NUTRITIONAL QUALITIES AND CONSUMERS ACCEPTANCE OF PROVITAMIN A BIOFORTIFIED AMAHEWU COMPLEMENTED WITH DEFATTED BAMBARA FLOUR

BY

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PREFACE

The work described in this thesis was carried out in the Department of Dietetics and Human nutrition, University of KwaZulu-Natal under the supervision of Dr Muthulisi Siwela.

Signed: ______________________ Date: ___________________

Temitope Deborah Awobusuyi (candidate)

As the candidate's supervisor, I agree to the submission of this thesis.

Signed: ______________________ Date: ___________________

Dr Muthulisi Siwela (Supervisor)

DECLARATION

I, Temitope Deborah Awobusuyi, declare that:

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

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

Signed:

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Finally, I thank God, for the strength and the blessings that have made this work possible.

DEDICATION

This thesis is dedicated to God Almighty.

He alone sustained me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Amahewu, a fermented non-alcoholic maize-based beverage, is popular in Southern Africa. It serves as a refreshing drink for adults, preschool children and school-going age children. The aim of this study is to evaluate the possibility of producing an acceptable provitamin A-biofortified maize amahewu complemented with defatted bambara groundnut flour, which can be used to alleviate protein energy malnutrition and VAD among the nutritionally-vulnerable groups.

In this study, Bambara groundnut grain was pre-treated by roasting and germination prior to their addition to amahewu. The bambara groundnut grain was germinated for 72 hours and roasting was done at 180°C for 20 minutes. Germinated, roasted and raw bambara flours, separately, were added at 30% (w/w) level of substitution to either white maize or provitamin A-biofortified maize and then the composite flours were processed into amahewu porridges. Amahewu samples were prepared according to a traditional method, whereby a mixture of maize meal and water (ratio: 1:7) at 90°C was boiled with occasional stirring for 15 minutes. The resulting porridge was left to cool to approximately 40°C, followed by inoculation and fermentation at 37°C. Wheat bran (at 0.5% w/w) was used as reference inoculum. Amahewu prepared with either provitamin A maize or white maize without bambara flour served as controls.

Amahewu samples were analysed for nutrient composition, including protein, total carbohydrate, fibre, ash, amino acid, and mineral contents. *In-vitro* protein digestibility of the samples was determined. Consumer acceptability of amahewu samples was evaluated using regular consumers of amahewu (n=70), who rated their liking of key sensory attributes, including overall acceptability, of the amahewu samples on a 9-point hedonic scale (1: disliked extremely, 9: liked extremely).

The proximate composition of amahewu made with provitamin A maize and white maize was similar, which suggested that maize type did not significantly influence the nutritional composition of amahewu. The protein content of amahewu (34%, dry weight basis) almost doubled with the inclusion of bambara at 30% substitution level. However, pre-

treating bambara by germination and roasting significantly influenced the protein contents of the resulting amahewu. Amahewu with added germinated bambara flour had a slightly higher protein content compared to amahewu with added roasted bambara flour. As expected, carbohydrate was the major nutrient of amahewu samples ranging from 63-83%. The carbohydrate content of amahewu did not vary with maize type. However, the addition of bambara slightly diluted the carbohydrate content as a small decrease was observed. Fat and ash contents in amahewu samples were generally low without the addition of bambara. Major minerals in amahewu samples were potassium followed by magnesium. The addition of Bambara in the preparation of amahewu increased some minerals, especially zinc and iron.

The addition of bambara significantly influenced the amino acid profile of amahewu. The major effect of adding bambara was noted on lysine, which almost doubled in both white maize and provitamin A biofortified maize amahewu. When comparing the lysine content to the WHO requirement for infants, the lysine accounted for 60% of the recommended intake for children (compared to 50% with no bambara added), which indicates about 10% increase at 30% level of inclusion of bambara.

In general, the *in vitro* protein digestibility (IVPD) of amahewu with added bambara was high compared with that of un-composited amahewu (controls). The protein digestibility of amahewu (65%) increased by almost 45% with the inclusion of bambara. Pre-treating bambara seemed to have a major effect on protein digestibility of amahewu as amahewu composited with germinated bambara flour recorded the highest IVPD value (93%). The type of maize used also seemed to influence protein digestibility.

The addition of bambara to amahewu did not negatively affect the sensory acceptability of the fortified amahewu. The types of maize used in the preparation of amahewu had a major effect on the acceptability of the products. In general, provitamin A-biofortified amahewu samples composited with bambara flour were very much liked for their sensory attributes of colour, aroma and taste when compared to their white maize counterparts. The effect of pre-treating bambara was also significant. Provitamin A-biofortified

amahewu composited with germinated bambara flour recorded the most liked for its colour, whilst amahewu samples with roasted bambara flour were the most liked for their taste, aroma and overall acceptability. Principal component analysis (PCA) indicated the first component explained 96% of the variation and provitamin A-biofortified amahewu samples were clearly differentiated from white maize amahewu samples. Yellow colour and taste resulting from roasting and germination were somehow strongly perceived in provitamin A amahewu compared to white maize amahewu samples.

The study findings indicate that provitamin A amahewu complemented with germinated and roasted Bambara would be acceptable to target consumers and may have the potential to contribute to addressing protein malnutrition due to its superior nutritional quality, including high protein nutritional value compared to white maize amahewu.

KEYWORDS: Bambara groundnut flour, Amahewu, Protein Energy Malnutrition, Germination, Roasting, Sensory acceptability, Vitamin A Deficiency.

CHAPTER 1

INTRODUCTION

1.1 Study background

Amahewu is a sour, non-alcoholic beverage made from the fermentation of boiled thin maize porridge. The alcoholic content of amahewu is less than 1%, and hence can be regarded as non-alcoholic. Amahewu is commonly consumed in the Southern region of Africa in countries such as Zimbabwe, South Africa and Botswana (Gadaga *et al*., 1999). The beverage is commonly consumed as a supplementary food by farm labourers. However, very often, amahewu is consumed as a substitute for food due to poverty, neglect of health needs and lack of knowledge. Mothers also give the beverage to their weaning babies as a complementary food (Simango, 1997). Research carried out by Simango (1997) based on the potential use of traditionally fermented foods in weaning babies indicated that 95% of the mothers fed amahewu to their children. Although amahewu has been commercialised, traditionally prepared amahewu is fermented with the aid of an inoculum found in millet, wheat bran or wheat flour. Amahewu consists of about 8-10% solids, the rest being water. Therefore a substantial amount would have to be consumed in order to meet the nutritional requirements of the body (Blandino *et al.*, 2003).

