrient density would increase and viscosity decrease. The sensory evaluation results showed that there was no significant difference ($p>0.05$) in the taste, colour, aroma, texture, appearance and overall acceptability of the composite complementary instant porridges made with BGN and PABM.

Conclusion

The results indicate that BGN improved the nutrient content of a PABM complementary instant porridge with respect to several nutrients, including protein, fat and total mineral content. Thus, the BGN-PABM complementary instant porridge would contribute to the alleviation of nutrient deficiencies, including PEM and mineral deficiencies, which are prevalent in SSA. Previous studies on the consumer acceptance of PABM in different parts of SSA have generally indicated low acceptance of PABM, however, in this study, the BGN-PABM composite complementary instant porridge was acceptable to the consumers. It is likely that the BGN masked the presumably undesirable sensory characteristics of the PABM. However, there is a need to conduct the sensory evaluation of the BGN-PABM composite complementary instant porridges with the caregivers whose children are vulnerable to PEM and VAD. Overall, the findings of this study suggest that there is good potential for combining BGN and PABM to produce complementary foods that would contribute significantly to addressing child malnutrition, including PEM, in countries in the SSA region, including South Africa.

Preface

The work described in this dissertation was carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, from May 2015 to July 2016, under the supervision of Dr Muthulisi Siwela.

Signed: _____

Date: _____

Sandra Denhere (candidate)

As supervisor of the candidate, I agree to the submission of this dissertation.

Signed: _____

Date: _____

Dr Muthulisi Siwela (Supervisor)

Declaration

I, Sandra Denhere, declare that:

1. The entirety of the work contained in this dissertation is my original work, except where otherwise stated.
2. This dissertation, or any part of it, has not been submitted for any degree or examination at any other university.
3. Where other sources have been used they have not been copied and have been properly acknowledged.
4. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the relevant reference section.

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Sandra Denhere (candidate)

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Dedication

I want to dedicate most of this thesis to my late mother, Angeline Denhere and to my beloved brother Munyaradzi Raphael Denhere. To my late mother I say thank you for all those sacrifices, giving your all just to make sure that I got a decent education for my better future. I am proud of you and may your soul rest in peace. To my beloved brother Munyaradzi Raphael Denhere thank you for the financial support, may God bless you abundantly. A share of this thesis is also dedicated to the rest of the Denhere family, I say thank you for all the bundles of prayers pressed between the pages of your hearts and all the anxious moments we have shared. A lot would not have been achieved without you.

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Abbreviations

AOAC	Association of Official Analytical Chemists International
BAMNET	International Bambara Groundnut (BGN) Network (BAMNET),
BGN	Bambara groundnut
CIE	Commission Internationale de' Eclairage
DOH	Department of Health
FAO	Food and Agriculture Organization
HIV	Human Immune Virus
HQBF	High Quality Bambara Flour
HTC	Hard To Cook
KZN	KwaZulu-Natal
NCFS	National Consumption Food Survey
OFSP	Orange Fleshed Sweet Potato
PABM	Provitamin A-biofortified maize
PVA	Provitamin A
PEM	Protein Energy Malnutrition
PMB	Pietermaritzburg
QPM	Quality Protein Maize

RR	Reconstitution Ratio
RtHC	Road to Health Charts
SANHANES	South African National Health and Nutrition Examination Survey
SAVACG	South African Vitamin A Consultative Group
SI	Solubility Index
SSA	sub-Saharan Africa
SV	Swelling Volume
UNICEF	United Nations Children's Emergency Fund
VAD	Vitamin A deficiency
WAC	Water Absorption Capacity
WHO	World Health Organization

Chapter 1- Introduction, the problem and its setting

1.1. Introduction

The primary causes of morbidity and mortality among the under-five children of the sub-Saharan Africa (SSA) region are: pneumonia, diarrhoeal diseases, low birth weight, asphyxia, malaria and human immune deficiency virus (HIV) infection (UNICEF 2007b). One out of every two of such deaths has malnutrition as the underlying cause although it is rarely cited as the major cause (UNICEF 2007b). The number of children suffering from malnutrition in SSA has rose by 6% between 1990 and 2010 (Zweudi 2012). Childhood malnutrition in SSA region ranges from chronic and moderate to mild and silent forms. However, protein-energy malnutrition (PEM) and vitamin A deficiency (VAD) are the two major forms of malnutrition that are highly prevalent in children and have been declared public health and nutritional problems (DOH 2013a). An estimate of 38% of under-five children in SSA have stunted growth while 28% are underweight as a result of PEM (UNICEF 2007). PEM is also associated with approximately 5 million deaths of children every year (WHO 2000). About 42.4% of under-five children are at risk of VAD in SSA (Dary and Mora 2002) and one out of every three children was also found to have a serum vitamin A concentration of less than 20 g/dl which also signify VAD (Low *et al.* 2007). An estimated 5.2 million pre-school children show signs of night blindness caused by VAD (Sherwin *et al.* 2012) and VAD is also commonly seen in children with PEM (Bentley and Lawson 1988).

Children living in poor rural areas have been found to be at more risk of PEM and VAD than those in urban areas as their diets are nutritionally inadequate and lack diversity (UNICEF 2010). According to the 1999 National Food Consumption Survey (NFCS), in South Africa, one in every five children aged between one to nine years living in poor rural areas were stunted and one in ten were underweight as a result of PEM (Labadarios *et al.* 2008). Children living in poor rural parts of Kwa-Zulu Natal (KZN), South Africa, were also found severely affected by VAD due to poverty and high rates of illiteracy among their mothers (Nojilanai *et al.* 2007).

The complementary feeding stage is the period when additional foods other than breastmilk are given to the infant that is still being breastfed (PAOH/WHO 2003). However complementary foods that are given to the infants by caregivers in SSA have been found to be nutritionally inadequate. Children are usually given unfortified starchy-based porridges from staple crops such as cereals, roots and tubers. These porridges lack diversity and are nutritionally inadequate as they are characterized by high viscosity, poor protein quality in terms of the two essential amino acids: lysine and tryptophan and are also deficient in several micronutrients including vitamin A. Such as diet can lead to PEM and VAD (Faber 2005). In fact, the complementary feeding stage is the most recognized period in which VAD and PEM occurs (Faber & Wenhold 2007).

Various strategies have been put in place in different parts of SSA to address PEM and VAD. Dietary diversification, amino acid fortification of cereals, biofortification and complementation are the strategies that are being used to combat PEM. In protein nutrition, dietary diversification approach includes increasing commercial production of protein-lysine rich foods e.g. meat, poultry, and dairy products, fish farming, keeping small livestock, and home gardening of legumes (Gershoff, McGandy, Suttapreyasri, Nondasuta, Pisolyabutra and Tantiwongse 1975). Dietary diversification reduced stunting rates among the toddlers of Democratic Republic of Congo and Zambia (Gershoff *et al.* 1975). However, it is anticipated that dietary diversification will be successful if it is accompanied by behaviour change as well as adequate supply of foods that are affordable (Faber & van Jaarsveld 2007).

Biofortification of staple crops with PVA carotenoids is currently being evaluated as a complementary strategy of addressing VAD. It can either be achieved by agronomic, conventional breeding or transgenic breeding (Saltzman *et al.* 2013). The orange fleshed sweet potato (OFSP) have been shown to be efficacious and effective at improving the vitamin A status of children in Mozambique, Uganda and Nigeria (Low *et al.* 2007; Hotz *et al.* 2012). Biofortification is also an effective way of reaching the poor rural malnourished populations who may have limited access to foods commercially fortified with vitamin A (De Groote & Kimenju 2008; Hotz & McClafferty 2007). However, biofortification of staple crops with PVA

carotenoids causes some undesirable changes in the sensory attributes of the crop such as colour, flavour and texture, which may pose a challenge in consumer acceptance (Muzhingi *et al.* 2008).

In an effort to alleviate VAD Maize (*Zea mays* L.) in maize consuming parts of SSA, the HarvestPlus Challenge Programme has been targeted for biofortification with PVA carotenoids (Tanumihardjo 2008). Children aged between 6-23 months were identified by the HarvestPlus Challenge Programme to be more vulnerable to micronutrient deficiencies, including VAD than any other age group (HarvestPlus 2013). Biofortification of white maize with PVA by conventional breeding is viewed as a potential long term sustainable strategy to alleviate VAD, because is the predominant staple food for more than 300 million people in SSA especially among the poorer communities with white maize constituting more than 90% of SSA's total maize production (Nestel *et al.* 2006). It is mainly grown for subsistence, across a range of latitudes, moisture regimes, slopes and soil types and is also cheap, thus easily available to consumers. It therefore plays an important role in food security (Khumalo *et al.* 2011). However, biofortification of maize changes the colour of the maize from white to yellow or orange (Chapman 2012; Nuss & Tanumihardjo 2011). The flavour and aroma of the maize are also altered. The changes in the sensory properties of maize due to biofortification have been found to generally have a negative impact on consumer acceptability of the maize (Meenakshi *et al.* 2010; Nestel *et al.* 2006; Stevens & Winter- Nelson 2008). Consumer acceptance studies on PABM conducted in different parts of SSA have shown a preference for white maize over orange/yellow PABM. Caregivers were however willing to give their children PABM-based food product if they had nutritional health benefits (Amod *et al.* 2014). The acceptance of PABM was also found to vary with the age of consumers. PABM was found more preferred over the normal white by pre-school children as compared to the older children and the adults (Pillay *et al.* 2011).

It has been shown that cereal-legume complementary foods have high nutritional value and has proved to be effective in preventing PEM in Nigeria (Osundahunsi and Aworh 2002). Cereal-legume complementation is also cost effective as it makes use of locally available low cost ingredients, which are culturally and organoleptically acceptable to consumers (Siebel 2007). Since biofortification is complementary strategy that can be combined with other strategies, it is

therefore vital to complement a PABM with a legume in foods intended for complementary feeding in an effort to combat PEM and VAD in children in SSA. Bambara groundnut (BGN) (*Vigna subterranea* L.) could be a suitable legume to complement with PABM.

BGN is currently receiving interest from researchers because of its high yield and resistance to diseases as well as its adaptability to poor soils and rainfall (Hepper 1970; Gwekwerere 1995). It is an underutilized crop that originate in West Africa and is ranked the third most important legume after peanuts and cowpeas (Sellschop 1962; Doku & Karikari 1971; Rachie & Silvestre 1977; Linnemann 1992). BGN serves as a source of cheap protein in semi-arid regions of SSA and is also regarded as a complete food (Massawe *et al.* 1999). People can live on BGN alone because it contains less oils and proteins, but more carbohydrates when compared with other legumes such as peanuts. The amino acid lysine, which is limiting in cereals, is found in relatively high amounts, and the total sulphur amino acids are found in fair amounts in the BGN (Mkandawire 2007). The levels of the essential amino acids lysine, methionine and cysteine in BGN are also comparable to that of soya bean (Fetuga, Oluyemi, Adekoya, Oyenuga 1975). People like its flavour, preferring it to cowpea, and food preparations have changed very little since the crop first gained popularity in the Middle Ages. BGN is also used in the fortification of cereal-based foods to improve the protein content for both infant and adult foods such as in the preparation of pap and pottage (Lewick 1974). Consumer acceptance case studies conducted in different part of SSA on BGN has also indicated a preference for BGN due to its desirable sensory characteristics especially its flavour. Hence, it can be a tool for addressing PEM among the vulnerable populations.

BGN could be combined with PABM to develop a complementary instant porridge that would contribute to combating PEM and VAD among children in SSA. It is likely that the undesirable sensory characteristics of the PABM such as colour, taste and flavour will be masked by the desirable sensory characteristics of BGN. It, however, appears that the acceptance of an instant porridge made with BGN and PABM together with its nutritional composition as well as its functional properties have not been investigated.

1.2. Summary of research focus

The effect of adding BGN on the nutritional, functional and sensory quality of a PABM complementary instant porridge will also be investigated. This study will provide useful data on the consumer acceptability of a BGN-PABM composite complementary instant porridge in SSA.

1.3. Aim of the study

The aim of this study was to determine the effect of adding BGN on the nutritional, functional, and sensory quality of a PABM complementary instant porridge. It is thought that the PABM-BGN complementary instant porridge will contribute significantly to addressing malnutrition, especially PEM and VAD, among children aged 0.5-23 months in the SSA region.

Study objectives

The objectives of this study were:

To produce composite complementary instant porridges by partially substituting PABM flour with different levels of BGN flour.

To determine the effect of BGN on the nutritional composition of a PABM complementary instant porridge.

To determine the effect of BGN on the functional properties of a PABM complementary instant porridge.

To determine the effect of BGN on the consumer acceptance of a PABM complementary instant porridge.

1.4. Hypotheses

The following hypotheses were tested in the study:

- 1.4.1 The nutritional composition of the complementary instant porridge made with BGN and PABM will increase with an increase in BGN concentration.
- 1.4.2 The functional properties of the complementary instant porridge prepared with BGN and PABM will improve with an increase BGN concentration.
- 1.4.3 The sensory attributes of the complementary instant porridge prepared with PABM and BGN will be enhanced as the level of BGN increases.

1.5. Study parameters and general assumptions

The sensory evaluation was done at Agriculture campus of the University of Kwa-Zulu Natal (UKZN), Pietermaritzburg (PMB), South Africa. It was assumed that these subjects would represent the rural populations of SSA. Assumptions relevant to specific sub-problems are discussed in the relevant sections of this thesis. The study was limited to two BGN varieties, one yellow PABM variety and one white maize variety. The yellow PABM variety was developed by conventional breeding and then produced in bulk at agricultural research stations in KwaZulu-Natal province, South Africa, whereas the BGN grains were obtained at Mbare Musika market in Harare, Zimbabwe. The effects of BGN on the nutritional composition, consumer acceptability, and functional properties of a PABM complementary instant porridge were investigated.

Definition of terms

To clarify the terminology used in this study, the following definitions are provided:

Biofortification: The process of breeding nutrients into crops as well as the delivery of micronutrient dense crops (Saltzman *et al.* 2013; Mayer *et al.* 2008).

Caregiver: A family member, sibling, friend of the family, neighbour or community member who provides care and assistance to an infant or child (Smith *et al.* 2005).

Complementation: The term complementation is used with respect to proteins when the relative deficiency of an amino acid in one is compensated by a surplus from another protein consumed at the same time (Bender 2005)

Complementary food: These are semi-solid or solid foods that are given to infants after six months of age to transit them from breast milk (PAOH/WHO 2003).

Complementary feeding: This is the transition from exclusive breastfeeding to complementary foods and typically covers the period from 6-24 months of age (PAOH/WHO 2003).

Cereal-legume complementation: A combination of a cereal and a legume where one supplements the other with the deficient amino acid thereby creating a mutual balance resulting in nutritional complementation (Young and Pellet 1994).

Infant: An infant is a young baby aged between six and 12 months, inclusively (Rudolf *et al.* 2011).

Protein-energy malnutrition: The term protein-energy malnutrition applies to a group of related disorders such as marasmus, kwashiorkor and marasmic-kwashiorkor which result from inadequate intake of protein and energy (PAOH/WHO 2003).

Provitamin A carotenoids: These are the β -carotene and α -carotene and β -cryptoxanthin carotenoids, which are converted to vitamin A in the human body (Preedy 2012, pp143- 157).

Provitamin A-biofortified maize: Maize varieties which contain higher levels of provitamin A carotenoids than white maize (HarvestPlus 2013).

Vitamin A deficiency: Is a form of malnutrition which exists when there is a chronic failure to eat sufficient amounts of vitamin A or beta-carotene rich foods resulting in levels of blood-serum vitamin A that are below a defined range.

Xerophthalmia: Refers to the abnormal dryness of the conjunctiva and cornea of the eye, with inflammation and ridge formation, typically associated with vitamin A deficiency.

1.6. Outline of thesis

This thesis is laid out as follows:

Chapter 1: Introduction, the problem and its setting

Chapter 2: Literature review

Chapter 3: Grain properties and nutritional quality of BGN and PABM varieties

Chapter 4: Effect of adding BGN on the nutritional, functional, and sensory quality of a PABM complementary instant porridge

Chapter 6: General Discussion

Chapter 7: Conclusions and Recommendations

The referencing style used in this thesis is according to the guidelines used in the Discipline of Dietetics and Human Nutrition, UKZN, PMB, South Africa.

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Chapter 2- Literature review

2.1. Introduction

Protein-energy malnutrition (PEM) and vitamin A deficiency (VAD) are the two major forms of malnutrition that are prevalent among children in SSA and have been declared nutritional and public health concern. The causes of PEM and VAD are many and interlinked. Various strategies such as dietary diversification, complementation, food fortification, supplementation and biofortification have been implemented in many parts of SSA as efforts of combating PEM and VAD. Bambara groundnut (BGN) and provitamin A-biofortified maize (PABM) could be combined together in formulating a complementary instant porridge that could be used to address these two forms of malnutrition. The BGN could also improve the undesirable sensory characteristics associated with PABM.

2.1.1 PEM and VAD with special focus on children in SSA

The World Health Organization (WHO) delineated malnutrition as a cellular disparity accompanied by insufficient supply of macro- and micronutrients resulting in impaired growth and development (Deen *et al.* 2003). Protein energy malnutrition (PEM) and vitamin A deficiency (VAD) are the most common forms malnutrition that pose serious nutritional and health threat among children in SSA (Brabin and Coulter 2003; Green and Watson 2005; De-Frias *et al.* 2010). PEM refers to all conditions attributable to inadequate dietary protein and energy, comprising mainly of kwashiorkor, marasmus and marasmic-kwashiorkor (Gurney *et al.* 1985; Okoye 1992). Children with mild or moderate PEM adapt to their inadequate diet by growing slowly and often become lethargic. They are usually susceptible to infectious diseases such as diarrhoea and may further develop other micronutrient deficiency disorders including VAD (Gurney *et al.* 1985). Estimates indicates that 35.8% of pre-school children in SSA are underweight, 42.7% are stunted and 9.2% are wasted due to PEM (de Onis *et al.* 2004). In Southern Sudan, approximately one out of every five children (22%) suffers from wasting (Ola *et al.* 2011) and about 42.9% of under-five year children in West Gojan, Ethiopia are reported to be underweight (Teshome *et al.* 2006). PEM has resulted in more than 10 million children being

physically and intellectually stunted in Nigeria (Babatunde, Olagunju, Fakayode & Sola-Ojo 2011). A study carried out in the country estimated a mortality rate of 40.1% which although lower than the WHO estimates of 60% is still very high and marasmic kwashiorkor was found to be associated with higher case fatality rate than other types of PEM (Babatunde *et al.* 2011). In Uganda PEM is also one of the greatest single contributors to childhood mortality, although it is rarely listed as a direct cause (UNICEF 2008). The prevalence of moderate underweight, severe underweight, moderate or severe wasting and moderate or severe stunting among children under five years of age in Cameroon in 2004 was 18%, 4%, 5% and 32% respectively (UNICEF 2008).

VAD occurs when body (liver) stores are depleted to the extent that physiological functions are impaired. In recent times, VAD is considered as one of the most frequent nutritional disorders in children all over the world. In SSA, about 42% of under-five year children are at risk of VAD (SCN 2004). In South Africa, one out of every three children was found to have a serum vitamin A concentration of less than 20 g/dl, which also signify VAD. The highest levels of VAD have been reported in KZN province (Labadarios *et al.* 2008). In parts of SSA, where immunization programmes are not widespread and VAD is common, millions of children die each year from complications of infectious diseases such as measles, diarrhoea and malaria with 2.8 million showing frank signs of xerophthalmia (Kennedy *et al.* 2003). In 1988, an estimated number of infants who became blind as a result of VAD was 100 000 (Bentley and Lawson 1988). About 250 000-500 000 million children with VAD in SSA die within 12 months of losing their sight (Kennedy *et al.* 2003).

2.1.2 Causes of malnutrition in children

The causes of malnutrition are best illustrated in the UNICEF conceptual framework of child malnutrition (Figure 1). The framework is used, at national, district and local levels, to help plan effective actions to improve nutrition. It classifies the causes of malnutrition as immediate, underlying, and basic, whereby factors at one level influence other levels (UNICEF 1998).

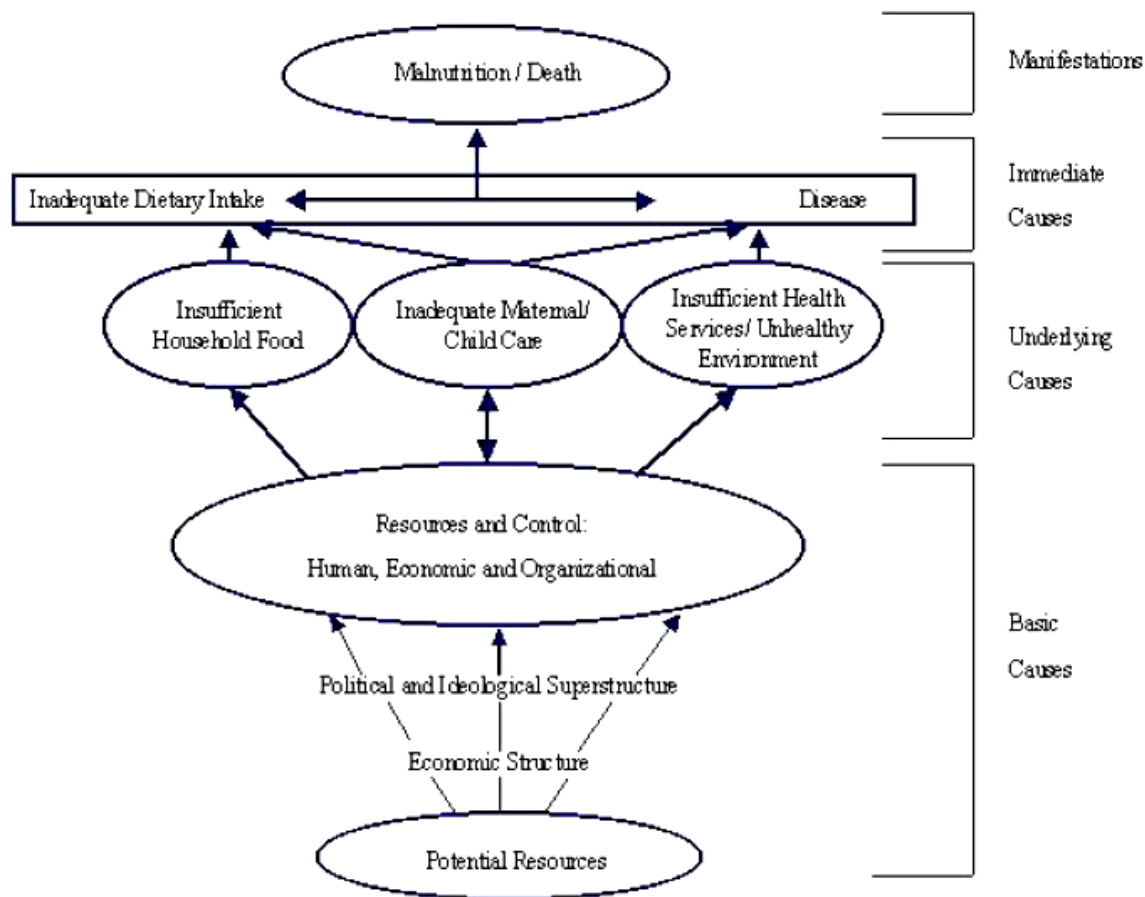


Figure 1. The UNICEF conceptual framework for child malnutrition (UNICEF 1998)

2.1.2.1 Immediate causes

PEM and VAD are directly linked to inadequate dietary intake of food rich in protein, energy and vitamin A. Factors that contribute to the inadequate dietary intake of protein, energy and vitamin A-rich foods include socio-cultural practices and socio-economic factors: vegetarianism and migration from rural to urban slums, as well as limited purchasing power (Torún and Chew 1994; Torún 2006; Piercecchi-Marti *et al.* 2006). Dietary choices which are influenced by the nutritional ignorance of parents', preference for alternative foods and true or perceived food allergies also contributes to inadequate dietary intake of protein, energy and vitamin A-rich foods

(Katz *et al.* 2005). Inadequate dietary intake in children is also caused by increased use of diluted milk and vegetable foods as well as early introduction of complementary feeding (Torún and Chew 1994; Kapur *et al.* 2005; Torún 2006). SSA's children are usually given a thin cereal porridge as a complementary food, which is inadequate in protein and energy, thereby causing the children to become susceptible to PEM. (Monckeberg 1991; Torún and Chew 1994; Golden and Golden 2000; Torún 2006). The consumption of fruits and vegetable in poor rural communities of SSA is also far below the WHO/FAO minimum recommendation for fruit and vegetables of 146 kg per person per year. This also makes the poor rural populations to be more vulnerable to VAD (West 2000).

Diseases and infections plays a major role in the aetiology of PEM and VAD because they result in increased needs and a high energy expenditure, lower appetite, nutrient losses due to vomiting, diarrhoea, poor digestion, malabsorption, utilization of nutrients and disruption of metabolic equilibrium (Golden and Golden 2000; NDoH 2005a; Williams 2005; Torún 2006). It takes time for a malnourished child to recover from respiratory and diarrhoeal diseases and therefore the risk of morbidity and mortality is higher compared to a well-nourished child. Repeated illnesses also contribute to ill health and compromised nutritional status (Pereira 1991).

2.1.2.2 Underlying causes

In poor SSA countries, many households usually set aside a large proportion of their income (around 40% or more) to buy food. For this reason, the rise in food prices has undesirable outcomes that affect the purchasing power by reducing the real income. When income decreases, the quality and quantity of food also decreases (UNICEF 2009b). There is evidence that when there is unemployment and wages are low, families will have insufficient food and usually eat cheaper food of less nutritious, leading to malnutrition. As food products derived from animals are more expensive, children's intake of proteins, energy and vitamin A from animal products decreases with poverty. Therefore, malnutrition among children will develop (Piercecchi-Marti *et al.* 2006).

There is also evidence that quality of care is linked to infant nutritional status. The quality of socio-psychological care is often determined by the interaction between mother and child (Carvalhaes and Benicio 2006). Hunger and food insecurity put extra stress on parents, which can lead to emotional problems and neglect, in turn leading to a decrease in the appetite of the child (Play Therapy Africa 2009). All these issues reduce the survival of the child, even when given enough food. Children that do survive these circumstances will have long-term cognitive disabilities and poor growth (Play Therapy Africa 2009). Maternal behaviours are also directly linked to the socio-psychological care of the child as children from low-income households have a high risk of PEM if the psychosocial environment is insufficient. The risk is also lower in households with a low-income and good psychosocial care, which shows that good psychosocial care, can almost protect the child against their poor socio-economic conditions (Carvalhaes and Benicio 2006).

PEM and VAD rates in SSA are still high due to lack of access to health services (NDoH 2003; Oyelami and Ogunlesi 2007). This is also worsened by patients not making use of the limited formal health services available to them (Müller and Krawinkel, 2005). Incomplete immunizations, lack of knowledge about the Road to Health Charts (RtHC) by some health workers as well as ignorance to growth monitoring and promotion among the caregivers also increases the risk of developing PEM and VAD in children (Ayaya *et al.* 2004). Many SSA health services are influenced by a loss and shortage of health staff, leading to a higher workload for those that stay behind and causing remote areas not being covered by health services. Poor performance of health services contributes to the high mortality rates of preventable deaths including PEM and VAD. Families that are also food insecure and reliant on inadequate health services develop a reduced resistance to infections, which may result in malnutrition (FAO 1996).

Unhealthy environments, overcrowding, lack of water and unclean water and poor sanitation, directly leads to PEM and VAD through infections (FAO 1996; Abate *et al.* 2001). Getaneh *et al.* (1998) found an association between PEM and poor housing conditions in Ethiopia, and also temporary housing in Kenya (Ayaya *et al.* 2004) or mud walled houses in Kampala (Owor *et al.*

2000). The household's economic position has thus a significant impact on the risk of a child being stunted and underweight (Zere and McIntyre 2003).