1.2 Importance of the study and problem statement

Protein-energy malnutrition (PEM) is one of the prevalent nutritional health problems in Africa. PEM results in diseases like Kwashiorkor and Marasmus (Muller and Krawinkel, 2005). White maize, which is the major constituent of the solids in amahewu is deficient in essential amino acids such as lysine and tryptophan (Shewry *et al*., 1995). Maize is also deficient in other micronutrients such as vitamin A. Another factor that contributes to the nutritional limitation of amahewu is that the frequently used source of the inoculum, millet, contains anti-nutritional factors like tannins, which interfere with the bioavailability of nutrients such as amino acids in the body (Towo *et al.*, 2006). A combination of these problems contribute to the nutritional inadequacy of amahewu. In a previous study carried out by Awobusuyi *et al* (2016) to improve the provitamin A content of amahewu through substitution of white maize with provitamin A-biofortified maize. The study reported that the β-carotene levels found in provitamin A-biofortified maize were well retained in all amahewu samples after fermentation. The product was however deficient in protein content and quality, hence there is need for the improvement of amahewu in that aspect.

Research has been done to improve the nutritional quality, especially protein content and quality, of cereal-based foods from legume proteins and other protein sources such as whey protein, soybean protein and okra seed protein have been used for fortification (Mugocha *et al*., 2000).

Legumes are generally rich in dietary fibre, protein and are of high nutritive value (Swapnil *et al.,* 2016). Legumes also contain considerable amount of vitamins and other micronutrients. The composition of legume materials is known to play a key role in preventing metabolic diseases such as diabetes mellitus (Boye *et al.,* 2010a, De Almeida *et al*., 2006, Simpson *et al.,* 1981) and coronary heart diseases (Simpson *et al.,* 1981, Boye *et al.,* 2010b).

In this study, Bambara is the legume of choice because of its many qualities, although under-utilised, it is drought tolerant, easy to cultivate, grows well on poor soils and contains high amounts of essential amino acid.

However, it appears that despite the high potential for use of bambara groundnut in the fortification of cereal grain-based foods, it has not been supplemented with amahewu. In this study, various processing methods such as germination and roasting of bambara groundnut will be employed. These processing techniques when implemented has been found to help improve the nutritive value of food products. This study will investigate the effect of adding bambara flour for the fortification of amahewu using provitamin A biofortified maize and white maize as control. How this addition bambara flour will affect the nutritional, functional properties and acceptability of amahewu is not known. Germination and roasting have been reported to improve the nutritional and functional properties of legumes (Alonso *et al*., 2000, Waldron 2000, Jirapa *et al.,* 2001 and Rahma 1988. Germination improves the nutritive values of cereals and legumes (Marero *et al.,*1989a. Marero *et al.,* 1989b, Hansen *et al.,* 1989). Germination and roasting have also been found to decrease the levels of anti-nutrients present in cereals and maximizes the levels of some of the utilizable nutrients (Nkama and Ikwele 1998). The addition of bambara flour is expected to improve the protein content and overall quality of amahewu.

1.3 Aim

To evaluate the potential of bambara flour for protein fortification of amahewu made with provitamin A-biofortified maize.

1.4 Hypotheses

1. Amahewu made of provitamin A-biofortified maize and bambara groundnut flour will have significantly improved nutritional value in terms of protein, lysine and mineral content compared to white maize and bambara. Bambara has a better nutrient composition with respect to protein, lysine and indispensable amino acids and minerals (USDA 2008). Fortified amahewu products will have significantly higher levels of bioavailable protein and lysine compared to unfortified amahewu. Protein and lysine that are deficient in maize will be increased by addition of bambara to the products.
2. Amahewu fortified with bambara will have better functional properties such as high protein digestibility, compared to white maize. Germination and roasting has been found to increase protein digestibility by reducing the effects of anti-nutritional factors in fermented foods thus increasing the bioavailability of proteins through the release of proteolytic enzymes that break down polyphenols (Oluwole and Taiwo, 2009).
3. The sensory properties of colour, taste and aroma of amahewu will be improved by the addition of roasted bambara and the product will be acceptable to consumers. This is because the fermentation enhances the organoleptic properties of foods such as the aroma, flavour and texture (Osungbaro 2009, Oyewole 2012a), thereby making it more acceptable to consumers. An improvement on aroma acceptability of porridge have been noted following the addition of roasted nuts in some previous studies (Mbata *et al*., 2009).

1.5 Objectives

1. To determine the effect that the addition of bambara groundnut flour will have on the nutritional quality of provitamin A-biofortified amahewu.
2. To determine the effect that the addition of bambara groundnut flour will have on the functional properties of provitamin A-biofortified amahewu.
3. To determine the effect that the addition of bambara groundnut flour will have on the consumer acceptability of provitamin A-biofortified amahewu.

1.6 Assumptions

The following assumptions were made:

- It was assumed that the study participants consumed maize on a regular basis.

- The food items used in this research project were safe for human consumption.

- The subjects answered all questions honestly and without bias.

1.7 Abbreviations

AOAC: Association of Official Analytical Chemists

NFCS: National Food Consumption Survey

SAVACG: South African Vitamin A Consultative Group

SSA: sub-Saharan Africa

VAD: Vitamin A deficiency

WHO: World Health Organization

PEM: Protein Energy Malnutrition

CHAPTER 2

LITERATURE REVIEW

The literature review covers the extent of malnutrition in South Africa and focuses on Protein energy malnutrition and vitamin A deficiency. The strategies employed to combat PEM and VAD in South Africa are reviewed. The proposal to use provitamin A-biofortified amahewu with the addition of bambara groundnut flour to combat PEM is evaluated.

2.1 Prevalence of malnutrition in sub-Saharan Africa with special focus on protein-energy malnutrition

Protein Energy Malnutrition continues to be a major nutritional problem resulting from under-nutrition that affects children in most of the developing world (Muller and Krawinkel 2005). The most recent estimates show that more than one billion people worldwide are undernourished (Food and Agriculture Organization (FAO) 2009). Africa is home to over 70 million undernourished children (World Food Programme (WFP) 2008). In this region, poverty causes food shortages and the most vulnerable populations survive predominantly on starchy staples such as maize, wheat, rice, sorghum, millet and cassava, with little or no meat and dairy products (Mayer, Pfeiffer and Beyer 2008). The protein nutritional quality of these staple foods is poor and lysine is the most limiting amino acid (United States Department of Agriculture (USDA) 2008). The health consequences most pronounced in children suffering from PEM include higher susceptibility to infectious and metabolic diseases, impaired physical and cognitive development and increased mortality rates because of their higher nutritional requirements due to high growth velocities (Stipanuk, 2006). The problem is further compounded by the Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) epidemic that has increased the number of vulnerable children to communicable and incommunicable diseases. An estimated 91% of new infections among children worldwide and 14.1 million AIDS orphans are in Sub-Saharan Africa (Joint

United Nations Programme on HIV/AIDS/ World Health Organization (UNAIDS/WHO) 2009).

2.1.2 Protein energy malnutrition (PEM)

Protein Energy Malnutrition refers to a group of diseases that result from under-nutrition and is a major public health problem in developing countries. It is a macronutrient deficiency disease resulting from an inadequate intake and/or utilization of protein and energy, and mainly affects children most because of their higher needs for protein and energy per kilogram body weight compared to adults (Stipanuk, 2006). It is estimated that approximately 27% of children younger than five years in developing countries are underweight and in Sub-Saharan Africa 38% have stunted growth while 28% are underweight (UNICEF, 2007). PEM is associated with the deaths of approximately 5 million children each year (WHO, 2000). The main cause of PEM in developing countries is dependence on a single starchy staple for virtually all the protein and energy requirements (Onis and Blossner 1997). The symptom of mild to moderate forms of PEM in children is inadequate growth (Shetty, 2006).The classic clinical syndromes of severe forms of PEM are Kwashiorkor, Marasmus and the mixed condition of Marasmic Kwashiorkor. Kwashiorkor arises from low protein intake and adequate energy consumption leading to reduced synthesis of visceral proteins. Hypoalbuminaemia develops because of short term protein deficiency and causes oedema (Furham, *et al*., 2004). The combination of a fatty and enlarged liver, because of impaired synthesis of hepatic proteins and fluid accumulation distends the stomach and disguises weight loss (Stipanuk, 2006). Other symptoms commonly observed are anaemia, hair discoloration, dry or peeling skin, diarrhoea, and fluid and electrolyte disorders.

Marasmus is a result of chronic deficiency of both protein and energy leading to protein loss in the skeletal muscle and adipose tissue (Gibney, *et al.*, 2002). There is an absence of oedema, severe muscle wasting, and shrivelled skin. The consequences are stunted brain development, depressed metabolism, stunted physical growth and development, anaemia, impaired immune system and fluid and electrolyte disorders. Marasmic Kwashiorkor occurs when a child has wasted muscles and untreated Marasmus can result in death from heart failure and dehydration (Thompson, *et al.,* 2008).

2.1.3 Functions of proteins in human nutrition

Proteins contribute to cell growth, repair and maintenance, act as enzymes and hormones, maintain fluid, electrolyte and acid base balance and also maintain a strong immune system (Thompson *et al.,* 2008). When fats and carbohydrates are not provided in adequate amounts in the diet, proteins also serve as an energy source, limiting their availability for the functions stated earlier (Gibson, 2005). Additionally, proteins act as carriers for other nutrients that include lipids, Vitamin A, iron, sodium and potassium. Consequently, protein deficiency in children is also accompanied by other nutrient deficiencies including micronutrient deficiency (Muller and Krawinkel, 2005).

Acute malnutrition causes wasting, low weight-for-height, while chronic malnutrition causes stunting and low height-for-age. Underweight, low weight-for-age reflects both stunting and wasting (Gibson, 2005). It has been shown that when school children consume animal source proteins, there is a positive impact on weight gain and increased lean body mass (Grillenberger, 2006). Protein helps maintain a strong immune system by supporting the increased production of antibodies in response to common infections such as colds, influenza and allergic reactions (Thompson *et al.*, 2008). Children who have PEM have greatly increased susceptibility to life-threatening infectious diseases such as HIV/AIDS, tuberculosis and malaria (Schaible and Kaufmann, 2007). There is also evidence that chronic PEM in 5 to 10 year olds impairs cognitive development (Kar, *et al*. 2008).

2.2 Vitamin A deficiency (VAD)

VAD affects 190 million pre-school children in the World Health Organization (WHO) regions of Africa and South East Asia. This section will focus on VAD trends in South Africa.

2.2.1 Trends of vitamin A deficiency in South Africa

In South Africa, in particular, 63.6 % of children aged between one and nine years were found to be vitamin A deficient in 2005 by the National Food Consumption Survey (NVASPGSA, 2012). In South Africa in 1994, a national survey done by the South African Vitamin A Consultancy Group (SAVACG) for the Department of Health showed that one out of three children under the age of six years in the country had poor vitamin A status. The provinces most seriously affected by VAD were the Northern Province, KwaZulu-Natal, Mpumalanga, North West Province and the Eastern Cape. Children living in rural areas and in low socio-economic environments were found to be more severely affected than those living in urban areas and in better socio-economic environments.

The Department of Health launched a national vitamin A supplementation (VAS) programme in 2001 following the 1994 SAVACG survey, which showed that VAD was a public health problem in South Africa. The 2005 National Food Consumption Survey (NFCS) indicated that other micronutrient deficiencies among women and children still persist and nutritional status may be deteriorating. Very recently, the HSRC (2014) reported that 11 in 25 (44%) of South African children under the age of five suffered from VAD.

Previous findings identified VAD to be a significant public health issue for young children in the country and that key intervention strategies were needed to alleviate this nutritional disorder. South Africa, like many other countries, has adopted multiple strategic approaches to prevent VAD, namely food fortification, vitamin A supplementation and dietary diversification.

2.2.2 Association of Vitamin A Deficiency (VAD) and Protein energy malnutrition (PEM)

PEM and VAD account for 250 million people globally, with 2.8 to three million people clinically deficient (FAO, 1997). Protein-energy malnutrition is one of the prevalent nutritional health problems in Africa. PEM results in diseases like kwashiorkor and marasmus (Muller and Krawinkel, 2005). Vitamin A deficiency (VAD) is one of the prevalent deficiencies resulting in poor eyesight in both the young and the old. High prevalence of poor diet and infectious diseases brought about by VAD and PEM contribute largely to malnutrition. In developing countries, diets are frequently deficient in macronutrients such as protein leading to PEM and micronutrients such as vitamin A and minerals leading to VAD. VAD can cause ailments such as measles, diarrhoea or malaria before causing complete blindness and improving the vitamin A status of children may decrease mortality rates by 25 percent, measles death rates by 50 percent and death caused by diarrhoea by 40 percent (UNICEF, 2007b). Between 100 and 140 million children globally, under the age of five, may have low vitamin A. Four million children globally show signs of severe deficiency (UNICEF, 2009).Different approaches have been taken in an effort to curb protein energy malnutrition. These include the bio-fortification of foods, supplementation as well as the dietary approach of fortifying foods using nutritionally richer foods (Pandey and Urquia, 2007).

2.2.3 Strategies employed to address Vitamin A Deficiency (VAD) and Protein energy malnutrition (PEM) in Africa

Malnutrition can be defined as a lack of proper nutrition (Fig 2.1). The nutritional status of a child, as with any individual, is assessed through dietary, anthropometric, biochemical and physical observation for signs of malnutrition. When there is a deficiency in the amount and nutritional value of the food consumed, the growth pattern of a child becomes disrupted owing to nutrient deficiencies (Faber and Wenhold 2007; Labadarios, 2005). About 854 million people are estimated to be undernourished in the world, with 820 million in the developing countries. 25% of this number is in Africa, and this number was expected to rise to 30% by 2015. Sub-Saharan Africa is the region that harbours the highest prevalence of under-nourishment. Ratios are estimated that 1 in 3 people is deprived of access to sufficient food (Pandey and Urquia, 2007).