2.1.2.3 Basic causes

Basic causes are also known as national or root causes. The basic causes of PEM at societal level include: poor availability and control of resources (political, social, ideological and economic), environmental degradation, poor agriculture, war, political instability, urbanization, population growth and size, distribution, conflicts, trade agreements and natural disasters, religious and socio-cultural factors (Torún and Chew 1994; Vorster and Hautvast 2003; UNICEF 2004; Torún 2006). In addition, landlessness and migrant labour are also considered to be basic causes of PEM (NDoH 2003). Other basic causes include: market failures due to economic decline, conflict and political upheavals that can lead to a reduction in food yields and increase in food prices (Mason *et al.* 2005). Loss of food after a harvest can also occur when storage conditions are poor and food is inadequately distributed (Torún and Chew 1994; Torún 2006).

2.1.3 PEM remediation strategies and their shortcomings

2.1.3.1 Dietary diversification in protein nutrition

In protein nutrition, dietary diversification approach includes increased commercial production of protein-lysine rich foods e.g. meat, poultry, and dairy products, fish farming, keeping small livestock, and home gardening of legumes (Gershoff *et al.* 1975). There is substantial evidence that consumption of animal-source foods by young children as a dietary diversification approach improves growth, cognitive performance, and motor function (Dror and Allen 2011). For example, in a large cross sectional study by Krebs *et al.* (2011) in Guatemala, Democratic Republic of Congo and Zambia meat consumption by toddlers was associated with lower stunting rates.

2.1.3.2 Amino acid fortification of cereals

Cereals can be fortified with the amino acid lysine by addition either as a protein such as milk, soy or fish or as the free amino acid lysine (mono-hydrochloride) in order to improve the biological value of cereal proteins for populations with lysine-poor diets (Jansen 1969). Recent studies by Zhao *et al.* (2004) indicate that lysine fortification of cereal flours can significantly improve the nutritional status of populations consuming cereal-based diets. A significant gain in weight and height in children was reported in a community that consumed fortified wheat flour (Zhao *et al.* 2004).

2.1.3.3 Biofortification of staple crops with lysine and tryptophan

Biofortification of cereals crops such as maize and sorghum with elevated lysine and tryptophan levels by chemically induced mutation has shown to improve the protein quality of the biofortified crops (Mertz *et al.* 1993). A 50 and 40% increase in lysine content was shown in biofortified white and yellow maize cultivars, respectively. The corresponding values for tryptophan were 40 and 30%, respectively. When compared to FAO/WHO standard protein, the Quality Protein Maize (QPM) chemical score ranges from 90-100% while that of common maize was 51%. The QPM also presented a sensible lower ratio of leucine to isoleucine when compared with common maize (Murray *et al.* 1994). Increasing the lysine and tryptophan content of staples crops by biofortification was found to be a cost effective method of improving the nutritional status of the poor rural populations. In addition, the sensory and physical properties are maintained without interference to the consumers eating habits. The crops are also adaptable to a wide range of environments (Murray *et al.* 1994).

2.1.3.4 Complementation

With respect to proteins, the term complementation is used when the relative deficiency of an amino acid in one is compensated by a surplus from another protein consumed at the same time

(Bender 2005). Bressani et al. (1972), which are as follows have classified the nutritional response of combining two proteins into four groups using rat bioassay:

- Type 1 - involves the complementation of two proteins with similar lysine deficiency such as maize and peanut. There is however no complementation effect.
- Type II - involves the complementation of two protein sources, which have the same limiting amino acids, but in different proportions e.g. maize and cotton grain flour.
- Type III - has the true complementary effect because one protein source has considerably higher concentration of the most limiting amino acid in the other, producing a synergistic effect e.g. maize and cowpea or sorghum and soy.
- Type IV - both protein sources have common amino acid deficiencies and the one with the higher provides the one without e.g. and beef proteins (Bressani *et al.* 1972).

However, in many SSA countries where cereal crops are a staple food, several researchers have implemented the type III (i.e. cereal-legume complementation) strategy in order to raise the energy density and protein quality of complementary foods in an effort to combat PEM (Kannan *et al.* 2001). In Nigeria, cereal-legume complementary foods have been shown to provide good nutritive value and has proved to be effective in preventing PEM (Osundahunsi and Aworh 2002). This is true in part, as there are no significant changes in the organoleptic qualities of the products (Annapure *et al.* 1998). Cereal-legume complementation is also cost effective because it makes use of locally available low cost ingredients, which are culturally and organoleptically acceptable by the consumers (Siebel 2007).

2.1.4 VAD remediation strategies and their shortcomings

2.1.4.1 Dietary diversification in vitamin A nutrition

As a strategy for combating VAD, dietary diversification aims at increasing production and consumption of vitamin A-rich foods, ensuring its bioavailability in the diet and making vitamin

A-rich foods available and accessible to populations in need. This can be achieved by behaviour change that aims at improving communication through social marketing and nutrition education, horticultural approaches e.g. home food gardens and ways of retaining vitamin A content of food by examining the preparation and cooking methods (Faber and Wenhold 2007). Dietary diversification has been successful in reducing VAD in South Africa, because of increased consumption of foods rich in vitamin A through home gardening together with awareness campaigns in affected areas (Faber *et al.* 2009). However, in poor rural consumers, dietary diversity is inhibited by the availability of resources at household level and seasonality of certain foods such as fruits and vegetables e.g. vitamin A intake among Kenyan pre-school children from low-income rural households considerably differed between post-harvest and lean months (Dary and Mora 2002). Dietary diversity has also been identified as a different and expensive strategy to maintain on a large scale (Faber *et al.* 2009).

2.1.4.2 Vitamin A supplementation

Vitamin A supplementation is another strategy used for combating VAD. It involves the provision of vitamin A capsules containing retinol, which is stored in the liver and is released, slowly in sufficient quantity to sustain vitamin A requirements for six months (Meenakish *et al.* 2009). A dose of 50 000 International Units (IU) is recommended for infants less than six months of age, 100 000 IU for infants six to 11 months of age and 200 000 IU for children aged 12 months to five years (DOH 2012). In areas where VAD is a health problem WHO recommends that children between six to 59 months should receive supplementation. Supplementation is the fastest way of controlling VAD in individuals or population groups that vulnerable. Supplementation also reduces child mortality by improving gut integrity thereby reducing diarrhoeal episodes and susceptibility to infections (WHO 2011).

There is however recurrent of VAD if supplementation is used for a long period of time The supplementation programme carried out in 103 priority countries has shown a coverage stagnated at 58%, with high annual fluctuation (WHO 2011). Some side effects associated with supplementation include headache, nausea and vomiting among children aged six to 59 months,

although the symptoms disappear within 24 hr of infant or child receiving the supplement (WHO 2011). The sustainability of supplementation has also been questioned. This is in support of a study conducted by van Stuijvenberg *et al.* (2011) which concluded that the blanket approach of administering vitamin A may not be appropriate for all areas of the country, even if the community is malnourished and poverty-stricken. Although the global cost of vitamin A capsule has been estimated to be \$US0.10, logistics and distribution cost makes the cost of the capsule to be more expensive, at \$US1.00 (Nestle *et al.* 2006).

2.1.4.3 Vitamin A fortification

Several food vehicles including vegetables oil, margarine, milk and other dairy products, cereal flours, sugar, infant formulas, and complementary foods for children have been used for fortification with vitamin A. Vitamin A is fat soluble hence high fatty foods can easily be fortified with vitamin A (Sherwin *et al.* 2012). Vitamin A fortification does also not require the target groups to change their dietary behaviours as in dietary diversification. However, to get the required level of vitamin A, it needs to be consumed in adequate amounts by a large proportion of the target individuals. In 2013, VAD in South African children less than five years of age decreased by 20% on a national level as a result of vitamin A fortification (Shisana *et al.* 2013). The retinol concentrations of children aged 6-59 months fed with vitamin A fortified milk and cereal was found to be higher than those fed non-fortified milk and cereal (Eichler *et al.* 2012). However, vitamin A fortification has limitations due to low accessibility of commercially vitamin A-fortified foods to poor people in rural areas (Faber and Wenhold 2007; Nestel *et al.* 2006). In South Africa the fortification of maize meal with seven micronutrients including vitamin A, faces the challenge of low compliance by food manufacturers with the SA regulations as well as poor adherence to the WHO recommendations. Food fortification also changes the sensory characteristics of food, which may pose a challenge on the consumer acceptance of these foods. Bioavailability of food is reduced because of food fortification (Papathakis and Pearson 2012).

2.1.4.4 Biofortification of staple crops with provitamin A carotenoids

Biofortification of staple crops with PVA carotenoids is currently being evaluated as a complementary strategy to address VAD in SSA (Tanumihardjo 2008). It is achieved by either agronomic, conventional or transgenic breeding (Saltzman *et al.* 2013). Currently, the HarvestPlus Challenge Programme has a biofortification programme in place that makes use of conventional breeding of staple crops such as maize, cassava, sweet potato and plantain with PAV carotenoids (Bouis *et al.* 2011b). Biofortification efforts are showing successes in improving the vitamin A status of the poor rural populations in several countries e.g. orange fleshed sweet potato (OFSP) have been shown to be efficacious and effective at improving the vitamin A status of children in Mozambique, Uganda and Nigeria (Low *et al.* 2007; Hotz *et al.* 2012). PVA-biofortified crops are not associated with vitamin A toxicity due to the body's ability to convert carotenoids to vitamin A as needed (Stevens and Winter-Nelson 2008). In plant breeding, biofortification is one-time investment as the investment pays by yielding micronutrient rich crops. Varieties bred for one country can also be evaluated and used in other countries which multiplies the benefits of the initial investment. It has been reported that the ongoing expenses for monitoring and maintaining these crops, are far lower than the initial costs of the crops (Bouis *et al.* 2011). Biofortification is also scientifically viable as well as cost effective because once the crop has been biofortified the cost of biofortification are far lower than industrial fortification or supplementation (Mayer *et al.* 2008).

Biofortified crops are however unable to deliver a high quantity of macronutrients as compared to vitamin A capsules as well as industrially vitamin A-fortified foods (Saltzman *et al.* 2013). Biofortification also results in an alteration of the sensory characteristics of the crop thereby presenting a challenge with consumer acceptance of the crop (Muzhingi *et al.* 2008). It was also predicted by the HarvestPlus Programme that it will take a decade for biofortified crops to be adopted in the Global South due to obstacles such as individual property rights, public and government acceptance and safety issues. As a result of these obstacles, transgenic approaches to biofortified crops are costly and take significant amounts of time before being released to

farmers in many countries (Meenakshi *et al.* 2012). The next section discusses the importance of maize as a human food in SSA.

2.2 The importance of maize as a human food in SSA

Maize/corn (*Zea mays* L.) originate in the Western Hemisphere and the word *Zea mays* comes from two languages (i.e. *Zea* comes from ancient Greek and is a genetic name for cereals and grains and *mays* comes from the *Tarino* language). Some scientists believe that *Zea* stands for ‘sustaining life’ and *mays* for ‘life giver’ (Smale, Byerlee & Jayne 2011). The cultivation of maize in SSA dates back to the 16th century when the Americans imported it to Africa along the western and eastern coasts and gradually moving inland as the slave trade expanded (Smale, Byerlee & Jayne 2011). The driving factors that propelled the rise of maize production in SSA include: agronomic suitability of maize, (i.e. it is cultivated across a range of latitudes, moisture regimes, slopes and soil types), the British starch market; milling technology; the integration of Africans into the settler wage economy and trade policies promoted by settler farm lobbies (Smale *et al.* 2011).

Unlike other continents that regard maize as livestock feed, the SSA continent depends on maize as a human food source (McCann 2005). White maize constitutes more than 90% of the SSA’s total maize production and grown by small- and medium-scale farmers who cultivate about 10ha or less (DeVry and Toenniessen 2001) under extremely low-input systems with average maize yields at 1.3 t ha⁻¹ (Bänziger and Lafitte 1997). South Africa, Tanzania, Uganda, Zambia and Swaziland were the top exporter of maize in 2005 and Zimbabwe (a maize exporter until the late 1990s), Angola, Ghana, Kenya and Mozambique were the top importers. Currently South Africa leads the continent’s maize production followed by Nigeria, and Ghana is the least (FAOSTAT 2010).

As a human food, maize serves as a staple diet for the majority of the poor rural populations. Due to the high prevalence of malnutrition in SSA, maize has been recommended for for micronutrient fortification (Labadarios *et al.* 2008). Maize provides substantial proportions not

only of calories but also of proteins (NRC 1988). In East Africa, maize is most important in Kenya, where more than 30% of both calories and protein in the diet comes from maize. In Tanzania, more than 25% of calories and protein comes from maize, and in Ethiopia, 20% of calories and 16% of protein come from maize (Hassan, Mekuria and Mwangi 2001). In Southern Africa, maize provides more than half of the calories as well as of protein in the food supply of Malawi, Zambia, and Lesotho, and more than 40% in Zimbabwe (McCann 2005). Generally, the average consumption of maize in SSA is 106.2 g/person/day and the annual per capita consumption ranges from 138 kg in Swaziland to 195 kg in South Africa, while in east Africa it ranges from 40 kg in Burundi to 105 kg in Kenya (CIMMYT 2007).

Maize is prepared and consumed into a wide range of food forms, which vary from region to region, or from one ethnic group to another (Alexander 1987). Some traditional foods made with maize include breads, porridges, snacks and beverages. However, porridge is the most dominant food form of maize in SSA (Nuss & Tanumihardjo 2010). It is eaten either as stiff or thin porridge, where thin porridge is usually the first complementary food given to infants. Maize based foods plays an important role in filling the scarcity and hunger gap between the raining season and dry season (Alexander 1987).

2.2.1 Nutritional composition of the normal white maize and its limitations

The maize kernel, just like that of other cereal grains, is composed of pericarp (6%), endosperm (82%) and germ (12%). Its nutritional composition is known to vary greatly due to genetic make-up, environmental factors as well as agronomic practices, which may also influence the weight distribution and individual nutritional composition of the endosperm, germ and pericarp (FAO 1992). The main structural component of the endosperm is starch, a complex carbohydrate that corresponds on average to 72% of the grain and is a source of concentrated energy. Maize starch is composed of two fractions: amylose (25-30%) and amylopectin (70-75). Other carbohydrates present include simple sugars such as glucose, sucrose and fructose in amounts that vary from 1-3% of the kernel's weight (Nuss & Tanumihardjo 2010; FAO 1992). The high starch content of maize is associated with high viscosity diets in populations whose diet depend solely on maize.

Several researchers have fortified maize based products with legumes in order to reduce the viscosity thereby increasing the nutrient density of the food especially in foods intended for complementary feeding (Kannan *et al.* 2001).

The average protein content of the maize kernel is approximately 9%, which is intermediary between that of rice and wheat and is found mainly in the endosperm. Recent studies by Machida *et al.* (2010) have reported protein levels of between 8.92-10.52% in a normal white maize kernel. However, its quality is poor in terms of the two limiting essential amino acids namely: lysine and tryptophan, and high concentration of leucine, which causes an imbalance of the amino acids (Bhatia and Rabson 1987). The lysine content of maize was found to be 2-3%, which is less than half of the recommended value required during complementary feeding of 5.2% (WHO/FAO/UNU 2005). The high consumption of maize in SSA as food, which is estimated to be around 70% of the total maize production is causing severe PEM in some parts of SSA (Aquino *et al.* 2001). The rate of stunting is reported to be over 40% in areas where maize is the only source of protein (Hyman *et al.* 2008). In addition, 65% of the population in the maize farming system of SSA is reported to live on USD 2 or less per day implying the difficulty of affording animal sources of protein (Wood *et al.* 2010). However, in order to achieve the maximum nutritional benefits, several researchers have complemented maize with legume crops such as soya bean, broad bean and cowpea as well as breeding quality protein maize (QPM) to yield the amino acid value that is closer to the FAO/WHO/UNU standards. These efforts have helped reduce the incident of PEM, which is more prevalent in populations whose diet depend solely on maize (WHO/FAO/UNU 2005; Kannan *et al.* 2001).

The fat content of maize (4%) is higher than that of rice and wheat and comes mainly from the germ (Nuss & Tanumihardjo 2010). It is also genetically controlled and is a good source of polyunsaturated fatty acids, being particularly high in linoleic acid (2%) which is an essential fatty acids and need to be provided by the body. The saturated fatty acids in maize are mainly palmitic (11%) and 2% stearic acid (2%). Small amounts of arachidonic acids have also been reported in the maize kernel. These fatty acids aids in the absorption of fat-soluble vitamins

(FAO 1992). A consumption of 100 g of maize provides 50% or more of the average adult daily requirement linoleic acid and 20% of vitamin E. Furthermore, maize oil is relatively stable since it contains high levels of natural antioxidants (Bouis 1996). Maize oil is also highly regarded because of its fatty acid distribution, mainly oleic and linoleic acids. In this respect, populations that consume degermed maize benefit less in terms of oil and fatty acids than populations that consume whole-kernel maize products (FAO 1992). The fat is important in the diet as it contributes over twice as many calories as does an equal weight of carbohydrate or protein and malnourished young children tolerate fat well, and fat is often used to increase calories (FAO 1992).

Maize contributes considerable amounts of dietary fibre, which confers a significant physiological role in the grain. The dietary fibre comes from the pericarp and the tip cap, although it is also provided by the endosperm cell walls and to a smaller extent the germ cell walls. Insoluble fibre makes a greater contribution to the total dietary fibre content as compared to soluble fibre (Nuss & Tanumihardjo 2010). Dietary fibre content in dehulled kernels would obviously be lower than that of whole kernels. It has been demonstrated that insoluble dietary fibre helps normalize intestinal obstipation, accelerating and increasing the faecal bulk whereas soluble dietary fibre lowers blood pressure, improves blood glucose control in diabetes mellitus, aids in weight loss and improves immune function (FAO 1992).

The concentration of ash in maize is about 1.3%, only slightly lower than that of the crude fibre content. The germ provides about 78% of the whole kernel's minerals and the most abundant mineral is phosphorus, found as phytate of potassium and magnesium (Brinch-Pederson, Borg, Tauris & Holm 2007). Hence, it may become an important source of phosphorus, potassium and magnesium for those who cannot afford better sources of these minerals such as meat and its derivatives. Calcium, iron and zinc are found in low quantities due to the presents of oxalic acid, which forms oxalate precipitates with dietary calcium and phytic acid which forms insoluble phytates with iron, zinc and possibly other metals thereby inducing deficiencies in these minerals (Okoye 1992). The low iron content of maize is also attributed to the absent of vitamin C of the maize kernel which activates its absorption (FAO 1992).

Due to its significant fat content, maize is also a good source of vitamin A and vitamin E. The endosperm contains a significant amount of the vitamin A precursor compared to the germ and pericarp (Brinch-Pedersen *et al.* 2007). The total vitamin E of the kernel varies from 0.3-0.7 mg/100g for most varieties (Nuss & Tanumihardjo 2010). In regard to water soluble vitamins, maize has relatively high concentrations of thiamine, riboflavin and folate. These are mainly found in the aleurone layer, followed by the germ and the endosperm. Variable amounts of riboflavin have also been reported. However, the thiamine and the riboflavin content are affected by environment and cultural practices rather than genetic make-up (Patterson *et al.* 2000). Although the niacin content of maize is even higher than that of rice and wheat, its availability is poor and this deficiency is a serious nutritive problem in maize. The low availability of niacin is further worsened by a marked deficiency of tryptophan, its precursor. In addition, the high amount of leucine in maize is known to inhibit the conversion of tryptophan to niacin (Murray *et al.* 1994). Therefore, the niacin deficiency disease, pellagra is associated with diets almost solely based on maize. Similar to other cereals, maize is deficient in vitamin C (ascorbic acid) as well as vitamin B12. Other vitamins such as choline, pyridoxine and pantothenic acid are found in relatively low concentrations (Coulter 2009).

2.3 Provitamin A biofortified maize as a tool for addressing VAD in children of SSA

Maize is among one of six staple crops that have been targeted for biofortification with PVA carotenoids by the HarvestPlus Challenge Programme (Tanumihardjo 2008; HarvestPlus 2010). In SSA, Zambia is among one of the countries targeted for biofortification of maize, due to its high prevalence of VAD. About 53% of Zambian children has been reported to have VAD. Other than the high prevalence of VAD, maize is also a staple food crop that is highly consumed by a larger population in Zambia (UNICEF 2008). The target level of PVA as set by the HarvestPlus is 15 µg/g (DW) (Ortiz-Monasterio *et al.* 2007). However, some PABM varieties contains between 0.5 and 1.5µg of β-carotene (Harjes *et al.* 2008) which is below the target level of the HarvestPlus.

PABM varieties exhibit orange and yellow colours (Figure 2), which are unfamiliar to most individuals as they are accustomed to white maize. This affects its acceptability. The orange PABM variety was however found to be more acceptable than the yellow PABM variety. This is because the orange PABM variety has a distinct taste which separates it from yellow PABM (HarvestPlus 2009b).



A (Yellow)



B (Orange)

Figure 2. PABM varieties (Source: CIMMYT)

Although PABM varieties are not readily available on the SSA markets (Khumalo *et al.* 2011) due to less farmers growing the crop on their farms, (e.g. Zimbabwean farmers only produce 7% of PABM), farmers are however willing to grow PABM because of its attractive attributes such as being drought- and pest-resistant as well as the fact that it matures at a faster rate than white maize. PABM may thus be viewed as a potential complementary strategy for combating VAD as it is also cost effective (Muzhingi *et al.* 2008).

2.3.1 The success of provitamin A-biofortified maize

The success of PABM can be measured through bioavailability when it is processed into food and consumed. Bioavailability refers to the proportion of carotenoids from a meal that is absorbed, present in circulation and available for utilization, metabolism or storage by the

organism (Howe and Tanumihardjo 2006). 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. The released carotenoids are then transferred to lipid droplets and incorporated into mixed bile salt micelles (Howe and Tanumihardjo 2006)

Howe and Tanumihardjo (2006) investigated the bioavailability of PVA carotenoids from maize using Mongolian gerbils with vitamin A deficiency. The vitamin A content of the liver of the gerbils fed on orange PABM was found to be significantly higher than that fed on yellow PABM. Muzhingi *et al.* (2008) reported that all the subjects used in their in-vivo experiment converted yellow maize β -carotene to vitamin A and was absorbed. The reference dose (retinyl acetate), was also converted to retinol. These results demonstrated that encouraging the consumption of PABM instead of white maize could be a sustainable strategy to combat VAD in SSA's populations where maize is predominantly used as the subsistence food (Muzhingi *et al.* 2008).

2.3.2 The nutritional composition of provitamin A-biofortified maize kernel

PABM varieties contains a significant amount of carotenoids, which are devoid in white maize variety (Nuss & Tanumihardjo 2010). PABM has also been reported to contain significantly higher starch, protein and fat content as compared to white maize (Pillay *et al.* 2011). The carotenoids in PABM are genetically controlled and the endosperm contains the highest amount of the carotenoids whereas smaller amounts are found in the germ (Ortiz-Monasterio *et al.* 2007). However, the fraction of the carotenoids with PVA activity is about 10-20%, which is very small (Pillay *et al.* 2013). The alpha-carotene are present in very small quantities whereas zeaxanthin and lutein constitute about 30-50% of the total carotenoids (Ortiz-Monasterio *et al.* 2007). Long storage of PABM is associated with loss/destruction of the carotenoids. The carotenoids and xanthophylls of PABM stored for 3 years was found to have decreased from 4.8 mg per kg to 1.0 mg per kg (Watson 2002). Improving the protein quality of maize was found to increase the conversion of beta-carotene to vitamin A (Pillay *et al.* 2013). In a study conducted by Oikeh *et al.* (2004), yellow PABM was shown to have higher concentrations of iron (2.05 mg/100g) and zinc

(2.12 mg/100 g) than the white maize varieties (i.e. iron and zinc concentrations ranged from 1.69-2.07 mg/100g and 1.85-2.04 mg/100g, respectively). The iron and zinc concentrations of the PABM samples from the International Maize and Wheat Improvement Center (CIMMYT) ranged from 1.1-3.9 mg/100g and 1.5-4.7 mg/100g, respectively (Ortiz-Monasterio *et al.* 2007).

2.3.3 Consumer acceptance of provitamin A-biofortified maize in SSA

In SSA's culture, there seems to be a preference for white maize over yellow/orange PABM. This began with the influence of the British starch market in the 1920's as well as the establishment of roller mills on a large scale in 1955 (McCann 2005). Yellow/orange PABM has undesirable sensory attributes, which poses a challenge on its acceptability (Stevens & Winter-Nelson 2008).

A study conducted by Stevens & Winter-Nelson (2008) in Mozambique, showed the acceptance of orange PABM to be greater in regions where children are at increased risk of VAD. In their study, the caregivers of the children with VAD were more likely to trade for the orange PABM as a substitute source of vitamin A. Consumers were also willing to make a change in their consumption habits when nutrition education programmes are implemented. The authors concluded that the orange PABM could be useful in combating VAD in Mozambique as it provides low-income caregivers with an affordable way to diversify their diets as well as to improve the diets of their children (Stevens & Winter-Nelson 2008).

In central and southern parts of Zambia, the negative perceptions of yellow PABM did not affect its acceptability. Consumers with nutritional knowledge on PABM were more willing to accept and pay for it than those without the nutritional knowledge. A discount was also needed, for PABM to be purchased. However, consumers were willing to purchase PABM for a higher price (Meenakshi *et al.* 2010).

In Ghana, consumers from Ashanti district showed a preference for *kenkey* prepared with white maize over that prepared with yellow PABM and lastly orange PABM. These consumers were willing to spend more on white maize than yellow PABM and a lower amount for orange PABM than

yellow PABM. The consumers of the Central district preferred *kenkey* prepared with yellow PABM over that prepared with orange PABM or white maize and were willing to pay more for yellow PABM over white maize and orange PABM. However, the consumers of the Eastern Ghana preferred *kenkey* prepared with both yellow and orange PABM to that prepared with white maize. These consumers were willing to purchase yellow and orange maize over white maize. The authors concluded that in Ghana, PABM is well accepted in areas where it is well known and nutrition education would influence its acceptability (De Groote *et al.* 2010).

Yellow PABM was more acceptable over white maize in Suraya and Vihiga districts of Kenya and would be purchased at discounted prices. A premium was, however, paid for fortified white maize. Nutritional education was also found to influence the purchasing of PABM and it could be easier to introduce it in areas where it is usually grown (De Groote *et al.* 2010; De Groote & Kimenju 2008).

In KZN, South Africa, a study conducted by Pillay *et al.* (2011) showed a preference for yellow PABM to white maize food products in pre-school children than in secondary school children and adults. The results of the focus group discussions indicated that the adults disliked the colour, flavour and the texture of the yellow PABM and men were also more favourable towards yellow PABM than women. Consumers were willing to consume yellow PABM if sold at a cheaper price and readily available in local markets. It was concluded that PABM could be used to combat VAD among pre-school children in South Africa (Pillay *et al.* 2011). However, a study conducted by Amod *et al.* (2014), showed a preference for a composite complementary food prepared with PABM among the rural KZN caregivers. The elderly had a negative perception as they associate it with food aid during drought as well as an animal feed. The caregivers were also willing to give their infants PABM if it had a positive effect on their health, such as improved digestibility and if the nutritional value was higher than that of white maize (Amod *et al.* 2014). In Limpopo province, Khumalo *et al.* (2011) found that the price of maize had an impact on the consumer acceptance of PABM and consumers of one village would consume yellow PABM for its nutritional benefit rather than cost whereas consumers of other village would purchase PABM if it was cheaper than white maize. The authors concluded that there would be better exposure to

yellow PABM if it was readily available, rather than only during drought periods (Khumalo *et al.* 2011).