Amongst the malnutrition problems, PEM and VAD have been the major public health problems for decades, particularly in Africa and parts of Southern Asia (Müller and Krawinkel, 2005). About 300 000 per annum die because of malnutrition and half of these are children. In developing countries, the diet is majorly composed of carbohydrate abundant foods such as maize, cassava, potatoes and cereals. The problem arises when these foods are consumed without protein supplementation which is often the case in developing countries. This is due to lack of knowledge and also inability to access expensive protein sources (Müller and Krawinkel, 2005). Many strategies have been set to increase the production, availability and access to foods rich in micronutrients and to increase the consumption of foods rich in micronutrients and the bioavailability of micronutrients from the diet. One of these strategies is through the biofortification of staple foods with protein rich legumes such as soybean and cowpea. Biofortification can deliver naturally fortified foods to people who may not have access to commercially fortified foods that are more readily available in urban areas (Nestel et al., 2006).

Fig 2.1: Manifestations of Protein Energy malnutrition

Source: Google pictures

2.3 Amahewu

2.3.1 Importance and utilisation of amahewu

Amahewu is a sour beverage which is prepared by boiling maize flour in water to produce a thin gruel (Fig 2.2). The maize can either be the conventional white maize or provitamin A maize. The amahewu gruel is then fermented with the aid of an inoculum. The inoculum source can either be malted millet or wheat bran (Simango, 1997). Commercially made amahewu is fermented with a bacterium. This makes the fermentation process faster as well as regulating the nature of the products that are obtained. The beverage is common in the Southern regions of Africa in countries such as Zimbabwe and Botswana (Gadaga *et al.,* 1999). Amahewu makes a large contribution to the diets of African people. The beverage is consumed by people of all ages at a rural household setting. It is used as a thirst quenching beverage after a long day's work. Some consume it as a snack whilst others consume it as a food substitute(Guyot, 2012). Breast feeding mothers also consume the beverage to increase the quantity of milk. Upon weaning, mothers also give the beverage to their babies as complementary feeding. Research by Simango, 1997 indicated that 94% of the interviewed women had knowledge of amahewu and indicated that the beverage was given to babies after the age of four months. This is because at the age of 6-12 months, breast milk no longer meets the needs of the baby as far as food is concerned. At this "food accustomed" or weaning stage, supplementary foods are required to satisfy the child as well as meet their nutritional needs (Egounlety, 2002, Joint and Organization, 2007).

Fig 2.2: Commercial amahewu
(Source: Wikipedia)

2.3.1.2 Limitations of amahewu and attempts to fortify it as a food/ complementary beverage

Amahewu, being a maize-based beverage is nutritionally limited. Maize is well known for its deficiency in the essential amino acids lysine and tryptophan. Also, the source of inoculum used, contains anti-nutritional factors that reduce the bioavailability of nutrients in the body. A combination of these factors renders amahewu an inadequate source of nutrients. Although large quantities of the beverage can be consumed, the quantities that are consumed do not translate to a better nutritional value. This is because 80% of the product is water (Egounlety, 2002). There have been attempts to fortify amahewu using protein rich legumes such as soybean and cowpea. Whey protein has also been used to improve the nutritional quality of amahewu. Granato *et al.*, (2010) added soybean flour to amahewu and recorded a 32% protein increase of the amahewu after fermentation. Hesseltine, (1983) fortified amahewu using whey protein and recorded a 60% protein increase after fermentation. Another researcher, Plahar *et al*., (1983) fortified amahewu using fish flour and recorded a protein increase of 13% after fermentation.

Although these protein sources have been used to fortify amahewu, their use is not very economical especially if the product is targeted at the poor communities. Soybean flour being a high protein legume is known to be deficient in methionine. Methionine is one of the essential amino acids that are required by the body. Also, soybean is a seasonal crop, implying that when it is out of season, amahewu cannot be fortified and people still revert to their old non-nutritious diet. The soybean plant is also usually farmed in commercial farms as a cash crop. This therefore makes it expensive and not readily available. Whey protein on the other hand, is a very rich protein source derived from milk. However, it is expensive due to the methods used to obtain it. This alternative would not be feasible especially if the fortified amahewu is targeted at the poor communities. Fish flour, is also another good source of protein but it is difficult to acquire in abundance hence this would make the product expensive as well. Also, if fish would be available, the first thought would be to use it as a relish rather than a supplement in amahewu. Another disadvantage of fish flour is that it imparts a fishy smell onto the amahewu which is generally not liked by the consumers. It is therefore imperative that alternative methods of fortification be found. Methods that can produce a cheap and sensory acceptable product. Bambara groundnut is one of the legumes that can be considered in fortifying amahewu.

2.4 Nutritional limitations of white maize grain and the possibility to replace it with provitamin A-biofortified maize

Maize (Zea mays), also known as corn, is one of the leading cereal grains in the world (Fig 2.3) (Nuss and Tanumihardjo, 2010). Worldwide consumption of maize is more than 116 million tons, with Africa consuming 30% and Sub-Saharan Africa 21%. Lesotho has the largest consumption per capita (174 kg per year). Eastern and Southern Africa uses 85% of maize production as food and Africa as a whole uses 95%. Some 90% of white maize consumption is in Africa and Central America (International Institute of Tropical Agriculture (IITA, 2009). Maize is processed into a wide variety of traditional and modern food products. Like any other food plant, seeds are the major storage organs. The maize kernel is made up of 72% starch, 10% protein and 4% lipids. The nutritional composition however is not balanced as maize lacks other essential components that are required by the body.

For instance, maize kernels lack the essential amino acids lysine and tryptophan, and is also deficient in ascorbic acid (vitamin C), B-vitamins, iron, and iodine (Nuss and Tanumihardjo, 2010). Food products made from maize include breads, porridges such as amahewu, steamed, roasted products, beverages and snacks. While white maize grain is staple, it is also devoid of provitamin A carotenoids (Li *et al.*, 2010). Due to the wide consumption of white maize, it has been used as a vehicle for biofortification. However, regardless of these efforts, white maize still remains the most preferred corn of choice as Provitamin A-biofortified maize is often labelled a “poor man’s food” (Muzhingi *et al.*, 2008). Hence, one way of promoting the consumption of provitamin A-biofortified maize is to process it into different products such as amahewu.

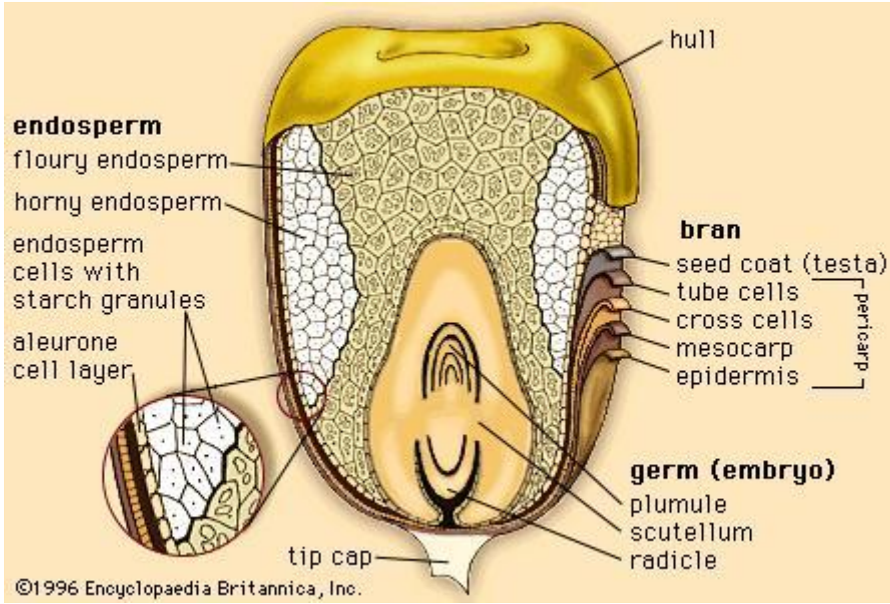


Fig 2.3: Structure of maize kernel (Encyclopaedia, 1996)

2.4.1 Biofortification

Strategies that have been used to address protein and vitamin A deficiencies include food diversification (FAO, 1997), fortification of food with indispensable amino acids, supplementation with good quality protein, improvement of protein quality by plant breeding and genetic engineering, and minimizing the damage to the nutritional value of protein during food processing and storage (Friedman 2004). Cereals constitute the most suitable vehicle for delivering proteins to at-risk populations because of their widespread consumption, stability and versatility (Bulusu *et al*. 2007). In developing countries where a single cereal is often the primary staple, they contribute 70 to 90% of the total dietary protein (Lasztity, 1984). The production of novel cereal-based food products designed to provide additional proteins to the daily diet has increased (Vitali *et al*. 2008).

Biofortification is the development of nutritionally richer foods through cross breeding as well as modern plant biotechnology (Nestel *et al*., 2006). Biofortification has been used extensively to improve the micronutrient contents in foods. Different plants have been biofortified with different minerals and nutrients in an effort to improve their nutritional quality. The biofortified sorghum using protein is also an effort to combat protein energy malnutrition. This has been achieved through chemically induced gene mutation and genetic engineering. A protein digestibility-corrected amino acid score (PDCAAS) for this variant of sorghum was found to be twice that of the normal sorghum (Taylor and Taylor, 2011). According to Li *et al.* (2010), biofortification programme targets the poor, vulnerable groups living in remote rural areas. One of the most important advantages of biofortification is that it is cost-effective (Nestel *et al.,* 2006). Research currently focuses on iron, zinc and provitamin A, which are three micronutrients that have been identified as limiting by the World Health Organization (Ortiz-Monasterio *et al.,* 2007). The food fortification approach, to fortify food with essential nutrients, is a highly effective strategy to address micronutrient deficiency in developing countries (UNICEF).

In order to combat vitamin A deficiency in developing countries, there has been need to develop crops that are rich in the micronutrient (Richardson, 1990). Such crops like the provitamin A maize containing provitamin A carotenoids will be a milestone in alleviating VAD in developing countries (Kristof, 2010). Provitamin A carotenoids are highly sensitive and can be destroyed by factors such as heat, light and oxygen.

Previous research has been done in order to evaluate the retention levels of the carotenoids contained in provitamin A-biofortified maize after various processing methods. Li *et al.* (2007) reported a retention level of 75.5% for fermented maize porridges and a 75.2 % for unfermented porridges. Awobusuyi *et al.,* (2016) also reported a 90% retention of carotenoids after processing in amahewu samples. A study carried out by (Mugode *et al*., 2014) on the retention levels of carotenoids after various methods of processing indicated that milling had no effect on the retention levels of carotenoids. However, cooking the maize meal into thin and thick porridges actually increased the retention levels whereas milling the maize into samp decreased the retention levels. These results agree with results obtained by(Pillay *et al*., (2014) who stated that the retention levels of β-carotene were much higher in maize meal than in samp. Pillay also indicated that cooking the maize into thin porridge and samp increased the beta carotene retention levels. Moreover, the levels of retention in the biofortified maize is dependent on the maize variant available. A study carried out by Bengtsson *et al*., 2008 on effects of traditional processing methods on orange-fleshed sweet potato indicated that boiling, steaming and deep frying reduced the carotenoid retention of the sweet potato. These studies indicated that processing indeed affects the retention levels of beta carotene in plant foods. Therefore, methods that possess high carotenoid retention levels should be recommended. Although biofortification of plants has not fully reach its maximum potential, it could be a solution to PEM and micronutrient deficiency. This is because families will be able to consume consistent and regular amounts of nutrients from the usually consumed staple foods (Nestel *et al*., 2006).

Biofortification through genetic modification will eventually be a cheap process as the germplasm can be distributed worldwide. Another benefit of biofortified crops is that they are highly sustainable and therefore nutritionally improved varieties will continue to be grown and consumed year after year. Biofortification will be able to reach even the most undernourished populations (Nestel *et al.*, 2006). Fig 2.4 shows types of provitamin A maize achieved through biofortification.

A (Yellow) B (Orange)

(Source: CIMMYT)

Fig 2.4: Provitamin A biofortified maize types

2.4.2 Food fortification

Food fortification has been defined as the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purposes of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups (Joint and Additives, 2000). Other terminology exists for the addition of nutrients to a food. Words such as restoration, which is the addition to a food of essential nutrients that are lost during the manufacturing or processing of a food product have been used. Enrichment has also been used interchangeably with fortification.

The addition of micronutrients to foods has been used as one of the ways of combating prevalent deficiencies such as PEM and VAD particularly in Africa. Although staple foods have generally been used as vehicles in food fortification, other accessory foods such as salt fortified with iodine, margarine fortified with vitamin A and milk fortified with vitamin D have also been used to deliver these essential nutrients to the body (Calvo and Whiting, 2003). Micronutrients such as iodine, iron and different vitamins have been added to various foods to improve their nutritional value. Essential amino acids have also been added to foods as they are important because they cannot be synthesised by the body (Hurrell, 1997).

The fortification of foods has a number of advantages in comparison to other interventions aimed at preventing and correcting demonstrated deficiencies. Fortified foods generally contain amounts of micronutrients that approximate to those provided by a balanced diet (Holick *et al.*, 1992). Fortified foods are able to reach a wide range of consumers hence is able to improve the nutritional status of vulnerable populations. The consumption of fortified foods does not require a change in consumption patterns and is therefore a guaranteed method of successfully delivering micronutrients (WHO, 2006). Many methods of food fortification have been adopted and these include mass fortification, market driven fortification, target fortification among others. Mass fortification is the addition of one on more micronutrients to foods commonly consumed by the general public such as staple foods and cereals. This form of fortification is mandated by the government and is a sustainable means of delivering micronutrients (WHO, 2006). Targeted fortification is the addition of micronutrients to foods consumed by a specific sub-group of a population such as school children or pregnant women. Market-driven fortification is a voluntary initiative taken by a food manufacturer to add one or more micronutrients to a food in order to increase sales. Although this form of fortification is regulated by the government, it is not a sustainable micronutrient delivery system as it only reaches a population that is able to buy the product (WHO, 2006).