In Zimbabwe, bread made with yellow PABM was more preferred over that made with white maize in terms of taste. 94% of the consumers would consume yellow PABM if it had nutritional benefits over white maize. However, only 2% of the consumers had knowledge on the nutritional qualities of yellow PABM. 50% of the poorest and 30% of the richest fifths of urban consumers would switch to yellow PABM at a price discount of 20%. However, the discount for yellow PABM over white maize in Zimbabwe has been estimated at 10%, based on a yield advantage of 13% for the former. This therefore poses a challenge on the consumer acceptance of yellow PABM as estimates of the consumer surveys are higher (Muzhingi *et al.* 2008). The previous sections have described the importance of maize as a biofortification tool for combating VAD in SSA, the potential of BGN in addressing PEM in children of SSA will be discussed next.

2.4 The potential of Bambara groundnut for use in addressing PEM in children of SSA

BGN is believed to have originated from central Mali, West Africa and is known by various names in many parts of SSA, such as *nyimo* (Zimbabwe), *aboboi* (Ghana), *okpa* (Nigeria) and *jugo* (South Africa) (Nwana *et al.* 2005). It is regarded as the third most important crop after groundnut and cowpea and it produces reasonable yields under drought conditions and on poor, pest and disease ridden soils, where the cultivation of similar crops such as peanut, common bean and cowpea would not grow well (Sellschop 1962; Doku & Karikari 1971; Rachie & Silvestre 1977; Linnemann 1992). It also stores well on farms.

BGN is an important source of edible protein, particularly lysine, and is complementary to staple cereals, which are low in this amino acid (Goli 1995). It makes a complete food as it contains sufficient quantities of protein, carbohydrate and fat and its gross energy exceeds that of other common legumes such as cowpea, lentils and pigeon pea (FAO 1982). It thus has a potential to improve the nutritional status of both rural and urban SSA communities (Jideani & Diedericks

2014). People like its flavour, preferring it to cowpea, and food preparations have changed very little since the crop first gained popularity in the Middle Ages (Lewicki 1974).

2.4.1 The anatomical structure of Bambara groundnut grain

BGN is herbaceous, intermediate and annual legume that self-pollinates. Its grain is round/oval shaped with the size of about 1.5 cm in diameter and; smooth and soft when immature, but as it dries, it becomes harder and sometimes wrinkled. It has two distinct parts namely: the seed coat and cotyledon (Figure 3) (Occeña & Uebersax 2003).

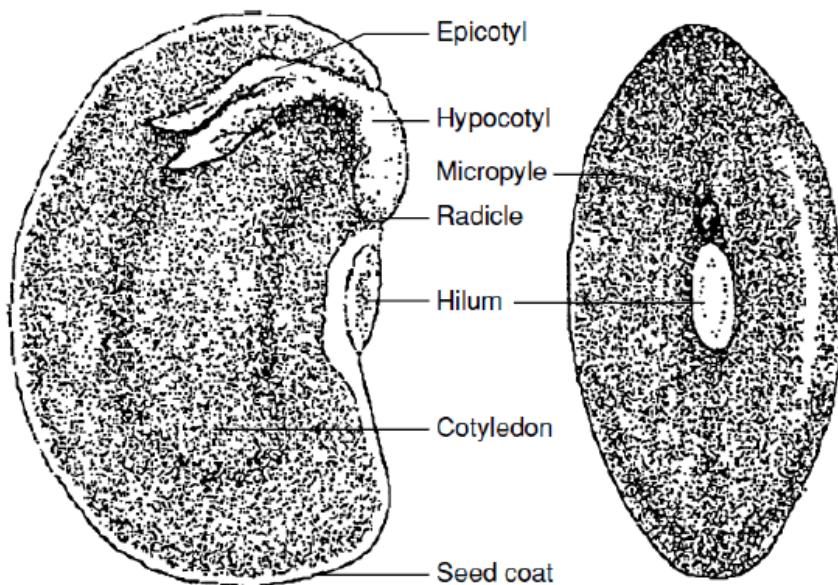


Figure 3. Anatomical structure of BGN grain (Occeña & Uebersax 2003)

2.4.1.1 Seed coat/testa

The seed coat/testa is the outer protective covering of the cotyledon. It develops from the integument of the ovule and has three distinctive cells namely: palisade, sub-epidermal, and parenchyma cells. These cells are compactly arranged to enhance the function as a barrier to the external environment. The seed coat has been identified to be rich in mineral nutrient, such as

calcium, magnesium, iron, zinc, potassium, and copper. Anti-nutritional factors such as phytate, tannins and phenolics have also been reported in the seed coat (Singh and Adebooye 2007).

2.4.1.2 Cotyledon

The cotyledon constitutes about 85% of the mass of the grain and it is the main food storage organ of the grain. It is an organized structure, composed of starch granules embedded in a protein matrix, with protein bodies surrounded by a lipoprotein membrane (Liu *et al.* 1992; Aguilera and Stanley 1985) and its cell wall is rich in cellulose (25.9-30.9%), hemicellulose (15.9-23.7%), lignin (0.4-0.6%), and pectin (28.5-41.2%). The middle lamella is found between cells cementing individual cells of the cotyledon, and mostly contains pectic substances, which dictate the physical strength of the tissue (Liu *et al.* 1992).

2.4.2 Bambara groundnut varieties

BGN is indigenous to SSA and there has been limited research into developing new varieties so all varieties are considered to be traditional. Recognizable morphological features such as colour and pattern of the testa are used for their identification. Figure 4 shows the different testa colours according to Brink *et al.* (2006) and Figure 5 shows the types of BGN eye and testa patterns according Goli (1995).

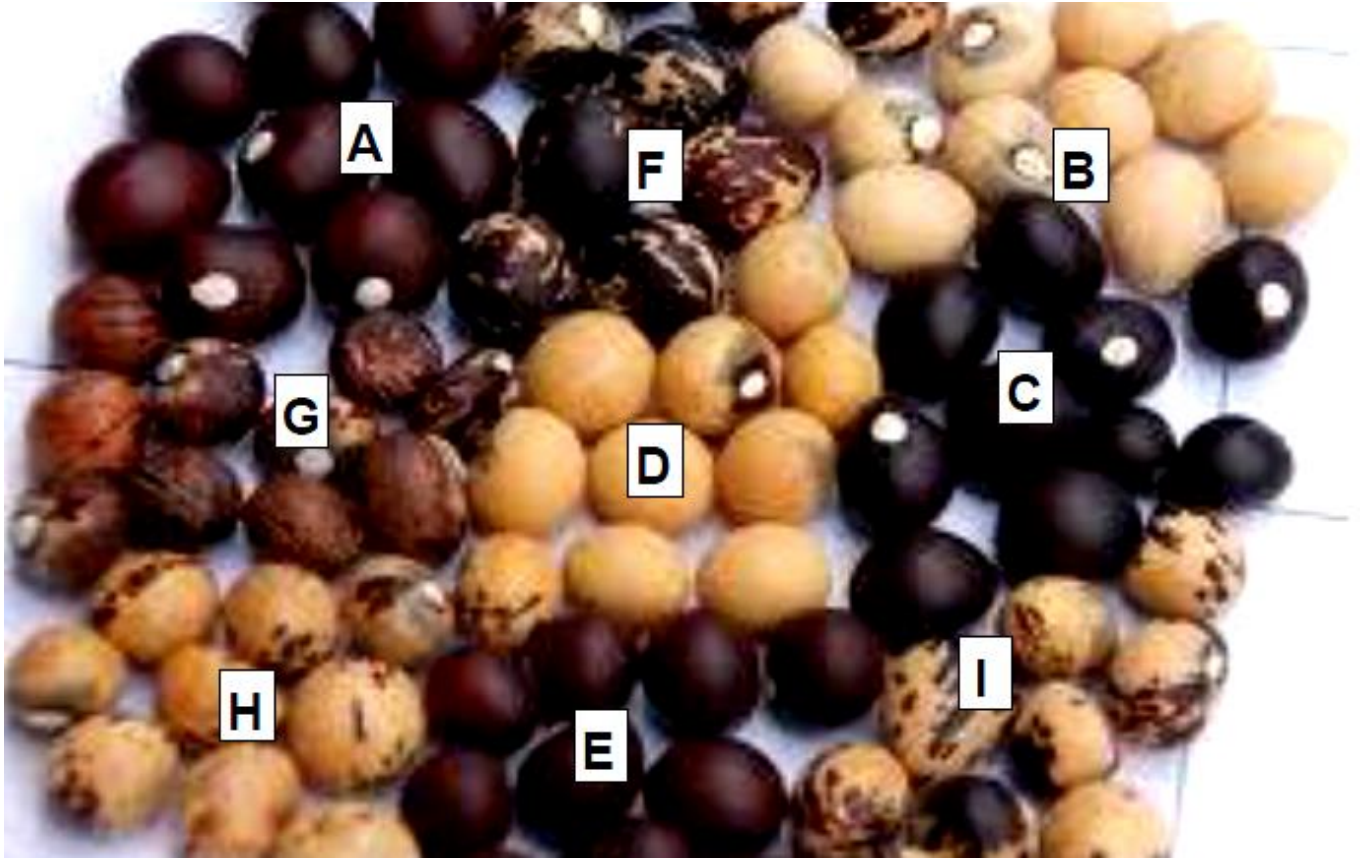
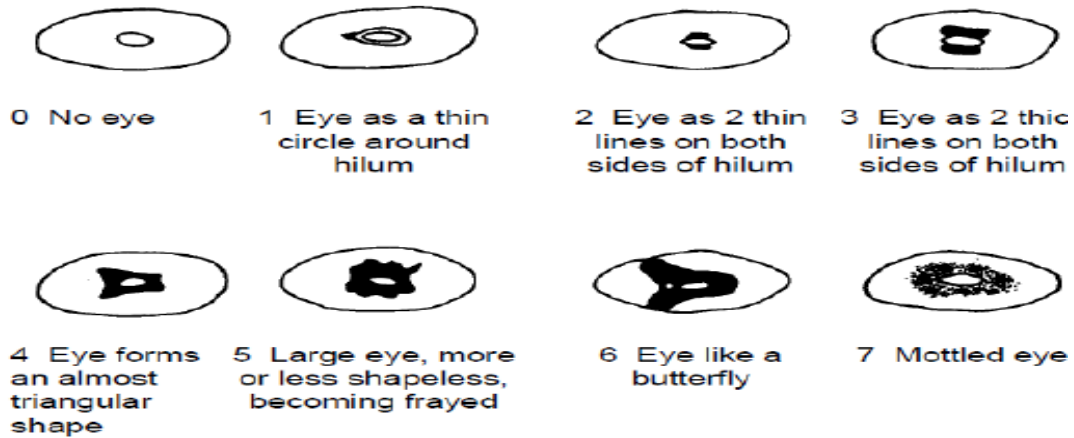
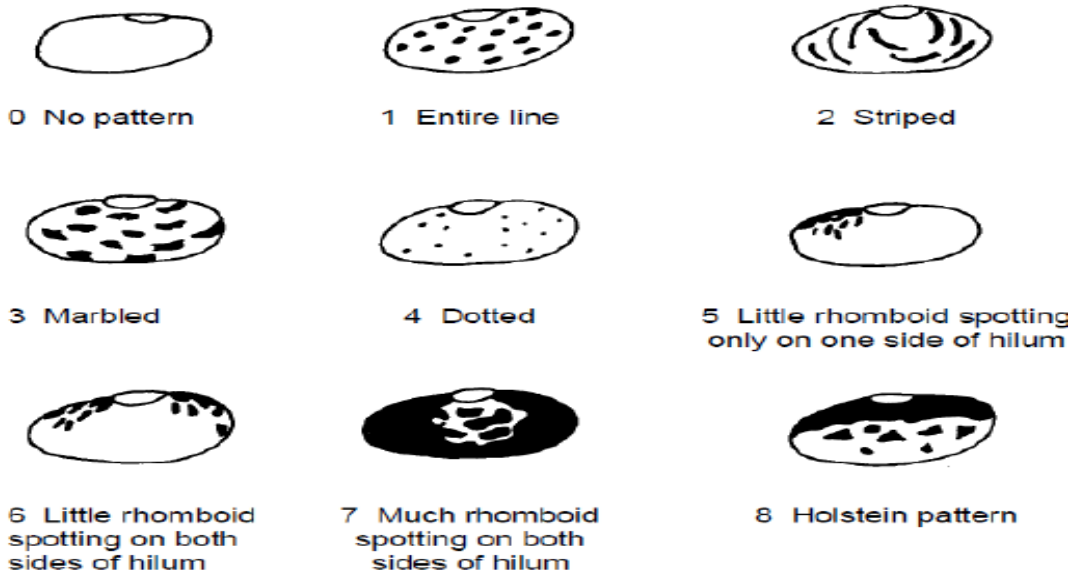


Figure 4. Different testa colours of the BGN grain: A-maroon, B-cream eye, C-black, D- brown and E-red (D and E where used in this study) while F-I named according to testa pattern (Brink *et al.* 2006)



(a)



(b)

Figure 5. Types of BGN (a) eye patterns and (b) testa patterns (Goli 1995)

2.4.3 The nutritional composition of Bambara groundnut

The total carbohydrates content of the BGN grain is about 60-63% and is predominantly composed of starch and non-starch polysaccharides, and lesser amount of reducing and non-reducing sugars (Minka and Bruneteau 2000). The protein content of the BGN grain differs with varieties and it ranges from 16-24% with the black and cream varieties having the highest and lowest protein content respectively (Temple and Aliyu 1994). Ojimekwe and Ayernor (1992) reported a protein content of 19.5% in the red variety and 19.0% in the brown variety. The amino acid lysine is found in high amounts whereas the total sulphur amino acids are found in fair amounts. Fetuga *et al.* (1975) observed that the essential amino acids content of lysine (6.82 g/16gN), methionine (1.85 g/16gN) and cysteine (1.24 g/16gN) to be comparable to that of soy bean lysine 6.24 g/16gN; methionine 1.14 g/16gN and cysteine 1.80 g/16gN. 94). However, the grain was found to be deficient in the amino acid tryptophan (Olomu 1995). The high protein content of BGN is an indication of its prospective use as a functional ingredient and the positive effects of processing techniques on the protein content of BGN is noteworthy, and could allow for utilization of BGN in the development of important food products such as complementary foods where high protein formulations are important (Nti 2009).

BGN is a non-oily leguminous grain which contains only about 6-8% (dry matter) of ether extract therefore could not give a cash crop status, which is of great importance in food industry (Aremu *et al.* 2006). The bulk of its fat content is unsaturated (about 59%) and is predominantly linoleic, palmitic and linolenic acids (Minka & Bruneteau 2000). The fat content of BGN grain was also found to be higher than that of the cereals (Goli 1995). BGN is also reported to have the highest concentration of soluble fibre among other legumes. Soluble fibre is a non-nutrient and is believed to reduce the incidence of heart diseases, diabetes mellitus and colon cancer (Minka & Bruneteau 2000).

The total ash content of the grains is 3.6% and is also a good source of iron, potassium and nitrogen (Mahala & Mohammed 2010). The red BGN variety has the highest iron content of 6.6 mg/100g, while 3.3-3.9 mg/100g was recorded for the cream-coloured variant. The red BGN

variety could thus be useful in areas where iron deficiency is a problem (Oyenuga 1968). In a study conducted by Omoikhoje and Arijeniwa (2004) that determined the proximate and mineral composition of raw, cooked, autoclaved and roasted BGN, the results on the mineral composition indicated that calcium ranged from 2.64-2.81%, phosphorus 0.06-0.08%, potassium 0.79-0.82%, sodium 0.004-0.005%, magnesium 0.65-0.68%, iron 151-160 ppm and copper 2.4-2.6 mg/kg (DM). On average, 100 g of the legume provide the following vitamins: 23% nicotinic acid, 50% thiamine, 15% riboflavin, 20% vitamin B6, 5% of the folate, and 30% of the pantothenic acid requirement of an adult based on US RDAs (Oyenuga 1968).

Studies have also shown that the nutritional value of the BGN grain is highly comparable to other commercialized legumes such as soya bean, cowpea, kidney, broad bean and chickpea (Table 1) (Caroline de Kock 2013).

Table 1. Comparison of the nutritional composition of BGN, soya bean, cowpea, kidney, broad bean and chickpea

	BGN	Soya bean	Cowpea	Kidney	Broadbean	Chickpea
Calories (kcal)	390	416	343	333	341	364
Proteins (g)	20.8	36.5	23.8	23.6	26.1	19.3
Carbohydrates (g)	61.9	30.2	59.6	60	58.3	60.6
Fat (g)	6.55	19.9	2.1	0.8	5.7	6.0

Source: De Kock (2013)

2.4.4 Utilisation of Bambara groundnut in SSA

The uses of BGN varies from country to country. Table 2 summarizes the uses of BGN in different parts of SSA

Table 2. A summary on the uses of BGN in different parts of SSA

Country	Uses	Reference
Kenya	<ul style="list-style-type: none"> - Among the Kambe and Giriama peoples of the Coast Province, dry grains are pounded to remove the seed coat, winnowed and boiled until they are cooked. It is then served with rice or <i>ugali</i> (a stiff maize meal porridge). - The Luo people make <i>nyoyo</i> (a mixture of BGN and maize boiled together), or plain boiled. This is served with tea, <i>nyuka</i> (porridge) or on its own. - Water from the boiled maize and BGN mixture is drunk to treat diarrhoea. - Among the Luhya tribe of Kenya, green pods are washed and boiled in salted water and eaten as a snack. The snack is rated higher than peanuts in terms of taste. - Dried grains are pounded/ground into flour and made into stew/sauce which is added to the traditionally prepared leafy vegetables and served with <i>ugali</i> or potatoes. - Dried grains are boiled with maize and served as a snack, especially with tea. 	Ngugi (1997)

	<ul style="list-style-type: none"> - Boiled BGN grains are also mixed with boiled sweet potatoes and mashed (a popular children's dish). It is also consumed plain, after being boiled and mashed. - Unshelled pods are boiled, fried and served with potatoes, bananas or <i>ugali</i>. 	
Nigeria	<ul style="list-style-type: none"> - Dried BGN is made into a paste then used to preparation various fried, or steamed products, such as <i>akara</i> (bean cake), <i>moin-moin</i> and <i>okpa</i> (bean pudding). 	Obizoba (1983); Uvere <i>et al.</i> (1999)
Ghana	<ul style="list-style-type: none"> - Commercial canning of BGN in gravy used to be a successful industry at GIHOC Cannery in Nsawam, Ghana, producing over 40 000 cans of various sizes annually. - In the northern parts, fresh BGN are boiled with a little pepper and salt/sugar to taste and served with fried ripe plantain. - In Southern parts, dry grains are also boiled and crushed to form cakes/balls. The cakes/balls are then fried and added to stews. - BGN is also made into a paste and used in traditional dishes such as <i>tubani</i> (steamed bean paste) and <i>koose/akla</i> (fried bean paste) - The cream variety are mixed with guinea fowl meat 	Swanevelder (1998); Nti (2009)

	and <i>dawadawa</i> for treating diarrhoea while black variety are mixed with water to treat sick children, ground to treat skin rashes or chewed to alleviate swollen jaw diseases.	
Cote d' Ivoire	- Grains are made digestible by being pounded to make flour, which will be used to make stiff porridge. The porridge keeps well and is traditionally used on journeys.	Goli (1995)
Cameroon	- Fresh grains are consumed as a complete meal by cooking with seasoning, or ground to prepare a traditional pudding sometimes with addition of taro leaves.	Ngugi (1997)
Angola and Mozambique	- In Angolan and Mozambican restaurants, boiled salted grains are often served as appetizers.	
Zambia	- Bread made from BGN flour has also been reported in Zambia.	Linnemann (1990)
South Africa	- <i>Seroma</i> (Sesutho), <i>tihove</i> (Shangaan) or <i>tshidzimba</i> (Venda) is prepared by adding BGN beans and peanuts, or just one of the two, to maize or millet-meal and boiling the mixture until it forms a stiff dough. This is salted and pounded into a ball, and will often keep fresh for several days. - Dry grains are boiled and then stirred, to make a thin porridge, which is known as <i>tshipupu</i> (Venda).	Swanevelder (1998)

	<ul style="list-style-type: none"> - Like maize, they may also be added to <i>lupida</i> (a porridge made from peanuts). - Immature or dried grains are boiled into a stiff porridge. - Dry grains are cooked with maize and pounded into a thick, sticky dough known as <i>dithaku</i> (in Sesutho). - Dried BGN are made into soup among the local white population. 	
Zimbabwe	<ul style="list-style-type: none"> - Ripe dry grains are roasted, broken into pieces, boiled, crushed and eaten as a relish with <i>sadza</i> (a stiff maize meal porridge). 	Zengeni and Mupamba (1995)
Botswana	<ul style="list-style-type: none"> - Immature grains are boiled in their pods, salted, and consumed, either on their own or mixed with maize grains. - Ripe, dry grains are pounded into a flour and used to make a variety of cakes, or are mixed with cereals to prepare several types of porridges. - The BGN stem and stalk are used as animal feed at end of the season, after the pods have been harvested. - Grains of the mature black landrace are used in traditional medicine. 	Bamishaiye <i>et al.</i> (2011)

Burkina Faso	<ul style="list-style-type: none"> - BGN constitutes the main source of vegetable protein for the rural population. - Its leaves, which are rich in protein and phosphorus, are used as fodder for livestock. 	Bamishaiye <i>et al.</i> (2011)
Democratic Republic of Congo (DRC)	<ul style="list-style-type: none"> - Some tribes in have been reported to have roasted the BGN grains and pounded them for oil extraction, despite the relative low oil content of the grain. 	Karikari (1971)

Despite its potential to improve the lives of the poor resource families with a reasonable yield and a source of edible protein in SSA, BGN is grossly under-utilized. Research on this crop has been very minimal, which contributes to its under-utilization (Linnemann 1992). Except in Ghana where canning of BGN used to be successful, it can be seen from Table 2.2 that BGN is only used at household level as food, medicine and as livestock feed. There are very limited products that are being processed to add value to the crop. Compared to other common crops produced in SSA, it is under-produced and has low yields - it is thus generally considered a lowly profitable crop (Mkandawire 2007; Begemann *et al.* 2002; Sesay *et al.* 1999). However, about 98% of farmers in Swaziland regard BGN as a profitable crop (Begemann *et al.* 2002; Sesay *et al.* 1999).

The crop is generally cultivated by poor rural subsistence farmers, particularly women in the semi-arid regions of tropical Africa for local consumption and only a surplus is sold. It is also given less priority in land allocation (Bamishaiye *et al.* 2011). In some parts of SSA such as Malawi, farmers are also failing to adopt the crop due to lack of BGN markets, shortage of land and seed, lack of knowledge on the importance and nutritive value of the crop as well as lack of knowledge on the different products that can be processed from BGN. Young farmers have misconceptions and attitudes towards the crop as they view it as for the elderly and someone planting the crop is seen as archaic and old fashioned (Pungulani *et al.* 2011).

The under-utilization of BGN in SSA is also attributed to its poor cooking characteristics (i.e. the hard to cook (HTC) phenomenon) which demand high energy, thus high cost of processing. A study by Plahar *et al.* (1998) in Accra, Ghana revealed that the main concern of farmers was not storage but rather processing, and farmers were reducing production, and had less for sale, because of processing constraints. The HTC phenomenon of the BGN grain is because of its thick testa. Hence, its water absorption capacity (WAC) is very poor relative to that of cowpea and peanuts (Plahar *et al.* 1998). Use of advanced technology in processing will thus allow BGN to be cooked more easily and efficiently.

Some taboos associated with consumption of BGN also affects its utilization. In Malawi, it is a common belief that BGN resemble bullets as such all people whose line of work predispose them to gunshot such as hunters, soldiers, police officers and criminals desist from consuming it. Traditional doctors also use BGN as part of herbal medicine to protect an individual or a household from being attacked by witches. In such cases, the households are advised not to cook or consume BGN. BGN, which have two seeds in one pod, are also used as part of a love potion so that a husband does not have extramarital affairs just as a pair of doves. As such, some men do not eat BGN, while those who beg are usually given shelled nuts (Pungulani *et al.* 2010).

Therefore, for BGN crop to gain popularity there is need to promote its utilization by increasing its production. This would benefit poor families, as surplus production would be sold. For example, in Bida, a central region of Nigeria, women have been reported to make pan cakes from BGN flour and reportedly enjoy a living selling them and in Mali, salted BGN are being sold by women, a premium production that is similar to macadamia nuts suitable for urban areas and possible for export as well (Begermann *et al.* 2002). It is also likely to find ready markets both at home and abroad as product that would add value to the crop would be developed as well. The consumer acceptance of BGN in SSA will be discussed in the next section.

2.4.5 Consumer acceptance of Bambara groundnut in SSA

Due to the under-utilization of the crop, there is little literature available on the consumer acceptance of BGN in SSA.

In Ghana, processing technique used in developing BGN flour was found to have a more profound effect on the sensory attributes than variety. The mean sensory scores for weaning porridges prepared with either roasted or improved flour indicated very much liking for the product, whereas those prepared with raw flour gave sensory scores that indicated only slight preference for the product. Dehulling was found to improve the consistency of the product while heat treatment, whether boiling or roasting, improved the taste, aroma and overall acceptability (Plahar *et al.* 2000). In another study conducted by Plahar *et al.* (2000), HQBF weaning food

had mean scores for all sensory attributes indicating very much liking whether the red or cream varieties of BGN were used in the preparation of the flour. However, when HQBF was used in other traditional food, (*akla/koose*) significantly lower scores for texture/consistency, mouthfeel and taste were obtained (i.e. moderate liking). When the suitability of HQBF for traditional food uses among the food processors/cooked food vendors in Northern Ghana was further examined, the results showed a good acceptance of HQBF over the traditional flour that the respondents were used to. HQBF was found to have good flavour and not possessing the typical raw beany flavour associated with the traditional flour (Plahar *et al.* 2002).

In Mozambique, Pungulani *et al.* (2010) found that most BGN consumers prefer the white variety with white bud (*otapo*), followed by white with black bud and brown variety. The *otapo* was reported to be sweeter whereas the brown bitter and its shell rigid, thus its cooking becomes difficult. Children fed on BGN for a relatively period of time were reported to be fat and stronger (Pungulani *et al.* 2010).

In Kenya, Brough *et al.* (1993) compared BGN milk with milks from other commercialized beans such as cowpea, pigeon pea, green bean and black bean in terms of preferences. Differences among the groups were shown, with the adults, both men and women ranking in this order: first cowpea, BGN, pigeon pea, green bean and lastly black bean, whereas in young people the preference changed a little where BGN gained the first to the detriment of cowpea and other legumes maintained the place rank (Brough *et al.* 1993).

Pungulani *et al.* (2010) determined the consumer acceptance of six cooked BGN varieties in Malawi. The results of the average acceptability is shown in Table 3.

Table 3. The acceptability rating of the six BGN varieties

BGN variety	Average acceptability rating
2768	6.15 ± 1.7
181 cream	5.87 ± 1.0
181 red	5.75 ± 1.1
<i>Chikope cha nyani</i>	6.44 ± 1.4
<i>Mazira a mpheta</i>	5.50 ± 2.0

The authors concluded that there is potential for BGN acceptance and consumption in Malawi (Pungulani *et al.* 2010). In another study Pungulani *et al.* (2010) also investigated the consumer acceptance of the following BGN dishes: *chuwa*, *chipere*, roasted, stewed and seasoned BGN, *mndavwa* and mixed BGN with cooked maize. In their findings, *chuwa* and stewed and seasoned nuts dishes were most preferred by the consumers. There was however a preference for small-sized nuts for the stewed dish as large-sized were reported to be mouthful and were disliked by the consumers Pungulani *et al.* (2010).

In Nigeria, fresh paste from BGN was found to produce *moin-moin* that was equally accepted in all attributes compared to *moin-moin* from fresh cowpea paste. The colour, flavour, texture, taste and overall acceptability scores of *moin-moin* made with BGN flour from steeped cold water and from blanched hot water were rated similarly to those of *moin-moin* from fresh pastes (controls). However, the *moin-moin* from steam blanching treatment had low rated colour, flavour, taste, texture and overall acceptance. Roasted sample produced less firm *moin-moin*. This could have been attributed to heat damage to its biopolymers constituents. Poor rating of the samples that were prepared by steam blanching and roasting was due to low dehulling ability and high least gelation concentration. The authors concluded that it is possible to produce acceptable *moin-*

moin from BGN (Olapade and Adetuyi 2007). It is likely that compositing BGN with PABM in developing a complementary food will improve the acceptance of the resulting blend. The complementary feeding process is discussed in the next section.

2.5 Complementary feeding

Complementary feeding refers to the introduction of solid foods to an infant's diet alongside breastfeeding from the age of 6 months, when breast milk alone is not sufficient to meet all nutrient requirements for the infant, up to 24 months (WHO/UNICEF 2002). The complementary feeding stage is determined by the neurological stage of development as it is expected that at six months the infants' gastro-intestinal tracts should be well developed to digest complementary foods in addition to breast milk. There is therefore need to undertake a careful assessment of the infant's growth and developments before initiating the complementary feeding process (WHO/UNICEF 2002).

Infants are especially vulnerable to malnutrition and infection during the complementary feeding period and nutritional needs for growth and development during the complementary feeding period are greater per kilogram of body weight than at any other time of life (WHO/UNICEF 2002). Good nutrition is essential at this stage to ensure healthy brain and body development. Insufficient nutrient intake and illness resulting from low quality food and poor complementary feeding practices are main causes of malnutrition. Consequently, growth faltering occurs in the first two years of life, especially in SSA. If not addressed, malnutrition and infection can lead to death or long term irreversible consequences on future learning ability, economic productivity, immune response and reproductive outcomes (WHO/UNICEF 2002).

On the other hand, the complementary feeding period is a crucial time of opportunity. Nutritional interventions during this stage can lead to great benefits. Feeding practices appropriate for the child's age, nutritionally adequate foods and continued breastfeeding can ensure optimal growth and development. Approximately 6% of deaths of children under-five could be prevented through improvements in complementary foods and feeding practices (WHO/UNICEF 2002).

WHO/UNICEF (2002) have recommended the use of home-based complementary foods that are easy to prepare, available affordable, safe and nutritious e.g. cereal-legume based complementary foods. Cereals generally are known to be relatively low in the amino acids: lysine and tryptophan, but fair in sulphur-containing amino acids i.e. methionine and cysteine. On the other hand, legumes are relatively rich in proteins (19-26%) and fat (40-46%), and contain moderate quantities of tryptophan and threonine. This class of foodstuff can therefore form a good supplement to cereals (WHO/UNICEF 2002). The next section looks more specifically at the popular complementary foods in SSA.

2.5.1 Traditional complementary foods used by caregivers in SSA

The composition of local complementary foods varies from place to place and from country to country. In Ghana the main traditional complementary food given to infants is fermented maize porridge (*koko*), while in Sierra Leone *ogi* prepared from maize or sorghum is used. Fermented maize or millet (*akamu*) and fermented *gari* (*foo foo*) are commonly used in Northern and Southern parts of Nigeria respectively and in Benin Republic a fermented maize, sorghum or millet (*ogi*) is used (Armer-Klemesu and Wheeler 1991). Malawian mothers were reported to give non-nutritive liquids, such as water to their children. Further investigations also found that children consume predominantly refined maize meal porridge which is bulky and low in energy and key nutrients for growth e.g. refined maize meal porridges contains about 10 g/100g of protein. The mothers were also reported to give their children foods prepared for the older family members such as *nsima* as complementary foods, which did not meet at least 80% of their estimated nutrient needs, as they were deficient in macronutrients and micronutrients (Hotz and Gibson (2001)

A study conducted by KNBS and ICF Macro (2010) revealed that the main complementary food given to the Kenyan infants is made up of only a cereal and water in 22% to 71% of the cases. The minimum of 4 food groups out of the 7 food groups recommended by WHO was achieved by less than 50% of the children. In their another study which assesses the complementary feeding practices of children aged 6-23 months of Korokogo Slum, Nairobi, Kenya, KNBS and

ICF Macro (2010), found that nearly all the children consume foods made from grains, roots and tubers mainly in the form of porridge. This was similar to studies done by Hussein (2005) in Tanzania, Rao *et al.* (2011) in Kenya and Owino *et al.* (2008) in Zambia. There was low consumption of vitamin-A rich fruits and vegetables as well as other fruits and vegetables. These findings compare with those of studies conducted in Kenya by Chelimo (2008). It was also highlighted that the low consumption of vitamin A-rich foods was contributed by the high poverty level in the slum and therefore limited income to purchase food. The intake of iron rich and iron fortified foods in the study was also reported to be low and showed consistency with past observations by Mamario *et al.* (2005) in Tanzania and Serlmitos & Fusco (2001) in Zambia which established that complementary foods are inadequate in iron.

In Burkina Faso complementary food diversification was found to be low and only limited to cereals and in Senegal, the most common foods given to infants are cassava, millet, and rice gruels. The grains are usually mixed with animal milks or water. Due to their deficiencies in vitamin A, iodine and iron, these foods represent poor protein sources and are inappropriate complementary foods when they are the primary source of nutrition (Sawadogo *et al.* 2011). The next section discusses the challenges of traditional complementary feeding in SSA.

2.5.2 Challenges of traditional complementary feeding in SSA

In many SSA countries, complementary feeding usually starts early. In Malawi Hotz and Gibson (2001) found that that all the 163 mothers in their study had introduced complementary food to their infants at four months of age. In Tanzania, Hussein *et al.* (2005) established that mothers in medium and high-income groups introduced complementary foods earlier than it is among lower income groups. These findings are also in agreement with those of a previous study conducted by Wandel *et al.* (1989) in the same country, which established that women participating in economic activities had less time for cooking and caring for their infants. Working mothers in Addis Ababa, Ethiopia were found to be more likely to introduce complementary foods before 6 months compared to mothers who stays at home (Gebru 2007). A systematic review by Wijndaele *et al.* (2009) shows that mothers with low maternal knowledge and perceptions about

infant feeding are more likely to introduce complementary foods early. A study assessing feeding practices of children in an urban slum of Kolkata in Nairobi, Kenya by Joshi *et al.* (2012) found that children below 6 months of age were introduced to complementary foods due to a perceived lack of sufficient breast milk by their mothers. The same was also demonstrated in a study conducted by Ochola *et al.* (2008) in the Kibera slum of Nairobi, Kenya.

Nutritional inadequacy of complementary foods in SSA is another challenge faced with the introduction of complementary foods by SSA mothers. From the studies reviewed in previous sections it is evident that the traditional complementary foods given to children in SSA are nutritionally inadequate as they are bulky (i.e. high viscosity and low in energy density) and have been found to provide low energy for the growing child (Akinyele and Omotola 1986). Mouquet *et al.* (2008) reported the solid content of cereal-based complementary porridges consumed in Burkina Faso to contain 5-8 g/100g solid content corresponding to energy density of 0.3 kcal/g. This energy density is marked below the minimum recommendations for complementary foods (0.8 kcal/g) (UNICEF 2008). At this low energy density, a child would need to be fed more than 5 times per day, which may not be feasible and low energy density weaning foods caused by these bulk gruels have long been implicated in PEM (Guthrie 1989; Okoye 1992; Devlin 1997).

The nutritional inadequacy of the traditional complementary food is attributed to the presence of toxins and anti-nutritional factors found in this food as well as poor processing, preparation and hygiene. Okoye (1992) reported a 90% loss of free folic acid content of cereals during boiling and a 7-12% reduction in available lysine was also observed by Anyanyo *et al.* (2011) in fermented sorghum porridges. Unfortified maize meal porridge given to infants in SSA as a complementary food is usually over-cooked and over-diluted. Although some studies indicate that margarine, peanut butter, eggs, sugar and formula powder are added to the porridge, the porridge however still lacks nutrients due to the loss of nutrients from over-dilution. The infants were found to be deficient in calcium, iron and zinc (Faber *et al.* 1999). An over-diluted formula is sometimes added to the porridge, making it more nutritionally inadequate. The next section reviews the proposal to composite BGN and PABM in developing an instant complementary food in order to address PEM and VAD.

2.6 Proposal to composite BGN and PABM in instant complementary foods to address PEM and VAD in SSA

As reviewed in previous sections, unfortified white maize porridge constitutes the most common starter complementary food given to infants in maize consuming parts of SSA. It is however nutritionally inadequate when consumed alone as it lacks vitamin A and its protein quality is also poor in terms of the amino acids: lysine and tryptophan. Therefore, compositing PABM with BGN in developing a complementary instant food could improve the nutritional quality of the resulting blend. It was also reviewed in Chapter Two that about 90% of white maize is produced in SSA. There is thus a potential for the locally produced white maize varieties in SSA to be developed into the PABM varieties. Home gardens that focused on the production of the PVA-rich vegetables, such as OFSP has shown to improve the vitamin A status of Mozambican, Nigerian and Ugandan children (Faber & van Jaarveld 2007). Hence, the use of PABM as a complementary food would be critical to address the risk of VAD associated with the use of white maize-based complementary foods.

On the other hand, BGN also has the potential to be composited with PABM in the preparation of complementary instant food since it is viewed as a women's crop and women at subsistence level (Bamishaiye et al. 2011) mainly do its production. It therefore easier to promote its cultivation in home gardens (Faber & van Jaarveld 2007). Furthermore, crops grown by women are mainly used for household consumption because women are usually responsible for cooking the food at household level. Given reports that BGN grain serves as a source of cheap protein in poor SSA's populations by being the least expensive and easily stored and transported non-processed protein source for rural and urban dwellers, it can thus substitute the expensive animal protein, which the majority of the poor SSA populations cannot afford. The surplus lysine will complement PABM in the complementary instant food (Rachie and Silvester 1977). This will help in combating PEM in children in SSA.

Other than the nutritional quality, the functional and sensory quality of a complementary instant food are also important (De Groote & Kimenju 2008). BGN has been reported to have poor

functional properties due to its hard testa. Use of advanced processing techniques has been suggested as a remedy of improving the functional properties of BGN (Plahar *et al.* 2000). In terms of sensory quality, PABM varieties have been reported to have undesirable sensory characteristics such as colour, flavour and texture and taste (Muzhingi *et al.* 2008). However, caregivers have been found to be willing give their infants PABM-based foods if it has some nutritional and health benefits (Amod *et al.* 2014). On the other had BGN has been reported to have desirable sensory characteristics such as flavour (Lewicki 1974). Therefore, using advanced techniques in compositing BGN with PABM when developing a complementary instant food will improve its functional and sensory quality as well as its nutritional quality. It is likely that the undesirable sensory characteristics of the PABM will be masked by the desirable sensory characteristics of BGN. The next section reviews the technology of processing instant porridge.

2.7 The technology of processing instant porridge

2.7.1 Instantisation

The process of developing instant porridges involves the instantisation of flours. Instantisation can be defined as the production of an agglomerated powder with greatly improved properties for dry handling and reconstitution (Peitsch 2005). It occurs as a result of gelatinization of starch and is facilitated by grain processing methods. Gelatinization can be described as the breaking of hydrogen bonds among and within starch molecules that opens the granules to hydration and enzymatic hydrolysis. Gelatinization of grains occurs at 90-100°C (Henning *et al.* 2006). The grain processing methods that are used to develop an instant porridge by facilitating gelatinization of starch include: cleaning, soaking, boiling, drying, milling, flour roasting, formulation, and reconstitution. These processes and their impact on nutritional, functional, and sensory quality of grains and instant porridge are reviewed next.

2.7.2 Grain processing

2.7.2.1 Cleaning

The process of cleaning aims at separating whole grains from split, broken and powdery ones, dirty, stones, chaff as well as other foreign materials such as metals. Traditionally grain cleaning is done manually by winnowing using sieving baskets as well as by hand picking of foreign matter and contaminants (Alonge and Adigun 1999). Traditional grain cleaning has however reported to be more laborious and time consuming. In food industries grain cleaning is done mechanically with machines such as winnowing fans, pre-cleaner, grain cleaner, grain grader and dry de-stoner. Grain cleaning result in reduced bulk of the material, safe and longer storage and high value products (Sahay and Singh 1994).

2.7.2.2 Soaking

Soaking is a simple technological treatment used by mothers to prepare complementary foods at home. It can be a simple prolongation of the obligatory washing of the grains and can have other advantages, such as facilitating dehulling or swelling of grains (Lestienne *et al.* 2005). The amount of water absorbed during soaking is affected by different factors such as the initial moisture content, grain variety, soaking duration, water to grain ratio, temperature and acidity level of the water (Hotz and Gibson 2001). Grain soaking is however, time consuming e.g. cereal grains are soaked for a period of 12-14 hr at 25°C or for 72hr prior to milling and dried legume grains are hydrated for 3-12 hr based on temperature of water (Hotz and Gibson 2001). Grain soaking results in retention of nutrients by removing some anti-nutritional factors thereby improving starch and protein digestibility as well as improving palatability of the product (Egounlety and Aworh 2003).

2.7.2.3 Boiling

Cooking by boiling is one of the commonest methods used to cook any form of comestible grain. It is generally done to produce a tender edible product, improve the sensory characteristics of

food and inactivate anti-nutritional factors present in grains (Negri *et al.* 2001). Cooking by boiling can be done in an open or a pressure cooker. Appropriate cooking times for grains are affected by genetic factors, physical structure, chemical composition and processing (Liu 2005). Consumers have been found willing to pay between 0.7% and 1.2% above the original price for a one-minute reduction in cooking time of grains (Faye *et al.* 2004). A reduction in cooking time is advantageous because it saves energy. Nutritional value is also improved by lower losses of leached nutrients as well as the destruction of heat labile vitamins during prolonged cooking (Akinyele *et al.* 1986).

2.7.2.4 Hot air oven drying

Currently hot air oven drying is the most widely used method in drying of grains and the majority of industrial drying installations rely on it since it is the simplest and most economical among other methods. (Akpapunam and Abiante 2006). The drying process involves both heating the product and removing water from the product surface. Inappropriate drying conditions results in grain injury and vast economic loss. A number of researchers have tried to determine the optimum drying condition for production of good quality grains. Different kinds of grains may have dissimilar optimum drying conditions. However, McDonald and Copeland (1997) suggested that a drying air temperature of 43°C is accepted as the safe upper limit for drying most grains without damage.

Hot air oven drying, results in a more uniform, hygienic and attractively coloured dried product as well as better effects on the nutritional and functional properties of flour (Ogunlakin *et al.* 2012). Despite the beneficial attributes of oven drying method over direct sun drying, it is an energy consuming operation and less-resourced communities, such as the majority of rural households in SSA, can hardly access this technology.

2.7.2.5 Conventional roller milling

In a conventional roller milling process, flour is produced by gradually reducing the particle size of the feed stock by a series of grindings (pairs of counter-rotating rolls), with intermediate

separation of bran, germ and endosperm flour streams by sifters and purifiers (Figure 6) (Posner & Hibbs 1997).

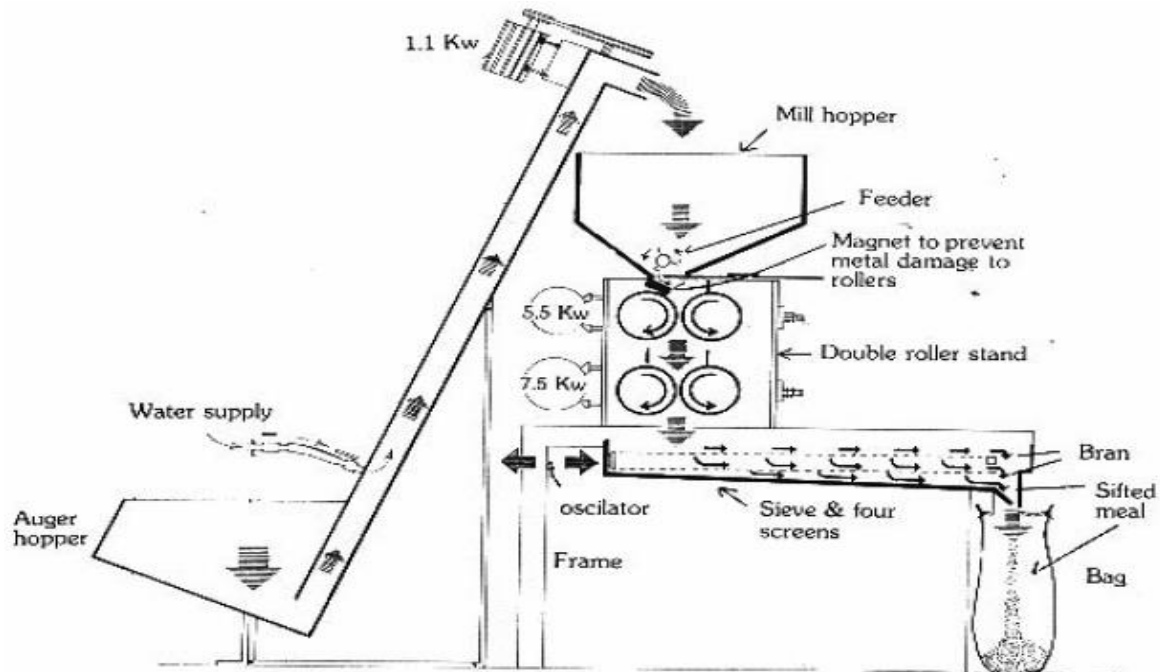


Figure 6. A typical double roll roller mill designed to process grain (Courtesy of Maximill, Kroonstad, South Africa)

During milling, the protein content of the cereal grain is decreases. Taylor *et al.* (1997) found that the germ and the pericarp, the parts normally removed during processing to be 3-4 times richer in lysine than the endosperm. In fact, they noted that with the exception of leucine, the protein composition of the germ conformed to high quality protein (Exam *et al.* 2002). Serna-Saldivar and Rooney 1995) also observed that nutrient digestibilities of mechanically milled sorghums were slightly higher than those of whole grains. However, nitrogen retention and protein efficiency ratios were lower due to removal of the germ. Milling may also decrease some anti-nutritional factors found in grains e.g. conventional roller milled brown sorghum was found to have a reduced amount of condensed tannins which may have deleterious effects on the nutritional quality of the product than traditional milled sorghum (Serna-Saldivar and Rooney

1995). Mwasaru *et al.* (1998) also observed that conventional roller milling removed 37% of the original grain weight and reduced tannins from 4.5-0.2 catechin equivalents.

2.7.2.6 Roasting of flour

Roasting of flour is done at a low temperature for a short period of time in an open pan or oven to avoid burning which might bring undesirable flavour and colour to the product. Roasting generally improves the flavour, colour and texture of the roasted flour (Fennema 1996). A roasted nutty aroma was found to be more intense in porridges with heat treated *marama* flour as compared to the porridges with unheated *marama* flour (Kayitesi *et al.* 2010). The flavours produced during roasting are as a result of Maillard reaction and Strecker degradation of amino acids and sugars. Maillard reaction is a non-enzymatic browning reaction that occurs between amino acid and a reducing sugar (Fennema 1996) whereas Strecker degradation occurs when carbonyl derivative from the Maillard reaction reacts with free amino acids in the food product. This causes a degradation of amino acids to aldehydes, ammonia, and carbon dioxide. The aldehydes that are produced contribute to the aroma. Strecker degradation of each specific amino acid produces a unique aldehyde with a unique aroma (Fennema 1996).

Other than improving the sensory attributes of the food, roasting has also been shown to enhance the digestibility of starch and proteins as well as other nutrients, thereby increasing the nutritional value of food. Roasting generally leads to a significant reduction in insoluble dietary fibre and total dietary fibre but an increase in soluble dietary fibre (Vidal-Valverde and Frias 1991). The heat can release vitamins such as vitamin B6, niacin, folacin, and certain carotenoids from poorly digested complexes and thereby enhancing the bioavailability of these vitamins. Roasting of flour has also been shown to lower trypsin inhibitor and hemagglutinin activities (Vidal-Valverde and Frias 1991). Therefore, roasting being a simple and cost effective processing method can be used in SSA in order to achieve maximum nutrient utilization from legumes and cereals flours such as BGN and PABM.

2.7.2.7 Compositing of cereal flours with legume flours

Several researchers have composited cereal flours with legumes flours in developing foods intended for complementary feeding. Opeifa *et al.* (2015) developed and evaluated 'ogi' prepared from yellow PABM and horse-eye bean (*Mucuna urens*). Yellow PABM variety and horse-eye bean were processed into flour, mixed in the following ratios 95:5, 90:10, 85:15 and 100% PABM flour (control). The result obtained revealed that the proximate parameters of the samples increases as the horse-eye beans added increase, which confirm the reality of the enrichment. However, in terms of overall acceptability the blend ratio of 95:5% horse eye bean was the most the acceptable to the panellist ($p < 0.05$). The authors concluded that apart from adding value and varieties to *ogi* meal due to its textural improvement, fortifying maize flour with horse-eye bean flour at 5% level would reduce the problem of food security especially among children where PEM is common and the utilisation of horse eye bean (Opeifa *et al.* 2015).

In a recent study, Mbata *et al.* (2009) enriched a maize based complementary food 'ogi' with BGN. It was shown that fortification of *ogi* with BGN protein at 10% and 20% replacement levels at the rate of fermentation and organoleptic product quality results in the nutritional enhancement of the product. The composite blends of maize and BGN with a ratio of 70:30 w/w gave a better nutritional and supplemental relationship in the production of BGN-maize 'ogi' in terms of the mineral content (zinc, calcium, magnesium, iodine and iron) as well as the essential amino acid content. The 70:30 blend also showed better sensory characteristics than other blends (Mbata *et al.* 2009).

The proximate composition, functional properties and sensory characteristics of a composite blend of germinated rice flour (GRF) and germinated Bambara-nut flour (GBF) in the ratio 100:0 (control), 90:10, 80:20 and 70:30 (w/w) were investigated by Adebayo-Oyetero *et al.* (2011) using standard processing technique. A decreasing trend with an increase in the level of substitution with respects to water absorption (10.38-5.12) and swelling capacities (6.85-4.91) was observed. However, the bulk density increases with increase in level of substitution (0.60-0.69). Proximate composition data indicated an increasing level of protein, fat, ash, crude fibre,

iron and gross energy (19.64-21.10%, 4.8-6.1%, 1.6-2.0%, 1.1-1.6%, 4.4-6.3% and 371.3-377.6% respectively, while carbohydrate content decreased (64.5 to 58.8%) with increasing level of substitution with BGN. Apart from adding value and varieties to the meal due to textural improvement, fortifying *Ofada* rice flour with BGN flour at 20% level would produce a more nutritionally balance and acceptable product, which will reduce problem of food security among children in Nigeria where PEM is prevalent (Adebayo-Oyetoro *et al.* 2011).

2.7.2.8 Reconstitution of composite instant flours

Instant porridges need to be reconstituted with hot water or milk. Reconstitution also known as rehydration can be described as the rate at which dried foods pick up and absorb water reverting to a condition which resembles the un-dried material, when put in contact with an excessive amount of liquid (Masters 1976). According to Hoge Kamp and Schubert (2003), there are four mechanisms that are involved in reconstitution namely: (i) wettability, (ii) sinkability, (iii) dispersability and (iv) solubility.

Wettability describes the capacity of the powder particles to absorb water on their surface, thus initiating reconstitution. It depends largely on the particle size as small particles, represents a large surface area to mass ratio and may not be wetted individually but may clump together sharing a wetted surface layer. This layer reduces the rate at which water penetrates into the particle clump. Clumping can be reduced by increasing the particle size and/or agglomerating the particles. Agglomeration is the production of several larger and heavier particles that are linked together. Therefore, agglomeration increases particle size, porosity and decreases density as compared to a non-agglomerated instant product (Peitsch 2005)

Another important property of reconstitution is sinkability, which describes the ability of the powder particles to sink quickly into the water. This depends mainly on the size and density of the particles. Larger denser particles sink more rapidly than finer, lighter ones. Particles with a high content of occluded air may be relatively large but exhibit poor sinkability because of their low density. Finally, dispersability describes the ease for a powder to be distributed as single

particles over the surface and throughout the bulk of the reconstituting water, while solubility refers to the rate and extent to which the components of the powder particles dissolve in water (Peitsch 2005).

Generally, the degree of reconstitution of an instant powder is influenced by structural and chemical changes caused by dehydration, processing conditions, sample preparation, as well as the sample composition. In a study conducted by Henning *et al.* (2006) reconstitution capability was found highly related to the density and porosity of the dried product. In their study, the rehydration index of four different classes of potatoes, subjected to variable drying conditions was found inversely proportional to the bulk density. Highest rehydration index was reported in extruded *beate*, followed by the non-extruded *beate*, pimperl and *mandel* potato varieties. This is because extrusion results in the formation of agglomerates in the product hence the high rehydration index reported in the extruded *beate*, pimperl, and *mandel* potato varieties than in non-extruded *beate*, pimperl, and *mandel* potato varieties because of non-agglomerated particles (Henning *et al.* 2006). The next section reviews the factors affecting the expected nutritional, functional and sensory quality of cereal-legume based complementary porridges.

2.8 Factors affecting the expected nutritional, functional and sensory quality of cereal-legume based complementary instant porridges

2.8.1 Nutritional quality

When formulating a complementary food, the following should be taken into consideration (i) feeding frequency, (ii) energy density and (iii) the energy intake from the milk. A concept that is used to calculate the recommended macronutrient composition of complementary foods for infants with medium to high intake of milk (600 mL/day; 375.5 kcal/day) is based on the calculated gastric capacity of 30 g/kg body weight/day and minimum energy density of foods of 0.6 kcal/g (Dewey & Brown 2003). This approach uses the recommended protein energy of 12% (Monte & Giagliani 2000). The fat content is of the cereal-legume based complementary instant porridge should contain at least 31% of its energy as fat for an infant and the remaining energy

requirement not provided by fats or proteins, is then used as the basis for calculating the carbohydrate content (Dewey & Brown, 2003; WHO 2004).

Given the relatively small amounts of foods that are consumed at 6-23 months, the nutritional density (ND) of the diet also needs to be very high i.e. the complementary food should provide substantial amounts of micronutrients (especially iron, zinc, calcium, vitamin A, vitamin C and folates) with relatively fewer calories (Monte & Giugliani (2001). The micronutrient density of complementary food is of particular important, due to the nutrient need per unit body weight and the limitations associated with ingestion, digestion and storage of nutrients during infancy (Bhutta 2000). The desired micronutrient densities of complementary foods (per 100kcal) for children aged 6-23 months assuming average breast milk intake according to WHO (2011) is shown in Table 4. Appropriate complementary foods should also include a low content of anti-nutrients (e.g. phytate and polyphenol) as only portions of ingested nutrients are biologically available Dewey 2001).