2.4.3 Supplementation by nutritionally richer foods

Cereal based foods are generally deficient in essential amino acids required by the body. As such, the use of cereals-legumes based foods has been advocated as an alternative protein and energy source for infants, young children as well as adults. Cereals are deficient in lysine and tryptophan (Baker *et al.*, 2010). Legumes are deficient in sulphur containing amino acids such as cysteine. The combination of these two crops complement each other to produce a nutritionally richer product (Aloys and Angeline, 2009).

In a research carried out by Ayo *et al*., 2014, bambara groundnut flour was added to acha based fura, which is a semi solid dumpling cereal usually made from pearl millet. Acha (*Digitaria exilis*) is a rice which is native to West Africa. The Bambara groundnut flour was added at 10% and the results obtained showed that the protein content of the composite meal increased from 6.14% to 9.3%. This increase was attributed to the addition of Bambara groundnut which is known to have substantial amounts of protein (Ayo *et al.*, 2014). Ibrahim *et al*., (2005) reported on the supplementation of fermented sorghum flour using whey protein. The whey protein was added at 3.25% dry matter basis. After fermentation, the results obtained showed that the protein content of the fermented sorghum increased by 40%. This was attributed to the addition of whey protein as well as the fermentation process which is known to enhance the nutritional value of foods.

Adelekan and Oyewole, 2010 carried out an investigation on the supplementation of ogi with soybean flour and concluded that the protein content of the composite flour increased. Work also carried out by (Aminigo and Akingbala, 2004) on the fortification of maize with okra seed (defatted and roasted) at 20% inclusions indicated that the protein content increased by 122% for defatted flour and 106% for roasted flour.

Baker *et al*., (2010), investigated the effect of soybean protein on sorghum and concluded that the protein content of the composite flour increased by two fold.

Mariam, (2005), carried out a research in the formation of a composite blend weaning porridge using dehulled rice, groundnut, bambara groundnut and carrot in the ratios 60:20:10:10% w/w basis. In comparison to a commercial porridge, Nestle Cerelac, it was found that the composite blend porridge contained more fibre, fat and energy than the commercial porridge. Also, the protein content of the composite blends were significantly higher than that of the commercial porridge. However, since amahewu is a fermented product, it is important to review the effect of fermentation of food products.

2.5 Nutritional potential of bambara groundnut

Legumes contribute significantly to the African diet. Grain legumes serve as cheaper sources of protein, particularly in Africa where meat protein is expensive. Bambara groundnut is a legume of African origin, first found in West Africa (Fig 2.5) (Swanevelder, 1998). Its occurrence has spread from the Sahara to Southern Africa and is also found in North Africa (Yagoub and Abdalla, 2007). Beyond Africa, Bambara has also been found in India, Brazil, Syria, Greece and Thailand and is cultivated by subsistence farmers.

Bambara groundnut is a herbaceous annual legume and grows for not more than 20cm in height. It belongs to the Fabacea family, subfamily *Papilionodease.* (Mkandawire, 2007);(Murevanhema and Jideani, 2013). Bambara groundnut is also known as nyimo in Shona, dithloo in Setswana, indlubu in Ndebele (Murevanhema and Jideani, 2013). The legume has two variants, *Vigna subterranean* variety *spontanea* is the wild variety whilst *Vigna subterranean* variety *subterranean* is the domesticated one (Swanevelder, 1998).

Bambara groundnut falls in the same family as peanuts *(Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Mkandawire, 2007). Bambara groundnut pods develop and mature underground or may be slightly visible above the ground. When unripe, the pods are yellowish in colour and gradually turn brown when maturing and become wrinkled upon drying (Nti, 2009a). The seeds are rounded and have a smooth surface. They are small with an approximate diameter of 2cm. The seeds can be identified with patterns and colours present on their testa. These include mottled, blotched, stripped and some have a hilum whilst other varieties do not have (Nti, 2009a). A hilum is commonly known as an "eye." The edible seeds vary in colour depending on the breed. Some seeds are cream in colour, others are maroon whilst others have speckles of cream, brown and maroon (Nti, 2009b). Bambara groundnut produces papilionaceous yellow flowers which emanate from the nodes of the stem.

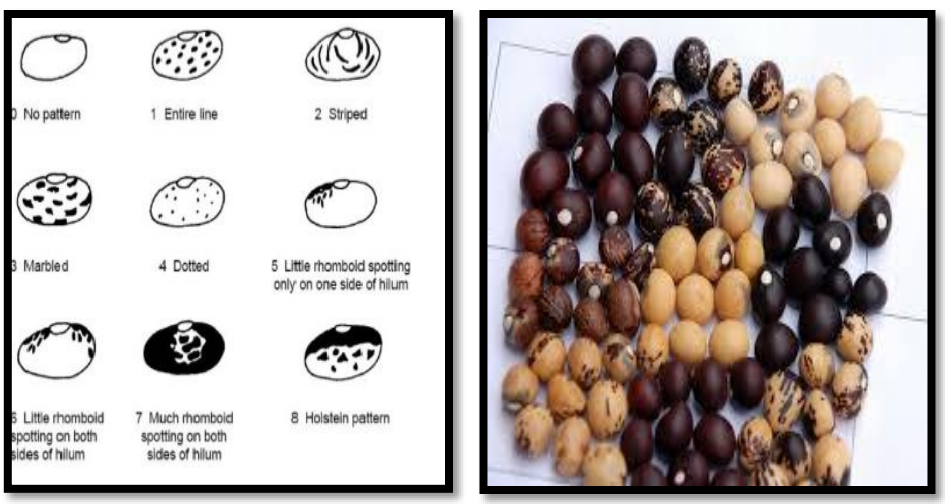


Fig 2.5: Physical appearance of BGN

Source: Google pictures

Bambara groundnut is a drought tolerant plant that is adapted to grow and survive in hostile environments of low rainfall. The available landraces have the capacity to tolerate biotic and abiotic stresses. Compared to other legumes, bambara groundnut often yields well in areas of low agricultural input (Baryeh, 2001). As such, subsistence farmers, who are the majority of the farmers are assured of a harvest even in cases of uncertain rainfall. Africa's hope now depends on such crops as bambara especially after the emergence of global warming and the unending cases of drought (Murevanhema and Jideani, 2013).