Table 4. The desired micronutrient densities of complementary foods (per 100kcal) for children aged 6-23 months assuming average breast milk intake (WHO 2011)

Vitamins	6-8 months	9-11 months	12-23 months
Vitamin A	26	22	20
Folate	9	7	17
Niacin	1.24	0.08	0.86
Pantothenic acid	0.24	0.18	0.16
Riboflavin	0.07	0.05	0.05
Thiamine	0.07	0.05	0.06
Vitamin B6	0.10	0.07	0.07
Vitamin B12	0.02	0.02	0.06
Vitamin C	1.3	1.2	1.3
Vitamin D	1.9	1.4	0.8
Vitamin E	0.5	0.4	0.6
Vitamin K	3.6	2.5	2.3
Minerals			
Calcium	88	64	58
Iodine	7	6	5
Magnesium	13	9	7
Selenium	0.0	0.0	1.0
Iron			
<i>15% bioavailability</i>	7.7.	1.7	0.6
<i>12% bioavailability</i>	2.5	2.2	0.8
<i>10% bioavailability</i>	3.1	2.6	0.9
<i>5% bioavailability</i>	3.8	5.4	1.9
Zinc			
Moderate bioavailability	1.3	0.9	0.4
Low bioavailability	1.7	1.2	0.4

2.8.2 Functional properties

The functional properties are considered as the fundamental physicochemical characteristics that influence the behaviour of raw materials and final product during processing, storage and consumption (Boye *et al.* 2010; Aremu *et al.* 2007). Based on a study by Kerr *et al.* (2000), when cereals flours are composited with legume flours, the functionality of the cereal-legume composite flour changes due to the increased protein content. These properties include colour, texture, water absorption capacity (WAC), solubility index (SI) swelling volume (SV) (yield) and reconstitution ratio (RR).

2.8.2.1 Colour of grains and porridge

The Commission Internationale de l'Éclairage (CIE) redefined the X, Y, Z model of describing any colour visible to the human eye by introducing CIE L*, a*, b* notation (Perez-Magarino and Gonzalez-Sanjose 2003). L* is the degree of lightness of the colour. This refers to the relation between reflected and absorbed light. L* values equals to zero for black and 100 for white, a* (red-green) is the degree of redness (0 to 60) or greenness (0 to -60) and b* (yellow-blue) is the degree of yellowness (0 to 60) or blueness (0 to -60) (Perez-Magarino *et al.* 2003). The CIE L*, a* and b* system is commonly used in food industry (Perez-Magarino and Gonzalez-Sanjose 2003) and the Hunterlab colorimeter could be useful in measuring colour of different grain varieties and colour of porridge on the basis of CIE L*, a* and b* values (Perez-Magarino, and Gonzalez-Sanjose, 2003). In cereal-legume based porridges, a high lightness (L*) and low Chroma (a*) is desired to meet consumer preference (Galvez and Ressureccion, 1993). However, flours usually contain other compounds resulting in higher a* and b* values (Ikegwu *et al.* 2010).

2.8.2.2 Texture of the grains and porridge

The hardness of grain is an important functional property for the production of instant porridges because it affects water absorption, seed-coat permeability, and overall texture and quality of the product. Grain hardness is affected by calcium content and water absorption, and affects

cookability (Chen *et al.* 1993). Calcium content in grains varies with cultivar but does not vary significantly with different environments. Generally large-sized grains are preferred in the development of instant porridges as small-sized grains requires more processing time because they have a tendency to become hard in texture which also causes undesirable ammonia gas especially in legume grains as well as higher cost of production (Bourne 1972). The instruments that are used to evaluate grain and porridge texture include puncture, shear and compression tests (Rhodes *et al.* 1972). A puncture test was described as one of the simplest methods and most commonly used in instrumental measurements for food texture (Bourne 2002), but it can give rise to large experimental errors when using single samples. The major disadvantages of shear test include lack of uniform protocol and multiple variable sources involved in the sample preparation and testing processes. A compression test is rarely used because gases are easily trapped in most products (Wheeler *et al.* 1994). There are however no official quantitative guidelines on the acceptable texture (consistency) of a porridge. The WHO guidelines only stipulate that the complementary porridges should not drip from the spoon, which is not a quantitative standard for texture (Okabe 1979).

2.8.2.3 Water absorption capacity (WAC) and solubility index

WAC can be defined as the ability of a product to associate with water under limited water conditions. It also indicates the amount of water available for gelatinization (Edema *et al.* 2005). The amount of water absorbed depends primarily on the availability of the polar chains and the imido group of the peptide, which binds water through hydrogen bond (Osungbaro *et al.* 2010). Thus a high WAC of a flour suggests the possibility of presence of some hydrophilic proteins or polar amino acid residue in the flour (Odoemelam 2000). Flours with no fortification have a higher WAC as compared to composite flours due to more hydrophilic constituents such as carbohydrates (Kaur and Singh (2005). The WAC of the BGN-*ogi* was found to be lower (41.5g/100g) as compared to that of maize fermented flour (44.5g/100g) (Mbata *et al.* 2009). The low WAC of the BGN-*ogi* flour was attributed to the presence of higher amount of protein, which is hydrophobic in nature (Mbata *et al.* 2009).

Solubility index (SI) is the ability of water to penetrate into the starch granules of flour. It depends on the type and origin of the starch (Ikegwu and Ekumankana 2010). The WAC of a composite flour influences its SI. The presence of protein or fat in cereal-legume composite flours results in a decrease in WAC of the flour, which may lead to reduced swelling and consequently reduced solubility (Appiah 2011). This assertion explains the reason for the high solubility in legume flours that are low in low fat content such as cowpea and BGN than cereal flours e.g. maize and wheat flours. The high solubility of legume flours suggests that they are more digestible and therefore can be suitable for use as ingredient in infant food formulations (Pomeranz 1991).

2.8.2.4 Swelling volume (SV)

Swelling volume (SV) also known as yield measures the cumulative effects of starch quality, specifically amylose/amylopectin ratio as reflected by the volume of gel produced when flour is heated with an excess of water (AACC 2000). It indicates the volume of the undissolved sediment obtained after centrifugation and the lower the SV the denser the nutrients. Rickard *et al.* (1991) reported an optimal yield of 80% (w/w) of dried cereal-legume composite complementary food extracted from oven drying.

2.8.2.5 Reconstitution ratio (RR)

Reconstitution ratio (RR) is the ratio between the dry material mass and water mass. It varies from 1:2 to 1:50 depending on the type of the dehydrated food (Peitsch 2005). In an experiment in which dried maize-bean porridges were tested under different temperatures, better RRs were obtained for samples dried at lower temperatures. By immersing the product during 60 seconds in a 90°C water bath higher rates were achieved than using water at 20°C. However, when instant porridges are reconstituted, they must show acceptable textural, visual, and sensory characteristics (Peitsch 2005).

2.8.3 Sensory quality

The sensory quality of food are their organoleptic appeals such as taste, aroma, colour and texture. Sensory evaluation is a scientific discipline used to evoke measure, analyse and interpret reactions to the sensory quality of food, as perceived by the senses of sight, smell, taste, touch and hearing (Seibel 2007). Consumers might buy a food product for nutrition, convenience and image, but most importantly, they buy the sensory properties (Dzung *et al.* 2004). It is therefore important to include sensory evaluation as integral to defining and controlling product quality. In developing, a cereal-legume based complementary foods; different proportions as well as grain processing methods used to produce the flours tend to change the sensory attributes of the complementary foods (Anyango *et al.* 2011).

Most cereal-legume composite flours used in complementary foods usually have a high score of 4 on a 5-point hedonic scale for colour, where, 5=golden brown, 4=brown, 3=neither brown nor dark, 2=slightly brown, 1=dark brown, but low acceptability scores for texture, flavour and overall acceptability (Barimalaa & Okoroji 2009). The brown colour of cereal-legume complementary foods is due to heat treatment such as roasting of flours, which causes Millard reaction that is associated with the browning of the flours.

The colour of the cereal-legume based porridges is also affected by the grain cultivar as well as the method of milling used to produce the flour. Light-coloured grains give light-coloured porridges, which seem to be preferred in most SSA cultures. In terms of flour yield Kebakile *et al.* (2008), found abrasive, decortication and hammer milling being superior to other milling processes as the flours produced were lighter in colour as compared to roller milling which produced dark-coloured flours. This is because dry abrasive action effectively removes bran from the endosperm without tainting, resulting in production of clean dry meal (flour) (Kebakile *et al.* 2003).

The low acceptability scores for flavour of cereal-based complementary foods is attributed to the beany flavour that is associated with legumes. A study by Asma *et al.* (2000) shows poor

acceptance of sorghum-cowpea complementary porridges by the caregivers due to the presence of beany flavour. Obatolu (2000) also reported low acceptability of soybean-maize porridges which was attributed to the presence of beany flavour. The beany flavour in cereal-based complementary foods is caused by the action of lipoxygenase enzymes on free fatty acids. Heat treatment of the legume grains during processing such as boiling at a higher temperature (75-100°C) or roasting will result in the denaturation of the lipoxygenase enzymes (Obatolu 2000). A roasted nutty flavour was found to be more intense in sorghum-based porridges with heat-treated *marrams* flour as compared to porridges with unheated *marama* flour (Kayitesi *et al.* 2010).

Generally, the texture of the porridge is directly influenced by the quality of flour used in the preparation of the porridge and the flour quality is strongly affected by the characteristics of the grain quality especially grain hardness and the type of the milling process used (Fliedel 1995). Kebakile *et al.* (2003) conducted a study on the comparison on the milling process of hand pounding, roller milling and abrasive decortication, followed by hammer milling on the textural properties of a cereal-legume based complementary porridge. The study revealed that hand pounding produced coarser meals with lower ash content and oil contents than the other two milling processes. Roller milling gave finer meals with slightly more ash and oil and produced the darkest meals but it had the greater advantage of giving the highest extraction (Kebakile *et al.* 2003). Therefore, a complementary porridge should be finer in texture. The possible effects of BGN and PABM on nutritional, functional and sensory quality of complementary foods is discussed in the next section.

2.9 Possible effects of Bambara groundnuts (BGN) and provitamin A-biofortified maize on nutritional, functional and sensory quality of complementary foods

2.9.1 Nutritional quality

PABM and BGN, the cereal and the legume choices of this study have the potential of giving a nutritious complementary food when blended together. As reviewed in previous sections complementing cereal and legumes improves the nutritional quality of the resulting blend as

compared to the individual food components. In addition, BGN has been reported to have better protein quality in terms of the two essential amino: lysine and tryptophan, which are limiting in maize. On the other hand, PABM also contains PVA carotenoids which are absent in both white maize and BGN varieties as well as significantly higher amounts of starch, protein and fat than white maize (Pillay *et al.* 2011). Therefore, compositing BGN with yellow PABM will improve the protein, energy and vitamin A content of the blend as well as other micronutrients such as calcium, phosphorus, magnesium, manganese, copper, zinc and iron.

Grain and flour processing such as soaking and boiling have also been reported to improve the bioavailability of nutrients such as vitamins (Egounlety and Aworh 2003). The grains in this current study are to be hydrated by soaking for an overnight period and boiling until tender. Soaking and boiling will also eliminate some anti-nutritional factors present in the grains. This is likely to improve the digestibility and bioavailability of the porridge's nutrients. Other than improving bioavailability of nutrients, heat processing of the grains and flours such as boiling and drying also has the potential to degrade heat-labile nutrients. However, PVA carotenoids have been shown to be stable to heat processing, although excessive exposure to heat may result in significant losses (Pillay *et al.* 2014). A substantially high carotenoid retention was observed when PABM was cooked into *phutu* and *samp* (Pillay *et al.* 2014). In this present study, quality control standards that minimises the reactions that destroys heat liable nutrients are to be done (i.e. use of low temperatures and short time methods).

2.9.2 Functional characteristics

Previous sections have also reviewed that composite flours have poor functional characteristics in terms of WAC and SI due to higher concentration of proteins that are hydrophobic in nature as compared to the flours with no fortification. In addition, the literature indicates that use of advanced techniques in grain processing improves its functional characteristics. The WAC and SI of the BGN-PABM composite complementary instant porridges in this study are to be improved by increasing the sorptive capacity of the grains through soaking and boiling. Roasting of flour at low heat for approximately 8-10 minutes will minimise the tightening of the starch

molecules that will improve the rehydration properties of the porridges. Roasting will also improve the colour and texture of the resulting blend. Since conventional roller milling has been reported to produce finer meal, the texture of the porridges in this study will be also be improved by conventional roller milling of grains to a particle size of 200 μm as well as reconstituting with hot water.

2.9.3 Sensory attributes

Consumer acceptance case studies conducted in difference parts of SSA has shown poor acceptance of PABM in terms of colour, texture and flavour. It was also demonstrated that the acceptance of PABM product depends with the type of food as well as the preparation method (Pillay *et al.* 2011). On the other hand, BGN has been reported to have desirable sensory characteristics, particularly its flavour. The acceptance of BGN-based foods was also found to be influenced by the technology used to develop the food rather than variety and use of advanced techniques in developing the food was shown to increase the acceptance of BGN-based food (Plahar *et al.* 2000). Therefore, fortifying PABM instant porridge with BGN will improve the acceptance of the blend as the desirable sensory characteristics of BGN are likely to mask the undesirable sensory characteristics of the PABM. The advanced techniques that are to be used in processing the BGN-PABM composite complementary instant porridge in this study will also improve its acceptance. Heat treatment such as boiling drying and roasting will improve the colour, texture and flavour of the porridge. It is likely that the beany associated with legumes will be removed because of heat treatment. The sugar added would also improve the flavour. Conventional roller milling has been reported to produce finer meals hence the texture of the porridge will improve because of conventional roller milling. Therefore, the overall acceptability of the BGN-PABM composite porridge is likely to improve due to the improvement in texture, colour and flavour of the composite blend.

2.10 Conclusion

PEM and VAD have been declared serious nutritional and health problems among the poor rural SSA's children. Biofortification and cereal-legume complementation are cost-effective nutritional strategies that could be used in addressing PEM and VAD. Unfortified white maize meal porridge constitutes the first and most common complementary food given to SSA's children whose staple diet is maize. This is nutritional inadequate as it low in energy, poor in protein quality and devoid in vitamin A as well as several micronutrients. This exposes the children at risk of PEM and VAD. Hence, maize could be an ideal medium for biofortification with PVA carotenoids. On the other hand, BGN could be combined with PABM to complement the protein quality of the PABM. The PABM will improve the PVA carotenoids and the overall energy content of the blend. Hence, PABM and BGN could be used for combating PEM and VAD.

However, it has been reported that cereal-legume based composite flours have poor functional properties due to an increase in the protein and a decrease in carbohydrate content, as several protein types are hydrophobic and hence bind less water than carbohydrates, which are hydrophilic in nature. Use of advanced techniques in developing instant porridges can improve the functional characteristics of the product.

Consumer acceptance studies on PABM and BGN had shown poor acceptance of the PABM in terms of colour, texture and aroma and a good acceptance of BGN particularly its flavour. Therefore, compositing BGN with PABM in developing a complementary instant porridge will improve the sensory characteristics of the porridge, as the undesirable sensory characteristics of PABM could be masked by the desirable sensory characteristics of BGN. There is however lack of data on compositing BGN and PABM in developing a complementary instant porridges and the nutritional, functional and sensory quality of these foods. It would be, therefore, highly beneficial to investigate the acceptance of BGN-PABM as a complementary instant porridge together with its nutritional and functional quality.

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Chapter 3 - Grain properties and the nutritional composition of Bambara groundnut and provitamin A-biofortified maize varieties

Abstract

A complementary food produced by combining Bambara groundnut (BGN) and provitamin-A biofortified maize (PABM) could contribute significantly to addressing protein-energy malnutrition (PEM) and vitamin A deficiency (VAD), which are prevalent in sub-Saharan Africa (SSA). The nutritional composition and food functional properties of different grain types of BGN and PABM may vary due to genetic and environmental factors, therefore, it is necessary to assess the properties and nutrient content of grain types intended for use in complementary foods. Therefore, the aim of this investigation was to determine the properties and the nutritional composition of grains of BGN and PABM varieties and thereby evaluate their suitability for use in a BGN-PABM complementary instant food. There was no significant difference ($p > 0.05$) in the texture (firmness and toughness) of the whole grains of each of the white maize (reference), yellow PABM (control), red and brown BGN varieties at 20, 30 and 40% moisture content, respectively. The CIE colour values (Hunter L^* , a^* and b^*) of the whole grains, oven dried grains and flours of each maize and BGN varieties were however significantly different ($p < 0.05$). L^* was significantly ($p < 0.01$) and negatively correlated to a^* , positively correlated to b^* and negatively correlated to toughness. a^* was significantly ($p < 0.05$) and positively correlated to toughness whereas b^* was significantly ($p < 0.05$) and negatively correlated to toughness. There was a significant difference ($p < 0.05$) in moisture, ash, fat, crude protein, calcium, magnesium, phosphorus, zinc, copper, manganese content among all the grains. Provitamin A (PVA) (as vitamin A equivalents) was measured in the yellow PABM and brown BGN grains, the PVA was not detected in these grain types. When compared with the reference (white maize grain), the control (yellow PABM grain) had higher moisture, ash and fibre content. The brown BGN variety had higher moisture, ash, fibre, protein, calcium, magnesium, potassium, zinc and copper as compared to the red BGN variety. Overall, the brown BGN was more superior in terms of ash, fat, fibre, crude protein, calcium, magnesium, sodium, phosphorus, zinc, copper, manganese and iron. The results of this investigation suggest that PVA carotenoids are susceptible to destruction

during storage. Being regarded as a complete/balanced food, the BGN grain has thus the potential to make a significant contribution towards meeting the nutritional requirements of poor communities whose diets depend solely on cereals including maize.

3.1 Introduction

Maize/corn (*Zea mays* L.) is the third among the world's leading cereal grains after wheat and rice. The grain is grown across a range of latitudes, moisture regimes, slopes and soil types (Smale, Byerlee & Jayne 2011). It serves as an important staple diet for the majority of poor rural SSA populations (McCann 2005). The average consumption of maize in SSA is 106.2 g/person/day (WHO 2003). In Southern Africa, maize provides more than half of the calories as well as of protein in the food consumed in Malawi, Zambia, and Lesotho, and more than 40% in Zimbabwe (McCann 2005). Maize is prepared into a wide range of food forms which vary from region to region and from one ethnic group to another (Alexander 1987). However, the most common traditional foods prepared with maize include porridges (thin and stiff), breads, beverages, and snacks (Ortiz-Monasterio *et al.* 2007).

The nutritional composition of white maize is unbalanced and inadequate for the consumers that depend largely on it (Nuss & Tanumihardjo 2010). Its carbohydrate content is predominantly composed of starch and its protein quality is poor in terms of the two limiting essential amino acids, lysine and tryptophan. The fat content is predominantly composed of the polyunsaturated fatty acid linoleic acid and low in saturated fatty acids. Maize is however, a good source of the following micronutrients: thiamine, pyridoxine and phosphorus (found as phytate of potassium and magnesium) (Brinch-Pederson, Borg, Tauris & Holm 2007). It is also a fair source of riboflavin, niacin, folate and biotin (FAO 1992). Calcium, iron and zinc are the micronutrients that are present in very small quantities due to the presence of oxalic acid, which forms oxalate precipitates with dietary calcium and phytic acid, which forms insoluble phytates with iron, zinc and possibly other metals thereby resulting in deficiencies in these minerals (Okoye 1992). The unbalanced nutritional composition of white maize, especially its poor protein quality and the lack of PVA carotenoids could partly explain why the prevalent of PEM and VAD in children

living in poor rural areas of the SSA region where maize is a staple food crop (Nuss & Tanumihardjo 2010). Estimates indicate that 35.8% pre-school children in SSA are underweight, 42.7% are stunted and 9.2% are wasted due to PEM (de Onis *et al.* 2004) and 42.4% of children aged between 0-59 months are at risk of VAD (Dary and Mora 2002). About 20-24% of child mortality from malaria, measles, diarrhoea and 3% mortality from infectious diseases are attributed to PEM and VAD (Rice *et al.* 2004).

In an effort to combat VAD, maize has been targeted by the HarvestPlus Challenge Programme among other staple crops for biofortification with PVA carotenoids (HarvestPlus 2009b). The breeding targets of PVA carotenoids as set by the HarvestPlus Challenge Programme is 15µg/g (DW) (Ortiz- Monasterio *et al.* 2007). The deep yellow/orange PABM has been reported to contain significantly higher amounts of carotenoids (15µg/g DW) than the yellow variety (0.25-2.5µg/g DW). PVA carotenoids are however highly susceptible to destruction during long period of storage. PABM varieties were also found containing significantly higher amounts of starch, protein and fat as compared to the white maize varieties (Pillay *et al.* 2011). Hence, PABM has the potential to be used as a tool for combating VAD in children of the SSA region. However, the grain properties and nutrient content of PABM varieties have been found to vary seemingly largely due to genetic factors (Pillay *et al.* 2014). These findings suggest that it is necessary to assess the grain properties in order to determine the suitability of PABM grain for food applications, such as processing of complementary foods.

Since biofortification is a complementary strategy that can be used with other strategies in combating malnutrition, it is vital to complement a PABM instead of white maize with legumes in order to combat VAD and PEM. One such legume which could be suitable for combining with PABM could be Bambara groundnut (BGN) (*Vigna subterranean* L.). The crop is of West African origin and is regarded as the third most important crop after groundnuts and cowpeas in Ghana. It is a drought resistant crop with much potential for enriching the diets of people living in the marginal areas (Sellschop 1962; Doku & Karikari 1971; Rachie & Silvestre 1977; Linnemann 1992). Recognizable morphological features such as colour of the testa (i.e. red, brown, cream, black, etc.) and the varying morphology of the testa (i.e. mottled, with or without

hilum (eye) coloration, blotched or stripped, with or without hilum coloration) are used for their identification (Goli 1995; Brink *et al.* 2006). As a human food, BGN grain is consumed either when immature or fully ripe and dry (Swanevelder 1998). When still immature, the grain is usually made into a snack by either boiling with salt and pepper or boiled mixed with maize or peanuts. Dried grains are made digestible by grinding into flour, which is used to make bread, stiff porridge, or fortifying cereal-based foods to improve the protein content for both infant and adult foods such as pap and pottage (Linnermann 1990; Swanevelder 1998). Roasting, pounding into pieces, boiling and crushing and served with sadza (a stiff porridge), also makes ripe grains into a relish (Zengeni and Mupamba 1995).

The nutritional composition of the BGN grain may differ across the several varieties existing, but the grain is regarded a complete food. Its total carbohydrates fraction is about 60-63% and is predominantly composed of starch and non-starch polysaccharides, and lesser amount of reducing and non-reducing sugars (Minka and Bruneteau 2000). The protein content ranges from 16 to 24% (Temple and Aliyu 1994). The black variety of BGN has been reported to have the highest protein content whereas the cream variety has the lowest (Nti 2009). In semi-arid regions, BGN is an important source of lysine, and is complementary to staple cereals, which are limiting in this amino acid (Lewicki 1974). The essential amino acids: lysine, methionine and cysteine of the BGN were found highly comparable to those of soya bean (Fetuga *et al.* 1975). The BGN grain maybe deficient in tryptophan (Olomu 1995). The fat content reported in BGN (6-8%) is higher than that of cereals (Aremu *et al.* 2006). The grain is also a good source of the minerals: iron, potassium, nitrogen, and the vitamins: thiamine, riboflavin, niacin, and carotene, but very low in ascorbic acid (vitamin C) (Oyenuga 1968). The reported variations in the BGN varieties with respect to grain properties (e.g. grain morphology and colour) and nutritional composition indicate that the grain types can be evaluated and the most suitable selected for use in specific food products, e.g. complementary foods.

Therefore, compositing flours made with PABM and BGN grains in developing a complementary instant porridge instead of using white maize flour may improve the nutritional quality of the resulting blend in terms of energy, protein and vitamin A. That contribute to addressing PEM and VAD in children in the SSA region. The aim of this study was to assess the grain properties and the nutritional composition of PABM and BGN grain types intended for use in a BGN-PABM complementary food.

3.2 Materials and methods

3.2.1 Bambara groundnut and maize grain varieties

The BGN grain varieties used in this research chapter were brown and red whereas the maize varieties were white (PAN 67) and yellow PABM (PVAH 94) (Figure 7). The white maize (PAN 67) was used as a reference and yellow PABM (PVAH94) was used as a control. The BGN grain varieties were obtained from Mbare Musika market, Harare, Zimbabwe, whereas the maize grains were produced as described in section 3.2.2.



Figure 7. Grain varieties used in this study. From left to right: brown BGN, red BGN, white maize and yellow PABM

3.2.2 Production of the yellow PABM and white maize varieties

The provitamin A maize hybrid grain (PVAH 94) (control) was produced by convectional breeding methods at Cedara Research Station, KZN, South Africa. The reference (white maize variety PAN 67) was also produced under the same conditions and location as the biofortified variety. Bulk grain of the developed maize hybrids was produced under the same environmental conditions and then harvested for use in the current study. The ears of the grain were harvested manually and then dried for 21 days at ambient temperature ($\pm 25^{\circ}\text{C}$). After manual threshing the grains were stored in a cold room (approx. 4°C) until needed. The grains were analysed for their physical properties and nutritional composition.

3.3. Sample preparation

3.3.1 Grain processing

The whole grains of yellow PABM, white maize, brown, and red BGN varieties were processed as described in section 3.3.1.1-3.3.1.2.

3.3.1.1 Cleaning

Each grain variety was cleaned manually by hand sorting to remove extraneous material and damaged grains.

3.3.1.2 Soaking

About 3kg of each cleaned grain was separately soaked in tap water in the ratio of 1:2 (w/v) at 25°C for an overnight period.

3.3.1.3 Decanting and washing

After an overnight soaking, water was decanted off and each grain washed separately thrice in tap water.

3.3.1.4 Boiling and decanting

Each grain was cooked separately in distilled water at 100°C in the ratio of 1:3 (w/v) in a stainless steel heavy bottom pot on a Defy Thermofan Stove (Model 731 MF) on high heat (plate control 6) until tender. Water was then decanted off.

3.3.1.5 Drying and cooling

Each cooked grain was then dried separately in a hot air oven drier (UL 453B The GS Blodgett Co. Inc. Burlington VT. USA) at 43°C (McDonald and Copeland 1997) to 14% moisture content. This was followed by cooling for a period of 1-2hr at 25°C.

3.3.1.6 Milling

Each oven dried grain (about 14% moisture) was separately milled into flour of particle size of 200 µm using a pilot plant roller mill (GB 12350 Leshan Dongchuan Machinery Co. Ltd).

3.3.2 Grain physical properties

3.3.2.1 Colour

The CIE colour values (L^* , a^* and b^*) of the cleaned whole grains, oven dried grains and flours of the grain varieties described in 3.1.2 were determined using the pre-calibrated HunterLab colorflex spectrophotometer (Hunter Associate Laboratories, Reston, VA). Before measuring the colour of the samples, the instrument was standardized by placing black and white standard plates and L^* , a^* and b^* colour values were recorded. The deviation of the colour of the samples to standard were observed and recorded in the computed interface. L^* values correspond to lightness/darkness and extend from 0 (black) to 100 (white) with higher values corresponding to more lightness. a^* and b^* values correspond to an object's colour dimensions, with a^* values describing a sample's redness (+a) to greenness (-a), while b^* values describe a sample's yellowness (+b) to blueness (-b). Higher a^* values indicate more redness and higher b^* values indicate more yellowness. The samples (3 g in a sample cup) were measured in triplicate.

3.3.2.2 Texture

The texture of the cleaned whole grains described in section 3.2.1 was determined at 20, 30 and 40% moisture content using a TA-XT Plus Texture Analyser. The samples were brought to the desired moisture content by using the procedure described by Yalcin (2007) with some modifications. Each sample was placed in a polythene bag, tightly sealed and kept in the cold room at 4°C for 5 days. The amount of water (Q) that was added to the grains before sealing was calculated using the following equation:

$$Q = \frac{W_i(M_f - M_i)}{(100 - M_f)}$$

Where: W_i = Initial mass of seed (g),

M_i = Initial moisture content of grains (% , db)

M_f = Final moisture content (% , dry basis),

The texture of the grains was then determined using the procedure described by Afoakwa and Yeniyi (2006) with some modifications. Three grains were placed longitudinal across the groove of the platform and the force required by the TA-XT Plus Texture Analyser probe, the Warner-Bratzler Blade to cut perpendicularly through the grains was measured at a crosshead speed of 1.5 mm/s. This measurement was repeated five times for each sample. The following parameters were set on the texture analyser; penetration speed 2.0 mm/s, penetration distance 30mm, return speed 10 mm and trigger level 5.099N. Higher readings of force corresponded to harder grains and vice versa.

3.3.3 Nutritional analysis

The whole grains described in section 3.2.1 were analysed for their nutritional composition using standard methods. Analysis for nutrient in a sample was done in duplicate. Due to the high costs

of grain PVA analysis in South Africa, only yellow PABM (PVAH 94) and brown BGN grain varieties were analysed for PVA composition in this study.

3.3.3.1 Sample preparation

The cleaned grain samples were packed into airtight containers and sent to Soil Science and Analytical Services, KZN Department of Agriculture in Cedara for analysis.

3.3.3.2 Moisture

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

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

3.3.3.3 Fat

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

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

3.3.3.4 Protein

The protein content of the samples was measured with a LECO Truspec Nitrogen Analyser (LECO Corporation, St Joseph, Michigan, USA) using the AOAC official method 990.03

(AOAC 2003). Controls and samples were measured in duplicate and placed into a combustion chamber at 950°C with an autoloader. The following equation was used to calculate the percentage of protein:

$$\% \text{ crude protein} = \% \text{ N} \times 6.25$$

3.3.3.5 Ash (total mineral content)

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

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

3.3.3.6 Fibre

Fibre was determined as neutral detergent fibre (NDF). The NDF of the samples was analysed using a Dosi-Fibre machine (JP Selecta, Abrera, Barcelona, Spain), according to the AOAC Official Method 2002.04 (AOAC 2002).

3.3.3.7 Individual mineral elements (calcium, magnesium, potassium, sodium, phosphorus, zinc copper manganese and iron)

Individual mineral elements were analysed using the Agricultural Laboratory Association of Southern Africa (ALASA) Method 6.5.1. The first step of this process was to freeze dry the samples in a freeze drier (Edwards, High Vacuum International, Sussex, England). Samples were ashed overnight at 550°C in a furnace. The samples were dissolved in HCl and then t HNO₃ added. The samples were analysed using an atomic absorption spectrophotometer. Calcium and phosphorus were determined using the Analytik Jena Spekol 1300 spectrophotometer (Analytik

Jena AG, Achatung, Germany). Iron was determined with the Varian Spectr AA atomic absorption spectrophotometer (Varian Australia Pty Ltd, Mulgrave, Victoria) and the zinc with the GBC 905AA spectrophotometer (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia).

3.3.3.8 Provitamin A-carotenoids

The PVA contents (as vitamin A equivalents) of the yellow PABM and brown BGN grain varieties was determined by high performance liquid chromatography (HPLC) using the procedures described by Lacker, Strohschein & Albert (1999).

3.4 Statistical analysis

Data was statistically analyzed using general linear model procedure of the SPSS program (22 version). Mean values and standard errors of means are reported. Statements of statistical significance were based on $p < 0.05$.

$$Y_{ijkl} = \mu + V_i + T_j + (V \times T)_{ij} + E_{ij}$$

Where:

μ = overall mean common to all observations

V_i = variety effect ($i = 1, 2, 3, 4$)

T_j = treatment ($j = 1, 2, 3$)

$(V \times T)_{ij}$ = Interaction between variety and treatment

E_{ijkl} = Experimental error

3.5 Ethical considerations

Ethical approval was obtained from the UKZN-Humanities and Social Sciences Research Ethics Committee (HSS/0534/013) (Appendix E).

3.6 Results and discussion

3.6.1 Grain physical properties

3.6.1.1 Colour

CIE colour values (Hunter L*, a* and b*) of the whole grain, oven dried grains and flours of each grain variety are presented in Table 5.

Table 5. The CIE colour values (Hunter L*, a* and b*) of the whole grains, oven dried grains and flours of white maize, PABM, brown and red BGN varieties

Sample	Mean \pm SD		
	L	a*	b*
Whole grains			
White maize (reference)	70.58 \pm 0.19 ^a	3.19 \pm 0.02 ^a	25.98 \pm 0.17 ^a
Yellow PABM (control)	65.68 \pm 0.21 ^b	13.86 \pm 0.17 ^b	38.42 \pm 0.47 ^b
Brown BGN	44.34 \pm 0.18 ^c	13.33 \pm 0.08 ^c	23.65 \pm 0.10 ^c
Red BGN	28.62 \pm 0.11 ^d	20.33 \pm 0.05 ^d	15.02 \pm 0.26 ^d
Oven dried grains			
White maize (reference)	68.91 \pm 0.12 ^a	1.67 \pm 0.05 ^a	33.70 \pm 0.02 ^a
Yellow PABM (control)	62.78 \pm 0.05 ^b	10.11 \pm 0.09 ^b	46.61 \pm 0.30 ^b
Brown BGN	30.85 \pm 0.12 ^c	10.60 \pm 0.06 ^c	14.14 \pm 0.14 ^c
Red BGN	23.95 \pm 0.14 ^d	11.04 \pm 0.08 ^d	7.11 \pm 0.24 ^d
Flour			
White maize (reference)	75.11 \pm 0.01 ^a	4.11 \pm 0.01 ^a	12.80 \pm 0.01 ^a
Yellow PABM (control)	67.25 \pm 0.02 ^b	6.17 \pm 0.01 ^b	15.33 \pm 0.01 ^b
Brown BGN	65.26 \pm 0.01 ^c	6.22 \pm 0.02 ^c	12.65 \pm 0.00 ^c
Red BGN	75.10 \pm 0.01 ^d	4.11 \pm 0.01 ^d	12.80 \pm 0.01 ^d

L* Measure of lightness (0 = black to 100 = white)

a* Measure of redness and (+a = redness; -a = greenness)

b* Measure of yellowness (+b = yellowness; -b = blueness)

^{a, b, c, d} denotes that there was significant difference ($p < 0.05$) amongst the means

There was a significant difference ($p < 0.05$) between the L^* , a^* and b^* of the whole grains, oven dried grains and the flours of each grain variety, respectively. Confirming visual appearance of the whole and oven dried grains, the reference sample (white maize) had the highest L^* values followed by the control sample (yellow PABM), brown BGN and red BGN grains, although the L^* values of the oven dried grains were slightly lower than those of the whole grains. However, in terms of a^* values, the red BGN variety had the highest L^* values whereas the reference sample grain had the lowest a^* values. The whole and oven dried grains of the control had the highest b^* values followed by the reference c, brown BGN and red BGN. When compared with whole and oven dried grains, the L^* values of the flours were higher. In general, all the flours had higher L^* values and lower a^* values which is desirable in developing instant porridges.

The L^* , a^* and b^* values of the yellow PABM of the current study were slightly higher than the values reported by Pillay *et al.* (2011) and Beswa *et al.* (2015). The L^* and b^* values of the white maize in this study were however higher whereas a^* values lower than those reported Beswa *et al.* (2015). The L^* , a^* and b^* colour values of the PABM grain studied by Pillay *et al.* (2011) ranged from 53.6 to 57.0; 16.5 to 25.7 and 29.3 to 37.5 whereas those studied by Beswa *et al.* (2015) ranged from 36.4 to 42.3, 11.5 to 19.8 and 24.0 to 33.6, respectively. The L^* , a^* and b^* values of the white maize studied by Beswa *et al.* (2015) were 46.3, 6.3 and 24.6, respectively. There was however no literature found on the L^* , a^* and b^* values for the whole grains, oven dried grains and flours of the BGN varieties and that of the oven dried grains and flours of both of the maize varieties.

The lower L^* and a^* values of the oven dried grains compared to those of the whole grains could have been attributed to the reactions that took place during drying such as evaporation and Millard reactions which causes some changes in the colour (i.e. browning) of the grains. The high L^* , a^* and b^* of the control, red BGN and yellow PABM is expected since the grains are white, red and yellow in colour, respectively. Hence the high degree of lightness, redness and yellowness in the respective grains. The yellow colour of the biofortified maize is due to PVA carotenoids. The high L^* values of the flours in this study could have been attributed to milling which reduces the particle size of the grain. The smaller particles of the flours cause low light

absorption and more spectral response contributing to the whiteness L* attenuation (Galvez and Resurreccion 1993). Hence, the flours in this study are suitable for developing instant porridges since they generally have high L* and low a* values which is desirable in porridge making.

3.6.1.2 Grain texture

The results for the texture of the whole grains of white maize, yellow PABM, brown and red BGN varieties determined at 20, 30 and 40% moisture content are shown in Table 6.

Table 6. The texture of the whole grains of white maize, yellow PABM, brown and red BGN grain varieties [Newtons (N)]

Grain variety	Moisture content	Firmness		Toughness	
		Mean \pm SD	P value	Mean \pm SD	P value
White maize (reference)	20%	19805.9 \pm 3474.9 ^a		19406.9 \pm 5686.8 ^a	
	30%	17688.7 \pm 7035.3 ^a	0.401	25095.3 \pm 3030.1 ^a	0.107
	40%	15327.3 \pm 3830.4 ^a		19759.1 \pm 3838.3 ^a	
Yellow PABM (Control)	20%	29101.2 \pm 6708.9 ^a		25529.1 \pm 7055.8 ^a	
	30%	19883.4 \pm 7296.6 ^a	0.027	26945.7 \pm 3072.8 ^a	0.564
	40%	17227.7 \pm 4461.4 ^b		23037.0 \pm 6203.2 ^a	
Brown BGN	20%	21908.2 \pm 2567.8 ^a		70148.9 \pm 14884.0 ^a	
	30%	15278.1 \pm 1654.0 ^a	0.139	52430.9 \pm 9447.5 ^a	0.097
	40%	16589.0 \pm 8362.8 ^a		66382.7 \pm 12105.2 ^a	
Red BGN	20%	19931.4 \pm 4123.2 ^a		64408.8 \pm 18205.9 ^a	
	30%	15605.8 \pm 2266.0 ^a	0.078	60208.0 \pm 6197.9 ^a	0.042
	40%	14306.3 \pm 4320.1 ^a		42003.2 \pm 11788.0 ^a	

^{a, b} Means marked with different letters in the same column are significantly different (p<0.05)

The results in Table 6 above show that there was a significant difference ($p < 0.05$) in the firmness of the yellow PABM (control) and in the toughness of the red BGN grains. The firmness of the white maize (reference), yellow PABM and red BGN decreased with an increase in moisture content of the grains from 20 to 40%. There was no clear-cut trend in the mean values for the firmness of the brown BGN grains with respect to an increase in moisture content from 20 to 40%. The toughness of the red BGN grain however decreased with an increase in moisture content whereas there was no cut trend in the toughness of the reference, control and brown BGN as the moisture content of the grain increases.

The findings of this study are different from those reported by Zhang *et al.* (2007) where there was a significant difference ($p < 0.05$) in texture of the grains. The variations in texture of the grains in this study could be mainly due to genetic make-up, different environmental conditions in which the grains were exposed such as growth conditions, soil type and storage conditions. In terms of the genetic make-up, it was reviewed in Chapter Two that biofortification of maize with PVA carotenoids changes the texture of the grain and BGN has a hard testa hence poor WAC which contribute to the hardness of the grain. Grain size could have also contributed to the variations in texture as small grains have a tendency to be harder in texture than large-sized grains and thus require more processing time in making instant porridges, which causes high cost of production (Chen *et al.* 1993).

The correlation between grain colour and grain texture is shown in Table 7.

Table 7. The correlation between colour and texture of the whole grains of white maize, yellow PABM, red and brown BGN varieties

		L*	a*	b*	Firmness	Toughness
L	Pearson Correlation	1	-0.836**	0.786**	0.357	-0.873**
	Sig. (2-tailed)		0.001	0.004	.282	0.000
a*	Pearson Correlation		1	-0.344	-.015	0.619*
	Sig. (2-tailed)			0.274	.964	0.032
b*	Pearson Correlation			1	.526	-0.632*
	Sig. (2-tailed)				.079	0.028
Firmness	Pearson Correlation				1	-0.134
	Sig. (2-tailed)					0.678
Toughness	Pearson Correlation					1
	Sig. (2-tailed)					

** . Correlation is significant at the 0.01 level (2-tailed).* . Correlation is significant at the 0.05 level (2-tailed)

As shown in Table 7, L* is significantly (p<0.01) and negatively correlated to a*, positively correlated to b* and negatively correlated to toughness. a* is significantly (p<0.05) and

positively correlated to toughness whereas b^* is significantly ($p < 0.05$) and negatively correlated to toughness. L^* and b^* had the highest correlation coefficient of 0.786 and L^* and toughness had the lowest correlation coefficient of $R = -0.873$ at $p < 0.01$. The lower correlation coefficient obtained in this investigation could have been attributed to the differences in texture and varieties of the grains. The findings of this investigation indicate that the genes controlling grain colour may be linked to those controlling grain texture. There was however, no literature found on the correlation between grain colour and grain texture. This suggest that grain texture which is tedious to measure can be predicted by measuring grain colour which is simple and rapid to do. However, the sample size ($n = 2$) of the BGN varieties is too small to make a firm conclusion.

3.6.2 The nutritional composition of the BGN and PABM grains

The nutritional composition of the whole grains of white maize, yellow PABM, brown BGN and red BGN varieties are presented in Table 8 and Table 9.

Table 8. Nutritional composition of the whole grains of white maize, yellow PABM, red BGN and brown BGN varieties [dry basis (db)]

Variables	Mean ± SD				
	Yellow PABM (control)	White maize (reference)	Red BGN	Brown BGN	P value
Moisture (g/100g)	16.69 ± 0.78	9.10 ± 0.40	3.33 ± 0.00	5.72 ± 0.23	0.000
Ash (g/100g)	1.50 ± 0.13	1.45 ± 0.09	3.79 ± 0.04	3.85 ± 0.06	0.000
Fat (g/100g)	3.42 ± 0.01	3.92 ± 1.03	7.14 ± 0.30	6.49 ± 0.04	0.005
ADF(g/100g)	7.30 ± 0.46	5.12 ± 0.06	8.91 ± 1.00	10.80 ± 0.20	0.002
NDF (g/100g)	14.35 ± 2.60	12.80 ± 0.59	13.33 ± 1.27	14.77 ± 1.10	0.617
Crude Protein (g/100g)	9.47 ± 0.74	10.47 ± 1.17	18.81 ± 0.05	19.87 ± 0.05	0.000
Calcium (mg/100g)	0.00 ± 0.00	0.01 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.002
Magnesium (mg/100g)	0.09 ± 0.00	0.10 ± 0.01	0.15 ± 0.00	0.17 ± 0.01	0.000
Potassium (mg/100g)	0.24 ± 0.00	0.33 ± 0.11	1.20 ± 0.00	1.21 ± 0.02	0.000
Sodium (mg/100g)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.121
Potassium/Calcium +magnesium (mg/100g)	0.80 ± 0.01	1.02 ± 0.24	2.11 ± 0.06	1.99 ± 0.12	0.001
Phosphorus (mg/100g)	0.21 ± 0.00	0.26 ± 0.02	0.25 ± 0.00	0.25 ± 0.00	0.038
Zinc (mg/100g)	15.00 ± 1.41	23.00 ± 2.83	22.00 ± 1.41	27.00 ± 0.00	0.010
Copper (mg/kg)	1.00 ± 0.00	2.00 ± 0.00	5.50 ± 0.71	6.50 ± 0.71	0.001

3.6.2.1 Moisture content

From Table 8, it can be seen that there was a significant different ($p < 0.05$) in the moisture, ash, fat, crude protein, calcium, magnesium, phosphorus, zinc, copper, manganese content of all the grains. The maize grains had higher moisture content as compared to the BGN grains, with yellow PABM (control) and red BGN having the highest and lowest mean values, respectively (Table 8). The lower moisture content of the BGN grain could be as a result of long storage which led to loss of moisture through evaporation. Grains that have a lower moisture content are difficult to mill hence there is need to increase the moisture content to about 14% by conditioning the grains so as to improve its milling quality.

3.6.2.2 Crude protein

The BGN grains had a higher crude protein content than the maize grains, with the brown BGN and yellow PABM having the highest and lowest mean values respectively (Table 8). The mean protein content of the yellow PABM variety in this study is slightly lower than those of Pillay *et al.* (2011b) and Govendor *et al.* (2014) but higher than those of Amod *et al.* (2014). The mean protein content of the white maize is however higher than 8.7 g/100 g, reported by Johnson (2000), but also within the range of 8.92-10.52 g/100g reported by Machida *et al.* (2010) in their study. The mean protein content of the red BGN is slightly lower whereas that of brown BGN higher than those of Ojimelukwe and Ayernor (1992) where the protein content of the red and brown BGN varieties were 19.5 and 19.0%, respectively. The high protein content of the two BGN grain varieties in comparison with that of the two maize varieties is expected because BGN is a legume whereas maize is a cereal and legumes naturally have more protein than cereals. The high protein content of legumes generally constitutes the natural protein supplements to staple diet. Hence, the findings of this study indicate that BGN can at least represent the legume of choice for many such populations SSA, which can assist in combating PEM.

3.6.2.3 Fat

The BGN grains also had a higher fat content than the maize varieties. Unlike in crude protein content, the red BGN variety had higher fat content than the brown BGN variety (Table 8). The fat content of the control (yellow PAMB) in this study is lower and that of the reference (white maize) is higher than those of Amod *et al.* (2014). The fat content of the red BGN is similar whereas that of the brown BGN is lower than those of Ojimekwe and Ayernor (1992). The high fat content of the BGN over maize varieties is also expected because the fat content of the BGN grain has been reported to be significantly higher than that of cereals, including maize. The high fat together with the high protein content of the BGN is encouraging, as it will contribute towards protein, fat and the overall energy intake of children. Infants requires approximately 100kcal of energy per day and about 30-40% of that, energy comes from fat (Mahan *et al.* 2012). Fat is also need for other functions, including absorption, digestion, and transportation of fat-soluble vitamins. Hence, the high protein and fat content of the BGN can contribute to combating PEM in children in the SSA region (De Onis & Blössner 2003) as well as improving the overall nutritional intake of the SSA populations who depend on low protein and energy staples such as maize.

3.6.2.4 Fibre

The brown BGN had the highest fibre content, followed by yellow PABM, red BGN and lastly the white maize sample (Table 8). The fibre content of the control is within the range of 8.1 to 23.1 g/100g reported by Beswa *et al.* (2015). The fibre content of the red BGN is higher whereas that of brown lower than that reported by Ojimekwe and Ayernor (1992). It has been reviewed in Chapter Two that insoluble dietary fibre normalises intestinal obstipation, accelerating and increasing the faecal bulk, whereas soluble dietary fibre lowers blood pressure, improves blood glucose control in diabetes mellitus, aids in weight loss and improves immune function (FAO 1992). From the results of this study, it can be inferred that the yellow PABM and the two BGN varieties would have better health benefits as compared to the white maize.

3.6.2.5 Total mineral (ash) and individual mineral element content

The BGN grains had a higher ash content as compared to maize grains with the brown BGN having higher mean values and the control sample having the lowest (Table 8). The ash content of the yellow PABM and the white maize varieties of the current study are higher than those reported by Amod *et al.* (2015). The two BGN varieties of the present study had higher ash values than the values reported by Mahala and Mohammed (2010) and Ojimekwe and Ayernor (1992) for BGN varieties studied.

In terms of the individual mineral elements assessed, it is shown in Table 8 that there was no calcium that was detected in yellow PABM grains and the reference sample had the lowest whereas the brown BGN had the highest mean values. There was no sodium that was detected in the two maize varieties and the two BGN varieties had similar mean values of 0.01 ± 0.00 . The potassium content of the maize grains was higher as compared to that of the BGN grains with the reference sample having the highest mean scores and the red BGN having the lowest mean scores. The BGN grains had higher magnesium, copper, iron and manganese values as compared to the maize grains. The brown BGN variety had higher values of magnesium and copper whereas the red BGN variety had higher values for iron and manganese. Highest mean phosphorus values were reported in white maize, followed by both BGN varieties which had similar mean values and lastly the yellow PABM. The brown BGN had the highest mean zinc values and the yellow PABM had the lowest.

The mean values of phosphorus, potassium, sodium, magnesium, copper and iron of the BGN varieties in this study are slightly higher whereas the calcium mean values are slightly lower than those of Omoikhoje and Arijeniwa (2004). The phosphorus and calcium mean values of the two maize varieties in this study were also lower than those of Amod *et al.* (2014). The iron content of the both the two maize varieties are higher and the zinc content lower than those of Amod *et al.* (2014). However the mean values for the iron and zinc content of the yellow PABM lower than those targeted by the HarvestPlus Challenge Program of $> 60\text{mg}/100\text{g}$ as well as those of Pillay *et al.* (2011b) and Ortiz-Monasterio *et al.* (2007).

The three major mineral deficiencies in infants identified by WHO (2008) are iron, zinc and calcium and these micronutrient deficiencies are important contributors to the burden of disease (WHO 2002b). This is compounded by the fact that maize, which is an important staple food in SSA, contains low levels of calcium, iron and zinc (Šimić *et al.* 2009; McCann 2005, p1). The low content of these minerals is due to the presents of oxalic acid which forms oxalate precipitates with dietary calcium and phytic acid which forms insoluble phytates with iron, zinc and possibly other metals thereby inducing deficiencies in these minerals. Furthermore, the absent ascorbic acid in maize inhibits the absorption of iron (White & Broadley 2005; Welch & Graham 2004; Mendoza 2002). Therefore, there is need to improve the levels of these minerals in order to reduce the incident of calcium, iron and zinc deficiencies in populations whose diets depends solely on maize.

3.6.2.7 Provitamin A carotenoids

The PAV carotenoids content of the yellow PABM, brown BGN and the composite complementary instant porridge with 30% (w/w) concentration of brown BGN flour is shown in Table 9.

Table 9. PVA content of yellow PABM and brown BGN grain (mg/g) [dry basis (db)]

Sample	Mean ± SD
Yellow PABM (control)	0.00 ± 0.00
Brown BGN	0.00 ± 0.00

As shown in Table 9, no PVA carotenoids were detected in yellow PABM and brown BGN grain varieties. The target PVA content a set by the HarvestPlus Challenge Programme that could alleviate VAD is 15.0ug⁻¹ (Nuss and Tanumihardjo 2011; Ortiz-Monasterio *et al.* 2007). The non-detection of the PVA carotenoids in brown BGN is expected because generally the BGN grain is deficient in PVA carotenoids (Uvere *et al.* 2010). In the case of yellow PABM grain, the PVA content could have been too low to be detected by the chromatography analysis or the

chromatography analysis was not sensitive enough to measure PVA as it is more suitable for measuring the active forms of vitamin A, namely retinol, retinal, and retinoic acid. PVA carotenoids are also susceptible to destruction during long storage as they are sensitive to light and oxygen in the air. Temperature of storage is also important, ideally the samples were also to be frozen, and stored in air-tight containers. Therefore, losses would have occurred as a result of long storage as the yellow PABM used in the current study was harvested in 2014 and used in 2016 and the samples were also stored without being frozen.

3.7 Conclusions

The investigation shows processing improves grain properties of BGN in terms of colour and texture. The variations in texture (firmness and toughness) of the grains in this study could be attributed to the difference in the genetic factors of the BGN varieties, including the genetic factors that resulted in differences in the size of the grains. Differences in the properties of BGN grains indicate that grains need to be characterised and carefully selected for different food applications. The yellow PABM was nutritionally superior in terms of moisture, ash and fibre as compared to the white maize. The brown BGN variety had higher levels of ash, fibre, crude protein, calcium, magnesium, potassium, zinc and copper than the red BGN. Overall, the brown BGN was more superior in terms of nutrient content. Thus, in terms of grain properties and nutritional composition, a combination of PABM and BGN variety would be, overall, a suitable food source than either of the two alone.

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Chapter 4 - Effect of adding Bambara groundnut on the functional, nutritional and sensory quality of a provitamin A-biofortified maize complementary instant porridge

Abstract

The effect of adding Bambara groundnut (BGN) on the nutritional, functional and sensory quality of a provitamin A-biofortified maize (PABM) composite complementary instant porridge was assessed. The composite complementary instant porridges were prepared with white maize, PABM, red BGN and brown BGN flours. Each, separately, of the two types of BGN flour was added to the PABM complementary instant porridge at 0, 10, 20 and 30% (w/w) level of substitution of the maize flour. White maize porridge was used as a reference and the yellow PABM porridge was used as a control. The nutritional composition of the instant porridges was determined using standard methods. The porridges were also analysed for their functional properties, including water absorption capacity (WAC), solubility index (SI) and swelling volume (SV). A 9-point hedonic scale was used to evaluate the sensory quality of the instant porridges. There was significant change in the protein, fat, fibre, and ash content, respectively, of the instant porridges. Provitamin A (PVA) carotenoids (in vitamin A equivalents) were not detected in the instant porridges. The WAC, SI, SV and firmness of the composite complementary instant porridges decreased with an increase in the concentration of either of the BGN flours. A decrease in SV has a positive effect on the functional properties of the porridge as it increases the nutrient density. The Hunter L* values of the instant porridges containing brown BGN ranged from 26.43 to 28.06, the Hunter a* values ranged from 11.37 to 12.44 and the Hunter b* values ranged from 21.08 to 25.6. The Hunter L* values of the porridges containing red BGN ranged from 24.68 to 26.64, the Hunter a* values ranged from 11.91 to 12.80 and the Hunter b* values ranged from 23.24 to 25.44. There was no significant difference ($p > 0.05$) in the taste, colour, aroma, texture, appearance and overall acceptability of the instant porridges. The findings of this investigation suggest that a BGN-PABM complementary instant porridge can deliver a significant amount of protein, fat, fibre, and minerals to nutritionally vulnerable

children, especially those found in sub-Saharan Africa (SSA). The porridge is also acceptable to the consumers. However, there is need to improve the functional properties of the porridges.

4.1 Introduction

The concept of cereal-legume complementation has been particularly applied to the development of infant complementary foods with high protein quality (Griffith *et al.* 1998). Nutritionally, the high protein content of legumes increases the protein content of cereal-based complementary foods and supplements the deficient amino acids. Apart from health significance, convenience foods are also a recent trend in the food market (Mbithi *et al.* 2002). Owing to the present demand for these foods, there is a need to develop convenient yet healthy cereal-legume based complementary instant foods. Due to several factors, including convenience, instant or ready to eat (RTE) cereal-legume based porridges are increasingly gaining acceptance in most SSA countries, and gradually replacing most of the non-instant traditional porridges (Egounlety 2002). The processing of instant porridges from cereal and legume grains involves the instantisation of flours, which is facilitated by grain processing techniques such as soaking, heat treatment (i.e. boiling, drying and roasting) and milling (Peitsch 2005; Henning *et al.* 2006). Before consumption, instant porridges may also need to be reconstituted with hot water or cold or hot milk depending on availability of resources and habit (Egounlety 2002).

In SSA, children are usually weaned on leading, unfortified starchy staple foods, such as white maize meal porridge, which contain low levels of poor quality protein as well as low energy density. Consequently, most of the children suffer from PEM and VAD, especially at the weaning stage (Dewey and Brown 2003). A complementary food made with PABM) and BGN could contribute significantly to addressing PEM and VAD as BGN is high in protein and biofortified maize provitamin A. Further, the protein quality of the PABM-BGN complementary food would be likely enhanced due to the fact that BGN is high in the essential amino acids lysine and tryptophan which are generally limiting in maize, while maize is generally high the essential amino acid methionine.