2.5.1 Production and utilization of Bambara groundnut

Bambara groundnut is an important legume in most African countries. It serves as a cheap source of protein particularly in developing countries where meat protein is expensive (Yusuf *et al.*, 2008). Bambara groundnut is farmed mainly for subsistence by rural farmers, a large percentage of them being women (Mkandawire, 2007). The optimum time of planting of this legume is November to December. Harvest time is between April and June. Bambara groundnut usually take 100-140 days to mature depending on the cultivar and climatic conditions. The time of harvesting of the crop is not critical compared to other legumes. This is because bambara groundnut can be harvested at the "green mature" stage or at the "fully mature" stage. The major bambara groundnut producer in Africa is Zimbabwe, followed by Nigeria, Ghana and Ivory Coast (Swanevelder, 1998).

Bambara groundnut has been underutilised in Africa and the world as a whole. This is because of lack of knowledge amongst the subsistence farmers as well as lack of research amongst the responsible agriculture authorities. The legume has many uses, from food for human consumption, animal feed, pharmaceuticals as well as in agriculture. Bambara groundnut is mainly farmed for its edible seeds which can be consumed in various ways. The immature seeds can be boiled and consumed as a salted snack (Bvochora *et al.*, 1999). The seeds can also be consumed, boiled and salted as appetisers (Ayo *et al*., 2014). In East Africa, the groundnuts are roasted, pulverized and used to make soup with the aid of spices. In South Africa, the groundnuts are cooked with maize and then pounded into a thick and sticky dough. This is known as "dithaku" in Sesotho. In Sudan, the seeds are pounded into flour and made into a stiff porridge (Yagoub and Abdalla, 2007). Roasted seeds can be crushed and mixed with other vegetables for consumption as relish. Bambara groundnut has also been used in the production of vegetable milk (Murevanhema and Jideani, 2013). In comparison with the recently emerging soymilk, it appeared that the

Bambara groundnut was preferred for its colour (Mkandawire, 2007). Commercial canning of the seeds in a gravy has been successful in Ghana.

Apart from being consumed as food, Bambara groundnut has known pharmaceutical properties. It has been found that drinking water that remains after boiling Bambara groundnut helps in treating diarrhoea. In Nigeria, the Igbos tribe are said to use Bambara ground nut seeds in the treatment of venereal diseases. The water obtained after boiling the seeds, can be helpful in treating internal bruising (Jideani and Diedericks, 2014). Pregnant women also chew the raw bambara seeds in order to alleviate the nausea associated with pregnancy. Mixing the crushed seeds and water can be used to treat cataracts, anaemia as well as ulcers (Jideani and Diedericks, 2014). Because bambara groundnut can thrive in harsh conditions, it then becomes very useful due to its ability to fix nitrogen into the poorly fed soils.

Many parts of Africa suffer drought spells year after year, and because of the economic restrains, fertilisers are not always available to the general population. Therefore, the use of such legumes as bambara can help in improving the fertility of the soil and thus contributing to the food security in Africa (Dakora and Keya, 1997).

2.5.2 Nutritional composition of bambara groundnut

Bambara groundnut is considered a balanced food because it contains a balanced content of carbohydrates, minerals, protein, ash (Oluwole and Taiwo, 2009). Although bambara groundnut has been underutilised, its nutritional value is comparable to that of other legumes. However, the exact nutritional quantities remain debatable. A proximate composition analysis carried out by Amarteifio and Moholo (1998), showed that Bambara groundnuts contain 4.4% ash, 63.5% carbohydrates, 5.2% fibre, 18.3% protein and 6.6% fat. A study carried out by Yusuf *et al.*, (2008) on the chemical composition of Bambara groundnut showed that the range of values obtained agreed to those of Amarteifio and Moholo, (1998).

Research carried out by Oluwole and Taiwo, 2009, showed that Bambara groundnut contained 1.63% moisture, 20% crude protein, 2% ash, 1.9% fibre, 68.5% carbohydrates and 6.0% fat. Based on the previous research done on this particular legume, it can be derived that proteins are present in Bambara groundnut. A study carried out by Adebowale *et al*., (2011) on two varieties of the Bambara groundnut showed that the protein content of the white variety contained 29.5% crude protein whereas the brown variety contained 30.4% crude protein. Although the quantities may differ from research to research, the valuable conclusion is that bambara groundnut is a source of protein which can be used to improve the health of those who consume the legume. The nutritional value of Bambara groundnut is comparable to that of other legumes. According to (Adebowale and Lawal, 2004), soybean contains 36.5% protein, cowpea has 23.8%, bambara groundnut has 20.8%, chickpea has 19.8% and kidney bean has 23.6% of protein. Moreover, the protein composition of bambara groundnut has been found to contain a more balanced methionine content than any of the legumes (Oluwole and Taiwo 2009). Table 2.1 shows the amino acid composition of the two legumes, soya bean and bambara groundnut. A comparison with soya bean protein was selected because amahewu has previously been fortified with soya bean protein.

Table 2.1: Comparison of Amino Acids in Bambara Groundnut Flour and Soya Bean Flour.

Amino Acid	BGF amino acid	SBF amino acid	RDA Values
Lysine	3.02	5.5	4.2
Histidine	2.27	2.3	2.4
Arginine	3.81	6.7	2.0
Aspartic acid	4.89	10.2	
Threonine	2.58	3.3	2.6
Serine	-	4.6	
Glutamic acid	15.83	16.4	
Glycine	3.60	4.5	
Proline	-	3.7	
Alanine	3.38	3.6	
Cystine	0.61	1.1	
Valine	-	4.4.	4.2
Methionine	1.78	1.2	
Isoleucine	3.81	4.3	4.2
Leucine	6.73	7.2	4.8
Tyrosine	3.11	3.3	
Phenylalanine	4.43	4.6	
Tryptophan	N.D	1.1	1.4

Where BGF: Bambara groundnut flour, SBF: Soya bean flour

Adopted from (BGF-(Oluwole Steve and Taiwo Ruth, 2009), SBF - (García *et al*., 1997).

However, the bioavailability of these nutrients is hindered by the presence of anti-nutritional factors in the Bambara groundnut such as phytic acid, tannins and polyphenols. If not appropriately treated, these anti-nutritional factors prevent the optimal utilisation of nutrients in the body.

2.6 Anti-nutritional factors and their effects on processed foods

Every living organism possesses some defensive mechanisms against factors that threaten its life. Anti-nutritional factors are one of the methods with which plants protect themselves against their predators, such as herbivores and insects. Kumar, (1992), defines anti-nutritional factors as substances generated in natural feed stuffs by the normal metabolism of species by different mechanisms such as inactivation of nutrients and thereby exerting effects contrary to optimum nutrition. Anti-nutritional factors are not inherently toxic but their toxicity is dependent upon the animal that is digesting them. This is because some anti-nutritional factors that cause deleterious effects in monogastric animals do not have an effect on ruminants as they are broken down in the rumen (Kumar, 1992).