Although PABM has more nutritional and health benefits than white maize, consumer studies conducted in several countries in SSA have found poor acceptance of PABM compared to white maize, e.g. in Mozambique (Steven & Winter-Nelson 2008) and South Africa (Pillay *et al.* 2011). These findings have been attributed to undesirable sensory attributes of the PABM (Tothova & Meyers 2006; Steven & Winter-Nelson 2008), especially the yellow/orange colour and unfamiliar, strong flavour and aroma, which are all largely due to carotenoid pigments, including PVA carotenoid pigments. On the other hand, BGN grain has been reported having desirable sensory attributes, particularly flavour (Lewick 1974). Application of advanced techniques in developing a BGN-based food was found to further improve consumer acceptance of BGN e.g. in Ghana (Plahar *et al.* 2000) and in Nigeria (Olapade and Adetuyi 2007). Other than improving the sensory properties of the BGN-based foods, use of advanced techniques was also reported to improve the functional properties of BGN-based foods, such as water absorption capacity (WAC) (Plahar *et al.* 1998).

Therefore, adding BGN meal (flour) to PABM meal (flour) coupled with advanced food processing techniques may enhance the nutritional, functional, and sensory quality of a complementary instant porridge. The undesirable sensory characteristics of the PABM could be masked by the desirable sensory characteristics of the BGN. However, it seems that there is no information on the nutritional, functional, and sensory quality of a BGN-PABM complementary instant porridge. The current investigation, therefore, aimed at determining the effect adding BGN on the nutritional, functional, and sensory quality of a PABM complementary instant porridge.

4.2 Materials and methods

4.2.1 Flour samples

The flours samples described previously (Chapter 3 section 3.3.1.6) were used in this investigation.

4.2.2 Sample preparation

The samples were further processed into instant porridges as described in the sections that follow (Sections 4.2.2.1 to 4.2.2.3).

4.2.2.1 Roasting and cooling

Each flour type (white maize, yellow PABM, brown BGN (BBGN) and red BGN flour (RBGN) (Figure 8) was roasted, separately, in a stainless steel pan on a Defy Thermofan Stove (Model 731 MF) on low heat for approximately 8-10 minutes until a golden-brownish colour was obtained and then allowed to cool for a period of 1-2 hrs at 25°C.



Figure 8. Instant flours, from left to right: white maize, yellow PABM, BBGN, RBGN

4.2.2.2 Formulation

The different weight-to-weight (w/w) ratios used to composite BGN instant flour with PABM instant flour are presented in Table 10.

Table 10. The weight-to-weight (w/w) ratios of the BGN-PABM composite instant flours

Composite flour	Ratio
Reference	0 BGN : 100 white maize
PABM (control)	0 : 100
Brown BGN: PABM	10 : 90
brown BGN: PABM	20 : 80
Brown BGN: PABM	30 : 70
Red BGN: PABM	10 : 90
Red BGN: PABM	20 : 80
Red BGN: PABM	30 : 70

4.2.2.3 Reconstitution of the instant composite flours

The instant composite flours made with BGN and PABM were reconstituted with boiling water (96°C).

4.2.3 The nutritional composition of the BGN-PABM instant porridges

The composite flours prepared as described in section 4.2.2.2 were analysed for their nutritional composition. Only the composite complementary instant porridge with 30% (w/w) of the brown BGN was analysed for its PVA content. The samples were prepared for analysis as described in section 3.3.3.1 using the referenced methods described in sections 3.3.2.2 to 3.3.2.8 of the previous chapter.

4.2.4 The functional properties of the BGN-PABM complementary instant porridges

The BGN-PABM composite complementary instant porridges were analysed for WAC, SI, SV, colour and texture as described in sections 4.2.4.1 to 4.2.4.5.

4.2.4.1 Water absorption capacity (WAC)

The WAC of the composite instant flours made with BGN and PABM was determined in triplicates according to the method of Yamazaki (1953) as modified by Medcalf and Gilles (1965). About 2 g of each flour sample was weighed into a pre-weighed centrifuge tube and 20 ml of distilled water added. Samples were vortexed with a vortex mixer (Model hs501, digital, Janke 7 Kinkel GMBH &Co. KG) and allowed to stand for 30 min at 25°C before being centrifuged at 2200 rpm with a centrifuge (Mistral 3000i, UK) for 15min. Excess water was decanted off by inverting the tubes over an absorbent paper and samples allowed to drain. The weights of the water samples were determined by difference.

4.2.4.2 Swelling Volume (SV) and Solubility Index (SI)

The SV and SI of the flours was determined in triplicates according to modified method of Leach, McCowen and Scotch (1959). One gramme of each flour sample was transferred into a weighed graduated 50 ml centrifuge tube. Distilled water was added to give a total volume of 40 ml. The suspension was stirred uniformly avoiding excessive speed, in order not to cause fragmentation of the starch granules. The samples were heated at 85°C in a thermostatically regulated temperature water bath (Grant instruments, England Ltd.) for 30 min with constant stirring. The tube was removed, wiped dry on the outside and cooled to 25°C. It was then centrifuged for 15 min at 2 200 rpm (Mistral 3000i, UK). The SI was determined by evaporating the supernatant in a hot air oven (BS Gallenkamp, England) and the residue weighed.

The SV was obtained by directly reading the volume of the swollen sediment in the tube. The sediment paste was weighed. The % solubility was then calculated as follows:

% Solubility = $\frac{\text{Weight of soluble sample}}{\text{Weight of sample on dry basis}} \times 100$

Weight of sample on dry basis

4.2.4.3 Reconstitution ratio (RR)

The RR [weight-to-volume (w/v)] of each instant composite flour was determined experimentally according to the serving suggestions of a commercial cereal-based instant porridge (brand Morvite, South Africa) by trial and error until the acceptable reconstitution consistency of each sample was achieved.

4.2.4.4 Colour

The CIE colour values (Hunter L*, a* and b*) of the reconstituted composite complementary instant porridges were measured using the method described in section 3.3.2.1 of the previous chapter.

4.2.4.5 Texture

The texture analyser described in section 3.3.2.2 was used to analyse the texture of the porridge samples. However, the probe was in the form of a rod with a disc attached to one end (0.5 cm diameter, 0.2 cm thinness). Samples were measured in the beaker in which they were reconstituted. All measurements were performed twice on samples prepared separately. The following parameters were set on the texture analyser; penetration speed 1.0mm/s, trigger level 49.04N, penetration distance and return speed. The force required for the probe to penetrate the porridge in Newtons (N) was measured to provide a measure of sample consistency (Song *et al.* 2003).

4.2.5 Consumer acceptance of the composite complementary instant porridges made with BGN and PABM

Evaluation of the acceptance of the composite complementary instant porridges made with BGN and PABM was determined through sensory evaluation.

4.2.6 Pilot study

A pilot study of the sensory evaluation was conducted before the main evaluation. Leon, Davis & Kraemer indicated the importance of a pilot study as it aims at determining whether or not the approach utilised is feasible for use on a larger scale. The purpose of conducting a pilot study is to detect and correct methodological problems. The pilot study is also conducted in order to refine the informed consent procedures as well as data collection tools (Leon *et al.* 2011).

All the five participants who participated in the pilot study were recruited from Rabie Saunders building, UKZN Agriculture campus, PMB, South Africa. Participation was voluntary and recruitment was done by *isiZulu*-speaking and English speaking research assistants, as the university is largely comprised of *isiZulu* and English speaking people. Subjects who participated in the pilot study were not permitted to participate in the main study in order to avoid bias. To ensure this, the researcher selected a different day and week to conduct the main study.

The outcomes of the pilot study were as follows:

1. The RRs of the porridges was standardized after many mixing trials.
2. The sensory evaluation questionnaires in English (Appendix A) and *isiZulu* (Appendix B) were modified.

The changes that were made to improve the main sensory evaluation are as follows:

1. The processing method of the BGN-PABM complementary instant porridge was modified by adding the sugar to the porridge so that it would be more acceptable to the consumers.
2. The porridge samples were to be reconstituted with hot water prior to be evaluated in order to be served hot and to avoid it from drying and becoming stiff.

3. In order to ensure sufficient recruitment of participants, the main sensory evaluation was conducted earlier in the day than was the pilot study.
4. All the panellists were allocated a number to assist with identification.
5. The consent form together with the sensory evaluation questionnaire were explained by the research assistant to ensure that all the participants understood what was expected of them.

4.2.7 The main sensory evaluation

4.2.7.1 Recruitment of panellists

The accepted sample size for consumer acceptance studies is 50 or more subjects (Sidel & Stone 2004, p247). In this study, a total of 55 students and staff members of UKZN, Agriculture campus, PMB, South Africa who are non-allergic and regular consumers of BGN and PABM, were recruited both verbally and by invitation letter posted on the notice boards in the Rabie Saunders building. Verbal invitations were done just before lectures of underground modules and lecturers were requested to allow the researcher to make the verbal invitations, which took about 5 minutes. Subjects who had participated in the pilot study a week before the main study were not allowed to participate in the main study. The potential study participants were informed that participation in the study was voluntary and that there was no payment for participation. Subjects were also required to abstain from eating food for 15 minutes before the evaluation. Subjects who had smoked 30 minutes prior to the study, who had flu, mouth sores or any other taste or smell abnormalities were excluded from participating.

4.2.7.2 Preparation of BGN-PABM composite complementary instant porridges

The porridges were prepared as described in sections 4.2.2.1 to 4.2.2.3. Sugar was added to the composite instant flours and the mixtures reconstituted into porridge with hot water using the RRs shown in Table 4.6. The ideal amount of sugar added in each sample as well as the

acceptable RR for each sample was determined during the pilot study. The porridges were reconstituted before evaluation to prevent them from drying, which would result in their stiffness.

4.2.7.3 Sample coding, serving order and sensory evaluation set up

The sensory evaluation was done at the Dietetics and Human Nutrition Department of the UKZN, Agriculture campus, PMB, South Africa. A total of 11 panellists participated in the sensory evaluation per session and were seated in cubicles to prevent them from influencing each other in the evaluation of the samples. All the panellists were provided with a questionnaire, either in English (Appendix C) or *isiZulu* (Appendix D) depending on the preference of the panellist, a pen, samples, polystyrene cup of water to rinse the palate between the samples, a plastic spoon and a serviette (Figure 9). The English version of the sensory evaluation questionnaire was translated into *isiZulu* by a translator who was proficient in both languages. The sensory evaluation questionnaire had a 9-point hedonic scale (1=dislike extremely, 2= dislike very much, 3=dislike moderately, dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much and 9=like extremely). The hedonic scale is a popular sensory evaluation tool that indicates the degree of liking (or disliking) of the food sample (Bergara-Almeida *et al.* 2002; Gacula & Singh 1984, p30).

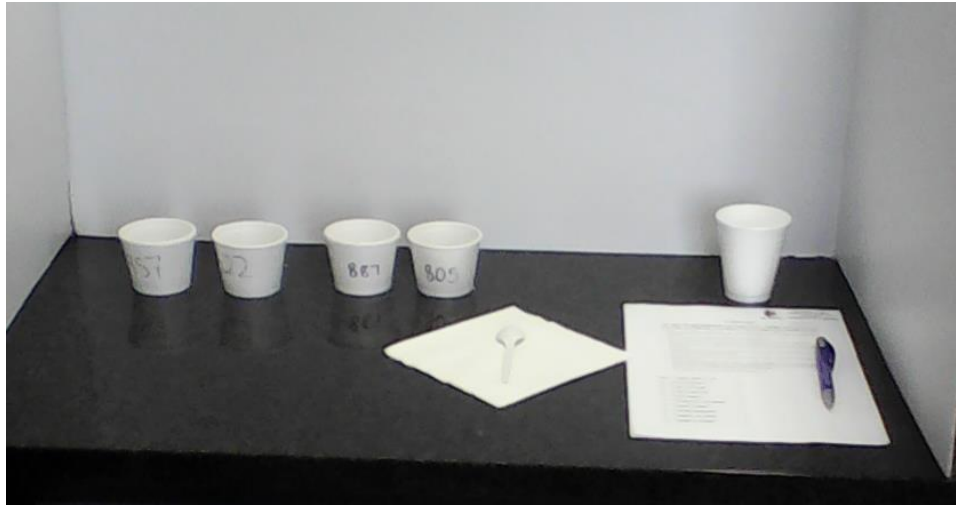


Figure 9. Sensory evaluation set up

The panellists were asked not to communicate with each other during the sensory evaluation session so as to avoid influencing each other's responses. The eight porridge samples were assigned a unique three-digit code obtained from a Table of Random Numbers (Heymann 1995). The serving order of the porridge samples was also determined by a Table of Random Permutations of Nine (Heymann 1995).

The participants were required to fill in a consent form before starting the sensory evaluation. A research assistant explained the consent form in detail in both English and *isiZulu* to all the participants. The participants were asked if they understood everything that was on the consent form before they signed the forms. If anything was not clear it was explained before the participant signed. The consent form was formulated in English (Appendix A) and was also translated into *isiZulu* (Appendix B). After the consent forms were signed, the research assistant also explained the questionnaire in *isiZulu* to the participants. All the sensory attributes (taste, colour, aroma, texture and overall acceptability) were explained to the participants. If the explanation for a sensory attribute was not clear, the research assistant repeated the explanation.



Figure 10. Panellists participating in sensory evaluation

4.2.7.4 Reduction of bias

In order to reduce bias during the research process, the following steps were taken:

1. The sensory evaluation questionnaire was developed using simple language and was translated into the local language (*isiZulu*) which *isiZulu* speaking panellists were able to understand.
2. During the preparation of the instant porridge, standardized equipment and apparatus were used. The pots in which the samples were cooked were also of the same brand, type, and size.
3. All the solid ingredients were measured using a calibrated balance and the liquid ingredients were measured using a standardized measuring jug.
4. The nutritional analysis was done in duplicate, whereas the functional properties were determined in triplicates using standard, referenced methods.

4.3 Results and discussion

4.3.1 The nutritional composition of the BGN-PABM instant porridges

The results of the nutritional composition of the composite complementary instant porridges made with BGN and PABM are presented in Tables 11 and Table 12.

Table 11. The moisture, protein, fat, fibre and ash content of the BGN-PABM composite complementary instant porridges [dry basis (db)]

Variable	BGN variety	Mean \pm SD				P value		
		0% (control)	10%	20%	30%	V	T	T*V
Moisture (g/100g)	BBGNF	1.56 \pm 0.06	1.61 \pm 0.01	1.71 \pm 0.04	1.79 \pm 0.02	0.000	0.029	0.017
	RBGNF	1.56 \pm 0.06	1.56 \pm 0.05	1.55 \pm 0.04	1.55 \pm 0.02			
Ash (g/100g)	BBGNF	1.28 \pm 0.16	1.44 \pm 0.14	1.61 \pm 0.13	1.77 \pm 0.11	0.970	0.004	1.000
	RBGNF	1.28 \pm 0.16	1.45 \pm 0.13	1.60 \pm 0.11	1.77 \pm 0.09			
Fat (g/100g)	BBGNF	3.58 \pm 0.00	4.07 \pm 0.01	4.55 \pm 0.01	5.03 \pm 0.02	0.060	0.000	0.472
	RBGNF	3.58 \pm 0.00	3.94 \pm 0.13	4.29 \pm 0.25	4.65 \pm 0.38			
ADF (g/100g)	BBGNF	9.60 \pm 0.08	11.34 \pm 0.04	13.10 \pm 0.16	14.84 \pm 0.28	0.000	0.000	0.057
	RBGNF	9.60 \pm 0.08	11.54 \pm 0.06	13.48 \pm 0.06	15.42 \pm 0.04			
NDF (g/100g)	BBGNF	21.68 \pm 0.82	23.77 \pm 0.70	25.86 \pm 0.59	27.94 \pm 0.47	0.630	0.000	0.986
	RBGNF	21.68 \pm 0.82	23.66 \pm 0.76	25.62 \pm 0.69	27.60 \pm 0.63			
Crude Protein (g/100g)	BBGNF	8.32 \pm 1.27	9.57 \pm 1.15	10.82 \pm 1.02	12.07 \pm 0.91	0.710	0.005	0.993
	RBGNF	8.32 \pm 1.27	9.71 \pm 1.13	11.10 \pm 1.00	12.50 \pm 0.87			

V=variety BBGNF

RBGNF

T= treatment 100% yellow PABM (control)

10% BGN

20% BGN

30% BGN

Table 12. The mineral element content of the BGN-PABM composite complementary instant porridges [dry basis (db)]

Variable	BGN variety	Mean \pm SD				V	T	T*V
		0% (control)	10%	20%	30%			
Calcium (mg/100g)	BBGNF	0.01 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00	0.000	0.000	0.124
	RBGNF	0.01 \pm 0.00	0.01 \pm 0.00	0.02 \pm 0.00	0.02 \pm 0.00			
Magnesium (mg/100g)	BBGNF	0.11 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01	0.12 \pm 0.00	0.720	0.053	0.931
	RBGNF	0.11 \pm 0.01	0.11 \pm 0.01	0.12 \pm 0.01	0.12 \pm 0.01			
Potassium(mg/100g)	BBGNF	0.21 \pm 0.00	0.28 \pm 0.00	0.36 \pm 0.00	0.43 \pm 0.00	0.213	0.142	0.452
	RBGNF	0.21 \pm 0.00	0.29 \pm 0.00	0.37 \pm 0.00	0.45 \pm 0.00			
Sodium(mg/100g)	BBGNF	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.01	0.01 \pm 0.00	0.010	0.023	0.025
	RBGNF	0.00 \pm 0.00	0.03 \pm 0.01	0.06 \pm 0.03	0.01 \pm 0.00			
Phosphorus (mg/100g)	BBGNF	0.22 \pm 0.01	0.22 \pm 0.00	0.23 \pm 0.01	0.23 \pm 0.00	0.440	0.023	0.878
	RBGNF	0.22 \pm 0.01	0.23 \pm 0.01	0.23 \pm 0.01	0.24 \pm 0.01			
Zinc (mg/100g)	BBGNF	29.00 \pm 1.41	29.00 \pm 1.41	28.00 \pm 1.41	28.00 \pm 1.41	0.030	0.855	0.290
	RBGNF	29.00 \pm 1.41	30.00 \pm 1.41	30.50 \pm 0.71	31.50 \pm 0.71			
Copper (mg/kg)	BBGNF	3.50 \pm 0.71	3.50 \pm 0.71	4.00 \pm 0.00	4.50 \pm 0.71	0.720	0.140	0.931
	RBGNF	3.50 \pm 0.71	3.50 \pm 0.71	4.50 \pm 0.71	4.50 \pm 0.71			
Manganese (mg/kg)	BBGNF	4.00 \pm 0.00	5.00 \pm 0.00	6.00 \pm 0.00	7.00 \pm 0.00	0.301	0.093	0.619
	RBGNF	4.00 \pm 0.00	5.00 \pm 0.00	6.00 \pm 0.00	6.00 \pm 0.00			
Iron (mg/100g)	BBGNF	30.00 \pm 2.83	28.50 \pm 2.12	27.50 \pm 2.12	25.50 \pm 2.12	0.240	0.502	0.819
	RBGNF	30.00 \pm 2.83	30.00 \pm 2.83	29.00 \pm 2.83	29.00 \pm 2.83			

V= variety BBGNF, RBGNF

T= treatment 100% yellow PABM (control), 10% BGN, 20% BGN, 30% BGN

From the results in Table 11, it can be seen that there was a significant different ($p < 0.05$) in moisture, crude protein, fat, fibre and ash content of the BGN-PABM composite complementary instant porridges. The mean moisture content of the samples containing brown BGN increased with an increase in BGN concentration whereas those containing red BGN flours decreased with an increase in BGN concentration. However, the crude protein, fat, fibre and ash content of the BGN-PABM composite complementary instant porridges increased with an increase in either of the BGN flour (Table 11).

In terms of individual mineral elements assessed, it is shown in Table 12 that there was a significant different ($p < 0.05$) in the calcium, sodium and phosphorus content of the BGN-PABM composite complementary instant porridges as the concentration of either of the BGN flour increases. There was no significant different ($p > 0.05$) in the magnesium, potassium, zinc, copper, manganese and iron content of the BGN-PABM composite complementary instant porridges as the concentration of either of the BGN flour increases (Table 12).

Results in Table 9 of the previous chapter section 3.6.2.7 indicate that PVA carotenoids were not detected in the BGN-PABM composite complementary instant porridge.

The literature on the nutritional composition of instant BGN-PABM based complementary foods that was not found. However, literature on other ingredients as well as on non-instant BGN-maize based complementary foods was found. The mean moisture values in this investigation are lower than those of Prapluettrakul *et al.* (2012) where the moisture content of the complementary instant rice was 5%; however, they are within the acceptable value for instant flours. The moisture, protein and fat content of the BGN-PABM in this investigation are lower than those of complementary foods developed from BGN fortified fermented raw or boiled BGN flour and white maize at 10 and 20% (w/w) BGN concentration by Mbata *et al.* (2007). The ash content of this investigation is lower at 10% (w/w) and higher at 20% (w/w) concentration of either of the BGN as compared to that of Mbata *et al.* (2009). The moisture, protein, fat and ash content of the BGN-maize complementary food produced from raw fermented flour ranged from 50.6 to 51.9, 12.5 to 16.2, 5.1 to 6.3 and 1.5 to 2.4%, respectively, whereas those produced from boiled

fermented flours ranged from 52.7 to 52.9, 12.6 to 16.4, 5.1 to 6.5 and 1.5 to 2.4%, respectively. The high moisture content of the fermented BGN-maize complementary foods of Mbata *et al.* (2007) as compared to the moisture content of this study is expected since the product was not instantised. The copper, zinc, magnesium, calcium and iron of the BGN-PABM composite complementary instant porridges of this study at 30% (w/w) concentration of either of the BGN variety are lower than those of (30/70) BGN-maize complementary food by Mbata *et al.* (2009) which were 24.6, 78.20, 475.20, 128.40 and 50.80 mg/100 g, respectively. The fibre, zinc, and iron content of this study are higher whereas the moisture, crude protein, fat, ash, calcium and vitamin A lower than those of maize-BGN complementary foods fortified with foods rich in calcium (beef bones), iron (Roselle calyces), zinc (potash) and vitamin A (red palm oil) by Uvere *et al.* (2010). The fat, ash and fibre of the composite complementary instant porridge with 20% (w/w) of either of the BGN flour in this study are higher whereas the protein content lower than those of a (80/20) yellow PABM-cream BGN composite complementary food by Oluwatofunmi *et al.* (2015).

The low moisture content of the composite flour in this investigation was attributed to roasting of the flours which caused some water to evaporate from the flours. A low moisture content in instant porridges is desirable as it increases the shelf stability of the product. An increase in protein, fat, fibre and ash of the BGN-PABM composite complementary instant porridges was due to the incorporation of the BGN into the PABM complementary instant porridge - the two BGN grain varieties were higher in these nutrients as compared to the yellow PABM grain variety. The results of this investigation indicate that the BGN-PABM complementary instant porridges are nutritious since the products provides about one third of the Recommended Dietary Allowance with respect to crude protein (10-12%) as recommended by FAO/WHO/UNU (1985) and the National Institute of Nutrition (1992) for combating protein deficiency in children living in poor rural parts of SSA. The high fat content of the BGN-PABM complementary instant porridges would result in an increase in the children's energy intake and thereby contribute to addressing energy deficiency which is prevalent among children in SSA. The non-detection of PVA carotenoids in the 30/70 (w/w) brown BGN-yellow PABM composite complementary

instant porridge was expected because from the results of the previous chapter (Table 9), it is shown that the PVA carotenoids were lacking in both the yellow PABM and brown BGN grain varieties. The reasons for the non-detection of the PVA carotenoids have been discussed in the previous chapter section 3.6.2.7. As reviewed in Chapter Two, VAD has been declared a public nutritional health problem affecting children in SSA whose diets depend solely on maize. Therefore, there is need to select PABM grain types that contain desired levels of PVA for use in the composite porridges so that the complementary instant porridges also make a significant contribution to addressing VAD among the children of low economic-status communities in SSA.

The three major mineral deficiencies in infants identified by WHO (2008) are iron, zinc and calcium and these micronutrient deficiencies are important contributors to the burden of disease (WHO 2002b). Therefore, there is need to improve the levels of these minerals in the BGN-PABM composite complementary instant porridges in order to reduce the incident of calcium, iron and zinc deficiencies among children in SSA, including South Africa.

4.3.2 Functional properties of the instant porridges made with BGN and PAMB

4.3.2.1 Water absorption capacity (WAC), solubility index (SI) and swelling volume (SV)

The results of the WAC, SI and SV are presented in Table 13.

Table 13. The WAC, SI and SV of the BGN-PABM composite flours compared to white maize flour (reference) and PABM (control)

Variable	Treatment Variety					P value		
		0% (control)	10%	20%	30%	V	T	V*T
WAC (mls/g)	BBGNF	57.86 ± 0.18	52.12 ± 0.02	50.03 ± 0.58	47.10 ± 0.12	0.000	0.000	0.000
	RBGNF	57.86 ± 0.18	49.13 ± 1.00	49.91 ± 0.36	41.50 ± 0.05			
	Reference	54.05 ± 0.32						
SI (%)	BBGNF	50.03 ± 0.07	40.06 ± 0.23	30.58 ± 0.75	23.02 ± 0.37	0.000	0.000	0.000
	RBGNF	50.03 ± 0.07	37.19 ± 0.20	27.96 ± 0.58	20.98 ± 0.06			
	Reference	49.54 ± 0.22						
SV	BBGNF	7.67 ± 0.58	7.00 ± 0.00	5.67 ± 0.58	5.00 ± 0.00	0.048	0.000	0.000
	RBGNF	7.67 ± 0.58	6.00 ± 1.00	5.33 ± 0.36	4.33 ± 0.58			
	Reference	9.67 ± 0.58						

V = variety

BBGNF = Brown Bambara Groundnut Flour

RBGNF = Red Bambara Groundnut Flour

T = Treatment

0 = PABM flour, 1= 10% BGNF, 2 = 20% BGNF, 3 = 30% BGNF

GLM test (p<0.05)

Table 13 shows that there was a gradual decrease in WAC, SI and SV mean values as the concentration of either of the BGN flour increased. Significant differences ($P < 0.05$) in WAC and SI were also observed among the composite flours. The highest WAC, SI and SV values were found in flours without added BGN flour. Amongst the two samples with no fortification (BGN addition), the control sample (100% yellow flour) PABM had the highest WAC and SI mean values as compared to the reference sample (100% white maize flour) whereas the reference sample had the highest SV value than the control sample. Although the WAC, SI and SV mean values of the samples with either of the BGN flour increased with an increase in BGN concentration, the samples with brown BGN flour had higher mean values than the samples with red BGN flour.

The findings of this investigation compare quite well with the result obtained by Mbata *et al.* (2009) when substituting *ogi* with BGN flour, whereby the WAC and SI values of the BGN-*ogi* decreases with an increase in BGN concentration. The results of this investigation are also in line with the report of Mbofung *et al.* (2002) which showed that most uncomposited flours had higher WAC and SI compared to composite flours. The mean values of the WAC of the composite instant flours containing 30% (w/w) of red BGN is similar to that of Mbata *et al.* (2009).

A low WAC and SI of the composite flours can be explained by their respective contents of hydrophilic constituents such as carbohydrates, which bind more water than protein and lipids (Mbaeyi 2005). Therefore, an increase in the concentration of either of the BGN flour led to an increase in protein content and a decrease in carbohydrate content thereby reducing the WAC and SI. The lower WAC and SI values of the composited flours of this investigation could also have been attributed to the small particle size of the composite flours as well as heat treatment. Heat treatment by roasting of flours causes the starch molecules to tighten/shrink there by reducing its ability to absorb water. With regard to milling, Enwere (1998) confirmed the fact that starch quality in terms of particle size equally plays an important role in the absorption of water as larger particle size have higher WAC than smaller particles. In the present investigation, the flour fraction used was 200 μ m in size obtained from conventional roller milling, hence the

low WAC and SI mean values. The differences in mean WAC and SI values of the composite flours made with either of the BGN flour suggests that variety and treatment had negative effect on the WACs and SI of the composite instant flours. However, a low mean SV value is desirable in a complementary food as it reduces the viscosity of the food thereby increasing its nutrient density and ability to be handled by the digestive tract of the child.

4.3.2.2 Colour of the BGN-PABM complementary instant porridges

Table 14. The CIE colour values (Hunter L*, a* and b*) of the composite complementary instant porridges made with BGN and PABM

Porridge sample	Mean ± SD		
	L*	a*	b*
100% white maize (Reference)	26.96 ± 0.08 ^{acdfg}	12.75 ± 0.16 ^{ac}	25.56 ± 0.13 ^{ad}
100% PABM (control)	27.92 ± 0.26 ^{bde}	12.95 ± 0.03 ^{ac}	26.31 ± 0.19 ^f
10% brown BGN	26.43 ± 0.25 ^{acfgh}	12.44 ± 0.06 ^{bd}	25.06 ± 0.10 ^{bcd}
20% brown BGN	27.20 ± 0.2 ^{abdg}	12.32 ± 0.06 ^{bd}	24.86 ± 0.08 ^{bce}
30% brown BGN	28.06 ± 0.08 ^{be}	11.37 ± 0.06 ^e	21.08 ± 0.18 ^g
10% red BGN	26.45 ± 0.39 ^{acfgh}	12.80 ± 0.06 ^a	25.44 ± 0.25 ^{abd}
20% red BGN	26.64 ± 0.43 ^{acfgh}	12.22 ± 0.09 ^{bd}	24.68 ± 0.08 ^{bce}
30% red BGN	24.68 ± 0.08 ^{bce}	11.91 ± 0.04 ^f	23.24 ± 0.27 ^h

Different superscripts in columns differ significantly (p<0.05)

The results in Table 14 above show that there was a significant difference ($p < 0.05$) in the Hunter L*, a* and b* values of the composite complementary instant porridges as the concentration of flour of either BGN variety was increased. The control sample (100% yellow PABM flour) had the highest Hunter L*, a* and b* values. The Hunter L* values of the samples containing brown BGN ranged from 26.43 to 28.06, the Hunter a* values ranged from 11.37 to 12.44 and the Hunter b* values ranged from 21.08 to 25.6. The Hunter L* values of the composite complementary instant porridges containing red BGN ranged from 24.68 to 26.64, the Hunter a* values ranged from 11.91 to 12.80 and the Hunter b* values ranged from 23.24 to 25.44.

Literature on L* a* and b* values BGN-PABM composite complementary instant porridges was not found. However, the literature on other ingredients was found. The findings of this investigation are in line with those of Mosha and Benning (2005) where there was a significant difference ($p < 0.05$) between colour of instant extruded sorghum-bean-sardine and corn-bean-sardine instant porridge compared to a non-instant (conventionally cooked) porridge containing either corn or sorghum. The Hunter CIE colour values (Hunter L*, a* and b*) of sorghum-cowpea extruded instant porridges by Pelembe *et al.* (2002) at various ratios of sorghum-cowpea was similar to that of an extruded commercial instant baby porridge. The colour changes in the BGN-PABM composite complementary instant porridges in this investigation could have been attributed to the roasting of the flours which causes the browning of the flours as a result of Millard reaction. The colour of the porridges was however desirable.

4.3.2.3 Texture of the composite complementary instant porridges

The results for the texture of the composite complementary porridges containing different concentrations of BGN and PABM are presented in Table 15.

Table 15. The texture (firmness) of the composite complementary porridges containing different concentrations of BGN and PABM [Newtons (N)]

Porridge sample	Mean \pm SD	P value
100% white maize (reference)	9.63 \pm 0.01 ^a	
100% yellow PABM (control)	8.85 \pm 0.11 ^b	
10% brown BGN	4.21 \pm 0.02 ^c	
20% brown BGN	2.61 \pm 0.03 ^d	0.000
30% brown BGN	1.40 \pm 0.03 ^e	
10% red BGN	4.48 \pm 0.04 ^f	
20% red BGN	1.57 \pm 0.02 ^g	
30% red BGN	0.68 \pm 0.03 ^h	

Tukey test (p<0.05)

Table 15 indicates that there was a significant difference (p<0.05) in the mean texture values of the composite complementary instant porridges. The samples with no fortification (no addition of BGN) were firmer (higher mean texture values) compared to samples with fortification. Among the samples with no fortification (no addition of BGN), higher mean texture values were observed in the reference sample (100% white maize flour) than in the control sample (100% yellow PABM flour). However, in samples with fortification, the mean texture values (firmness) decreased as the concentration of either of the BGN flour increased, with the samples containing brown BGN having higher mean values compared to the samples containing red BGN. Data on the texture of BGN-PABM complementary instant porridges could not be found in the literature. Literature of instant porridges made with other ingredients was however found. The mean texture

values of this study are higher than those of the instant porridges made with OFSP, cowpea, soybean, sorghum and maize flours by de Carvalho *et al.* (2014). In the current investigation, higher mean texture values in samples with no fortification (no BGN flour) compared to samples with fortification could be attributed to high starch content which increases the thickness/consistency, and hence an increase in firmness, of the porridges. However, as reviewed in Chapter Two, there is no official quantitative guidelines on the acceptable texture of complementary porridges, but WHO guidelines only stipulate that the complementary porridges should not drip from the spoon, which is not a quantitative standard for texture.

4.3.2.4 Reconstitution ratios (RR) of the composite complementary instant porridges

The results for the reconstitution ratios of each composite flour are shown in Table 16.

Table 16. The reconstitution ratios (w/v) of the BGN-PABM composite complementary instant porridges

Porridge sample	Reconstitution ratio (RR)
100% white maize (reference)	1:4
100% yellow PABM (control)	1:3
10% brown BGN	1:3
20% brown BGN	1:3
30% brown BGN	1:3
10% red BGN	1:3
20% red BGN	1:3
30% red BGN	1:3

From Table 16 it can be seen that the reference sample (white maize instant porridge) had the highest RR. The rest of the BGN-PABM composite instant porridges had lower and similar RRs compared to the reference. The RRs of the composite complementary instant porridges in this

investigation are within the range of 1:2 to 1:50, which were suggested by Pietsch (2005) as reviewed in Chapter Two. The better reconstitution properties of the composite complementary instant porridges of this investigation maybe attributed to the less damage of the starch granules as a result of low temperatures and short time methods used during the processing of the porridges (i.e. during drying grains and roasting of flours) thereby improving the reconstitution ability of the product.

4.3.3 Sensory acceptability of the composite complementary instant porridges made with BGN and PAMB

The consumer acceptability ratings of the composite complementary instant porridges made with different ratios of BGN and PABM are presented in Table 17, Table 18 and Table 19.

Table 17. White maize (reference)

Attribute	Mean
Taste	3.44 ± 2.21
Colour	4.75 ± 2.20
Aroma	4.49 ± 2.18
Texture	4.16 ± 2.52
Appearance	4.27 ± 2.35
Overall acceptability	4.05 ± 2.33

Table 18. Sensory acceptability of composite complementary instant porridges made with BGN and PABM

Attribute	Variety	Treatment				V	T	V*T
		0% (control)	10%	20%	30%			
Taste	BBGN	3.95 ± 2.31	4.96 ± 2.50	4.93 ± 2.46	4.59 ± 2.53	0.420	0.090	0.790
	RBGN	3.95 ± 2.31	4.30 ± 2.54	4.63 ± 2.65	4.65 ± 2.77			
Colour	BBGN	4.95 ± 2.16	4.91 ± 2.09	4.78 ± 2.13	5.00 ± 2.32	0.245	0.741	0.840
	RBGN	4.92 ± 2.16	4.30 ± 2.38	4.56 ± 2.65	4.65 ± 2.46			
Aroma	BBGN	4.36 ± 2.05	4.85 ± 2.16	5.07 ± 2.26	5.07 ± 2.32	0.461	0.271	0.902
	RBGN	4.36 ± 2.05	4.81 ± 2.42	4.59 ± 2.55	4.85 ± 2.49			
Texture	BBGN	4.36 ± 2.52	4.67 ± 2.40	4.64 ± 2.67	4.78 ± 2.59	0.212	0.987	0.876
	RBGN	4.36 ± 2.52	4.15 ± 2.74	4.26 ± 2.74	4.23 ± 2.49			
Appearance	BBGN	4.13 ± 2.41	4.71 ± 2.51	4.64 ± 2.30	4.61 ± 2.29	0.475	0.582	0.924
	RBGN	4.13 ± 2.41	4.22 ± 2.36	4.41 ± 2.98	4.54 ± 2.50			
Overall acceptability	BBGN	4.24 ± 2.18	4.95 ± 2.27	5.02 ± 2.30	4.83 ± 2.27	0.551	0.167	0.909
	RBGN	4.24 ± 2.18	4.52 ± 2.14	4.78 ± 2.64	4.88 ± 2.53			

BBGN=Brown Bambara groundnut

RBGN=Red Bambara groundnut

Table 19. The percentage of panellists who rated the overall acceptability of composite complementary instant porridge samples containing different concentrations of either red BGN or brown BGN and yellow PABM flours

Score	Brown BGN				Red BGN			
	0% (control)	10%	20%	30%	0% (control)	10%	20%	30%
Dislike extremely	18.2%	9.1%	13.6%	11.4%	18.2%	20.5%	9.1%	0%
Dislike very much	13.8%	20.7%	13.8%	20.7%	13.8%	13.8%	3.4%	0%
Dislike moderately	26.1%	13.0%	8.7%	8.7%	26.1%	10.9%	6.5%	0%
Dislike slightly	13.5%	18.9%	16.2%	27.0%	13.5%	5.4%	5.4%	0%
Neither like nor dislike	14.8%	11.5%	16.4%	9.8%	14.8%	23.0%	9.8%	0%
Like slightly	14.5%	20.0%	18.2%	12.7%	14.5%	12.7%	7.3%	0%
Like moderately	11.6%	16.3%	16.3%	23.3%	11.6%	18.6%	2.3%	0%
Like very much	13.6%	13.6%	22.7%	18.2%	13.6%	13.6%	4.5%	0%
Like extremely	5.9%	23.5%	17.6%	11.8%	5.9%	17.6%	17.6%	0%
Total acceptable	45.6%	73.4%	74.8%	66.0%	45.6%	62.5%	31.7%	0%

The samples were rated in terms of taste, colour, aroma, texture, appearance and overall acceptability, using a 9-point hedonic scale (Larmond 1977). The ANOVA test showed that there was no significant difference ($P>0.05$) in the acceptability of the samples in terms of all the sensory attributes evaluated, including the overall acceptability (Table 18). The acceptability ratings were generally lower for all the different sensory attributes rated by the panellists, the ratings generally ranged from '4=dislike slightly' to '5=neither like nor dislike' (Table 17 and Table 18). The mean values for aroma acceptability of the of the composite complementary instant porridges containing brown BGN flour increased with an increase in the concentration of BGN, whereas no clear-cut trend was observed in the mean values for aroma acceptability of the samples made with red BGN with regard to increasing BGN concentration in the blend. The reference (white maize flour alone) had a higher mean value for aroma acceptability compared to the control (100% yellow PABM flour), but had lower aroma acceptability than the samples fortified with either of the BGN flour (Table 17 and Table 18).

The samples made with brown BGN flour had higher mean values for both taste and appearance acceptability compared to the samples with red BGN flour. However, the mean taste and appearance acceptability values of the samples made with brown BGN flour decreased with an increase in BGN concentration, whereas those of the samples made with red BGN flour increased with an increase in BGN concentration, with the reference sample having the lowest taste mean values and the control having the lowest appearance mean values (Table 17 and Table 18).

Although the samples containing brown BGN flour had higher mean values for colour and texture acceptability than the samples made with red BGN flour, the mean values for colour and texture acceptability did not show any clear-cut trend. The mean values for both colour and texture acceptability of the samples containing red BGN flour increased with an increase in the concentration of the BGN flour. The reference samples had the lowest mean values for both colour and texture acceptability (Table 17 and Table 18).

In terms of the overall acceptability, the reference sample had the lowest mean overall acceptability values than the composite complementary instant porridges containing either of the BGN flour. There was no clear cut trend in the mean values for overall acceptability of the composite complementary instant porridges containing brown BGN flour, whereas the overall acceptability of the composite complementary instant porridges containing red BGN increased with an increase in BGN concentration (Table 17 and Table 18).

Table 19 shows the percentage of the panellists who gave different ratings for the overall acceptability of the composite complementary instant porridge fortified with either of the BGN flour at 0, 10, 20 and 30% (w/w) substitution levels. It is clearly shown in Table 4.10 that as the concentration of either of the BGN flour increased the number of panellists liking the BGN-PABM composite complementary instant porridge decreased, although the number of panellists who liked the composite complementary instant porridge containing brown BGN were higher than those who liked the samples with red BGN. None of the panellists liked the composite complementary instant porridge containing 30% (w/w) of red BGN and the composite complementary instant porridge containing 20% (w/w) of brown BGN was liked the most by the panellists.

The findings of this investigation compare well with those of Mbata *et al.* (2009) who reported that there was no significant difference ($p>0.05$) in the mean values of the sensory attributes of *ogi* complementary food samples containing different concentrations of BGN.

The sensory evaluation results of this investigation indicate that the composite complementary instant porridges prepared with different ratios of BGN and yellow PABM flours are acceptable to the consumers. As reviewed in Chapter Two, several studies have found yellow PABM-based foods products to be less acceptable to the consumers when compared with the corresponding white maize products, which has been largely attributed to the undesirable sensory attributes of the PABM. For example, Pillay *et al.* (2011) found the acceptance of yellow PABM food products such as samp, *phutu* and thin porridge lower than that of the corresponding white maize

products. The incorporation of BGN in the PABM complementary instant porridge in this investigation could have contributed to the improvement in the sensory attributes of the yellow PABM complementary instant porridge. It is possible that the presumably undesirable sensory attributes of the PABM were masked by the desirable sensory attributes of the BGN. BGN has been reported to have desirable sensory attributes (Lewick 1974). In Chapter Two, it was indicated that using advanced techniques in the processing of BGN-based food products has been reported to improve their acceptability. Similarly, in this investigation, the use of advanced techniques in the processing of the BGN-PABM complementary instant porridges could have resulted in the improvement in the sensory attributes of the product. Roasting of flours led to the enhancement of the colour, texture and flavour of the porridges thereby increasing its acceptance. A desirable golden brownish colour which was formed as a result of roasting made the BGN-PABM composite complementary instant porridges more visually appealing. It is also likely that the heat treatment reduced the undesirable beany flavour associated with cereal-legume based complementary foods, as it was not reported by the consumers. The texture of the porridges was improved by conventional roller milling to particle size of 200 μm .

4.4 Conclusions

The findings of the current investigation indicate that addition of BGN flour to PABM complementary instant porridge flour improves the nutritional composition of the product in terms of protein, fat, fibre and total mineral content (ash). The higher fat and protein content of the BGN-PABM composite complementary instant porridges would be useful in addressing the escalating levels of PEM in most of the SSA countries, including South Africa. The increased mineral content in the composite complementary instant porridge would contribute to addressing mineral deficiencies, which are prevalent. In most of the SSA countries, including South Africa. However, careful steps would have to be taken before and during processing of the complementary foods to ensure that the PABM supplies the desired concentration of PVA in the products so that VAD is also addressed by the BGN-PABM composite complementary porridge.

The functional properties of the BGN-PABM composite complementary instant porridges such as WAC, SI, SV and texture changed with an increase in the concentration of flour of either of the BGN varieties. A decrease in the SV of the composite complementary instant porridges with increasing concentration of BGN flour observed in this study would have a positive effect on the nutritional quality of the porridge, as the nutrient density of the product would increase. There is a need to improve the WAC and solubility (measured as SI) of the composite complementary instant porridges.

The composite complementary porridges were also acceptable to the study population with clear preference for BGN-PABM composite complementary porridges over the white maize composite complementary instant porridge with regard to taste, aroma, appearance and overall acceptability. It appears that the BGN masked the presumably undesirable sensory characteristics of the PABM. However, there is need to conduct sensory evaluation of the BGN-PABM composite complementary instant porridges with the caregivers whose children have or are vulnerable to PEM and VAD.

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Chapter 5 - Conclusions and recommendations

In this chapter comprises the main conclusions and recommendations of the study. The aim of this study was to determine the effect of adding BGN on the nutritional, functional and sensory quality of a PABM complementary instant porridge. The research objectives were to: (i) produce composite complementary instant porridges by partially substituting PABM flour with different levels of BGN flour (ii) determine the effect of BGN on the nutritional composition of a PABM complementary instant porridge (iii) determine the effect of BGN on the functional properties of a PABM complementary instant porridge (iv) determine the effect of BGN on the consumer acceptance of a PABM complementary instant porridge.

5.1 Conclusions

This work has provided useful and important baseline data on the nutritional, functional and sensory quality of a BGN-PABM complementary instant porridge, an area in which data were either lacking or scarce.

5.1.1 Nutritional composition

The results of the current study demonstrate that adding flour of either the brown or red variety of BGN improves the nutritional composition of PABM complementary instant porridge in terms of protein, fat, fibre and total mineral content. Thus, the findings of this study suggest that the BGN-PABM complementary instant porridge could significantly contribute to the alleviation of PEM and other nutrient deficiencies, including mineral deficiencies, which are prevalent in SSA. However, the effect of adding PABM on the PVA content of the instant porridge was not conclusive, since PVA carotenoids could not be detected. This could be have been due to destruction of PVA carotenoids during storage and processing. For instance, roasting or browning could destroy the PVA carotenoids, thus it is not the best processing method for instant cereal products, in PVA carotenoids need to be preserved. However, there would be also need to select PABM grain types that contain desired levels of PVA for use in the composite porridges so that the complementary instant porridges also make a significant contribution to addressing

VAD, especially among children of low economic-status communities in the SSA countries, including South Africa.

5.1.2 Functional properties

Adding flour of either the red or brown variety of BGN to the PABM complementary instant porridge led to deterioration of the functional properties of the PABM complementary porridges with respect to WAC and SI - therefore, these functional properties should be improved. Adding BGN resulted in the reduction of the SV of the complementary porridge, which would be desirable as the nutrient density of the porridge, would increase and its viscosity decrease. A porridge of low viscosity can be easily handled by the gastro-intestinal tract (GIT) of children.

5.1.3 Sensory acceptability of the composite complementary instant porridges

The composite complementary porridges were also acceptable to the study population with clear preference for BGN-PABM composite complementary instant porridges over the white maize complementary instant porridge with regard to taste, aroma, appearance and overall acceptability. An increase in concentration of either of the BGN flour improved the sensory acceptability of the PABM complementary instant porridge. The colour of the porridges was also desirable. Probably, BGN masked the undesirable sensory characteristics of the PABM, which has been reported in previous studies. The advanced processing techniques, including hot air oven drying, milling and roasting of flours, used to develop the instant porridge could have also contributed to reduction of the presumably undesirable sensory properties of the PABM by, for example causing production of strong desirable flavour and aroma substances, such as the Maillard reaction products (MRPS). However, the study was limited to a small sample of students and staff member of the Agriculture campus of the UKZN, PMB, South Africa. The caregivers whose children are affected by or are vulnerable to PEM and VAD should evaluate the sensory quality of the product. Furthermore, the study should cover a larger sample of caregivers.

5.2 Recommendations

5.2.1 Study limitations

- Due to financial constraints, only two grain samples and one composite sample of the complementary instant porridge were analysed for their PVA content. The amino acid profile of the samples was also not analysed.
- The sensory evaluation was done using UKZN students and staff. The findings of the study cannot be applied to other parts of South Africa and the larger SSA.

5.2.2 Recommendations for improvement of the study

- More detailed nutritional analysis should be conducted on the BGN-PABM complementary instant porridges. These analyses should include starch, PVA carotenoids, amino acid profile and mineral bio-availability.
- More functional properties of the BGN-PABM including bulk density (BD), water binding capacity (WBC), swelling power (SP) and pasting properties should also be conducted.
- There is need to assess the protein and starch digestibility of the BGN-PABM complementary instant porridge.
- The PVA content of the grains should be determined before use to ensure that only grain types with the desired levels of PVA are used.
- A cost-benefit analysis of using the BGN-PABM instant porridge in the target communities should be performed. The sensory evaluation should be conducted in the rural parts of SSA using caregivers whose children are at risk of PEM and VAD.

5.2.3 Implications for further research

- Similar studies should be conducted in other parts of SSA using the most popular BGN and maize-based composite complementary foods in those regions.
- Studies could be conducted to determine if marketing has an impact on whether or not BGN and PABM would be grown, purchased and used.

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Appendix A: Consent form in English



INFORMED CONSENT FORM

The effect of adding Bambara groundnut on the nutrition composition and consumer acceptability of provitamin A-biofortified maize instant porridge.

Aim: To determine whether the combination of Bambara groundnut and provitamin A-biofortified maize instant porridge will be acceptable to the target consumers.

I _____ (full name) of age _____ agree to voluntarily take part in this research project.

I, the undersigned, _____ (Full Name)

Participant, student number _____, Tel: _____,

I have been fully informed of:

- The purpose of this study.
- That my participation is voluntary.
- That I can withdraw at any time.
- That participation will cost me nothing.

- That all information given will be kept confidential.
- I have been informed that persons who are allergic to maize or nuts must not participate in the study- I declare that I have no allergic reactions to maize or nuts. However, in the case that I have an allergic reaction to the food samples I will have tasted, the researchers have informed me that, if the allergic reaction occurred during the study or while I am still at the study premises, they will assist me to get medical attention according to the UKZN standard operating procedure entitled “Reporting Structure of all Accidents/Incidents on Duty”, which is attached to this Consent form I am appending my signature on. In the event that an allergic reaction occurs during the study, while on the study site, the Researchers have informed me that according to the “Reporting Structure of all Accidents/Incidents on Duty”, they will take full responsibility to ensure that I receive medical care.

I agree to: Taste the food samples and answer a questionnaire.

This consent form was explained to me by _____ (Full Name), in _____ and I confirm that I have understood.

(Participant's signature or mark)

Signed at: _____ Date _____

Appendix B: Consent form in isiZulu



I-form lesivumelwano

Singabafundi basenyuvesi yaKwa-Zulu Natal senza i-Masters Degree kwezokudla (Human Nutrition). Njengengxenywe yocwaningo lwezifundo zethu sizama ukuthola ukuthi abantu bangalamukela yini iphalishi elakhiwe ngempuphu emhlophe elinomsoco owengeziwe ongu Provitamin-A kanye ne Bambara okuyihlobo la lwe kinati eligayiwe nanokuthi lizwakala linjani uma liqhathaniswe ne palishi elenziwe ngempuphu elengeziwe ngomsoco iprovitamin-A lilodwa. Imininingwane etholakele kulolucwaningo izogcinwa iyimfihlo, amagama abantu ababambe iqhaza azogcinwa eyimfihlo.

Mina, _____ (Amagama aphelele omhlanganyeli)

iStudent number _____

Ngiyavuma ukuba ngazisiwe:

- Ngenhloso yalolucwaningo.
- Ukuthi ukuhlanganyela kwami ngizikhethele.
- Ngingayeka noma ingasiphi isikhathi.

- Ukuhlanganyela kwami angeke kungibize mali.
- Ukuthi yonke imininingwane izoba imfihlo.

Ngiyavuma ukuba:

Ngizophendula imibuzo mayelana nokunambitha ngeqiniso.

Leli fomu ngilichazelwe ngu _____ (igama eliphelele), ngolimu lwe
_____ (lime) ngiyavuma ukuba ngikuzwile konke engikuchazelwe kona.

Mina _____ (Igama eliphelele) ngiyavuma ukuhlanganyela
kulolucwaningo.

Sayina lapha

ufakazi

Appendix C: Sensory evaluation questionnaire in English



SENSORY EVALUATION OF A COMPLEMENTARY FOOD MADE FROM PROVITAMIN A-BIOFORTIFIED MAIZE AND BAMBARA GROUNDNUT

Participant number:

Sample number:

INSTRUCTIONS

1. Prior to participation on the sensory evaluation, please read and sign the consent form provided.
2. Please answer the questionnaire honestly and truthfully.
3. Do not communicate with other panellists during the process of the evaluation.
4. Please take note not to consume any food products for at least 15 minutes prior to the evaluation.
5. Please rinse your mouth with water before you begin with the evaluation, as well as after each sample of the porridge.

6. Please rate the taste, colour, aroma, texture, appearance and overall acceptability of the samples according to the scale given below (Table 1). Kindly note that the samples must be evaluated in the order that they are provided (from left to right). The samples may be re-evaluated if necessary.

Table 1: 9-point hedonic scale

9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Sample code	Taste	Color	Aroma	Texture	Appearance	Overall acceptability
	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>
	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>
	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>
	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>
	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>	<u>Score:</u> <u>Comment:</u>

Thank you for taking part in this study

Appendix D: Sensory evaluation questionnaire in isiZulu



Participant number:

Sample number:

Imibuzo: Ucwangingo Lokunambitheka lwe phalishi elenziwe ngempuphu elinomsoco owengeziwe onguprovitamin-A elixutshwe ne Bambara okuyihlobo lwekinati eligayiwe.

Imiyalelo

1. Sicela ube nesiqiniseko sokuthi uyifundisisile imiyalelo, wayiqondisisa kahle bese wasayina ifomu lesivumelwano sokuba yingxenywe yalolucwangingo.
2. Sicela uphendula yonke imibuzo ngokuthembeka nangeqiniso.
3. Sicela ungadli lutho emizizwini eyishumi nanhlanu ngaphambi kokuba ingxenywe yocwangingo.
4. Sicela ungaxhumani nabanye ngesikhathi kuqhubeka ucwangingo.
5. Sicela uhlanze umlomo wakho emva kokunambitha i-sample ngayinye.

6. Sicela ukases (rate) ukunambitheka, ukuzwa, iphunga, umbala .Kanye nokuthi uyithanda kangakanani i-sample ngayinye usebenzisa i- 9 – point hedonic scale esingenzansi (Itafula lokuqala). Sicela uqale ukunambitha i-sample engakwesokunxele kuya kwengasokudla.

Itafula lokuqala: 9-point hedonic scale

9	Ngiyithanda kakhulu kakhule
8	Ngiyithanda kakhulu
7	Ngiyithanda ngokuphakathi nendawo
6	Ngiyithanda kancane
5	Mhlawumbe ngiyayithanda noma angiyithandi
4	Angiyithandi kancane
3	Angiyithandi ngokuphakathi nendawo
2	Angiyithandi kakhulu
1	Angiyithandi kakhulu kakhulu

Iminyaka nobulili:

Inombolo yobambe iqhaza:

Inombolo ye-sample	Ukunambithe ka	Umbala	Ukuzwa	Iphunga	Ukubukeka	Uyithande Kangakanani
	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:
	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:
	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:

Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:
Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:	Imiphumela: Umbono:

Ngiyabonga ngokuzibandakanya kulolu cwaning

Appendix E: Ethical approval letter from the University of KwaZulu-Natal



11 March 2016

Dr M Siwela 640271
School of Agricultural, Earth and Environmental Sciences
Pietermaritzburg Campus

Dear Dr Siwela

Protocol reference number: HSS/0217/016CA
Project Title: The effect of adding bambara groundnut on the nutritional composition and consumer acceptability of provitamin A-biofortified maize instant porridge

Full Approval – Expedited Application

In response to your application received 3 March 2016, 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 discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of 3 years from the date of issue. Thereafter Recertification must be applied for on an annual basis.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....
Dr Shenuka Singh (Chair)
Humanities & Social Sciences Research Ethics Committee

/pm

Cc Supervisor: Dr M Siwela
Cc Academic Leader Research: Prof Onesimo Mutanga
Cc School Administrator: Ms Marsha Manjoo

Humanities & Social Sciences Research Ethics Committee

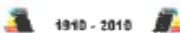
Dr Shenuka Singh (Chair)

Westville Campus, Govan Mbeki Building

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