Despite the detrimental effects of anti-nutritional factors, they have also been found to contain anti-cancer and anti-microbial properties but this is in variation with the amounts consumed (Khokhar and Chauhan, 1986). If consumed without treatment, either by humans or animals, these anti-nutritional factors are capable of causing adverse physiological effects (Khokhar and Chauhan, 1986). Huisman *et al.*, (2001) classified anti-nutritional factors on the basis of their effects on the nutritional value of feed stuffs as well as how ruminants respond to them in the gut. Anti-nutritional factors are divided into several groups depending on their mode of action and effects. Anti-nutritional factors with a depressive effect on the protein digestion and the utilisation of protein. Into this class falls the protease inhibitors, tannins, saponins and lectins.

Protease inhibitors are responsible for the reduction of trypsin and chymotrypsin activity which may result in impaired growth (Aletor and Adeogun, 1995). Another class of anti-nutritional factors is the anti-nutritional factors that affect the digestion of carbohydrates. These are grouped into amylase inhibitors, phenolic compound and flatulence factors. Other factors affect mineral utilisation and these factors such as phytates, oxalates and glucosinolates (Thorpe *et al*., 2001).

Some anti-nutritional factors have actually been reported to be poisonous to health. Glycoalkaloids in potatoes have been associated with gastrointestinal upsets and neurological disorders. Tannins bind dietary protein and digestive enzymes forming complexes that are not readily digestible (Deol and Bains, 2010). Phytates are responsible for the binding of minerals such as calcium, iron, magnesium and zinc. Oxalates such as phytates also bind minerals as well as interfering with metabolism. In extreme cases, very high concentrations can result in muscular weakness and paralysis (Deol and Bains, 2010).

Although not much is known about the anti-nutritional factors in legumes, they also have the same effects of diminishing the bioavailability of nutrients for assimilation in the body. Raw kidney beans are known to contain anti-vitamin E compounds which has been evidenced by liver necrosis in rats (Soetan and Oyewole, 2009).

Uncooked soybean has also been found to contain high levels of vitamin $B_{12.}$ Other components that are present in legumes include alkaloids which have been reported to cause foetal malformation particularly in animals (Mulvihilli, 1972; Soetan and Oyewole, 2009). Some plant alkaloids are reported to cause infertility and glycoalkaloids have been reported to cause haemolysis in humans (Aletor and Adeogun,1995). The total destruction or partial removal of these anti-nutritional factors is dependent on how heat-liable they are. The correct application of heat and other processes such as soaking, roasting and germination have been found to reduce the effect of the anti-nutritional factors in legumes and thus their assimilation in the body (Liener, 1962). Work carried out by (Yagoub and Abdalla, 2007), he concluded that roasted bambara groundnuts had the lowest content of tannins and the germinated seeds had the lowest amount of phytic acid. This is however contrary to what Oluwole and Taiwo, (2009) found. The difference in results could be due to use of different germination and roasting times and temperatures. Oluwole and Taiwo, (2009) also recorded an increase in crude protein from 20.00 to 20.20 after roasting and germination increased the crude protein from 20.20 to 20.24.

Although information is limited on the effects of germination and roasting of bambara on nutritional value, other methods of pre-treatments have been reviewed and they indicate that pre-treatment processes do have a bearing in improving the nutritional value of foods. A study carried out by Deol and Bains, (2010) showed that pressure cooking increased the protein digestibility of cereals, and further indicated that cereals that were pressure cooked for longer periods (3 minutes) showed improved digestibility than cereals pressure cooked for 1 minute which led to the conclusion that general cooking methods such as boiling also had an effect on improving nutritional value. The extent to which nutritional improvement occurred was also attributed to the form in which the heat was applied. Wet heat, in the form of boiling and pressure cooking was found to significantly increase the nutritional value of cereals as compared to dry heat methods Wang *et al.*, (1997).

These results were in agreement to the findings by Tromp *et al.*, (1995) who indicated that elevated temperatures and longer holding times significantly destroyed the trypsin inhibitors present in soybean. The results also indicated that the use of a heat transfer medium such as water also contributes to the extent to which anti-nutritional factors are destroyed (Egounlety and Aworh, 2003).

From the knowledge obtained by the various literature reviewed, processing is indeed helpful in reducing anti-nutritional factors. Although the methods of analysis differ as well as the processing controls, it can be concluded that a combination of these methods would be effective in reducing the anti-nutritional factors. The consumption of legumes through the use of these traditional processing methods can significantly contribute to alleviating food security in Africa.

2.7 Fermentation as a method of enhancing the nutritional value of foods

Fermentation is an ancient method used in the preservation and production of food particularly in Africa and Asia (Mensah, 1997). Chelule *et al*.,(2010) defines fermentation as a desirable process of biochemical modification of primary food products brought about by micro-organisms and their enzymes.

Apart from being a cheap and economic method of processing foods, fermentation is responsible for improving the nutritional quality of foods and this becomes very helpful especially with the fermentation of cereals which generally have a poor nutritional value (Svanberg and Lorri, 1997). There are different ways by which the fermentation process can affect the nutritional quality of foods and these include improving the nutrient density and increasing the quantities as well as the bioavailability of nutrients. The latter may be achieved by degradation of anti-nutritional factors, pre-digestion of certain food components, synthesis of promoters for absorption and by influencing the uptake of nutrients by the mucosa (Svanberg and Lorri, 1997). It has been shown that fermentative bacteria are very useful in the conversion of vitamins to functional components like D-pantothenic acid (Mehta *et al*., 2012). Fermentation reduces the amount of anti-nutritional factors in cereals leading to increased bioavailability of nutrients and micronutrients such as Fe, Zn, Ca and Mg to the body (Chelule *et al*., 2010). It achieves this by providing an optimum pH for enzymic degradation of phytates in the cereals.

A study carried out by Sanni *et al*., (1999) on the nutritional value of soybean and cereal composites indicated that fermentation of the composite flour increased the crude protein level by 25%. The fermented composites also had higher levels of vitamins as well as amino acids but there was a general decrease in the amount of alanine, proline and glutamic acid. Oluwole and Taiwo, (2009) indicated that fermentation increased the protein content of bambara groundnut seeds from 20.00 to 20.49. Towo *et al.*, (2006) also carried a similar study on the fermentation and enzyme treatment of tannin sorghum gruels. From the results obtained, fermentation resulted in a decrease in the amount of phytates and phenolic compounds and this caused an increase in in-vitro accessibility of iron in tannin sorghum gruels. Although limited information is available on how fermentation improves the nutritional value of cereals, a number of scholars believe that the decrease in phenolic compounds during fermentation could be due to the acidic environment that may result in the abstraction of hydride ions and rearrangement of the phenolic structures (Beta *et al*., 2000). Another group of scholars also believe that fermentation results in reduced extractability of the phenolic compounds which may be due to self-polymerisation and integration of macromolecules such as proteins (Ezeji and Ojimelukwe, 1993). However, the valuable conclusion still remains; fermentation can be used to improve the nutritional value of cereals.

2.7.1 Safety of fermented food products

Fermented foods are those which have been subjected to the action of micro-organisms or enzymes, so that the desirable biochemical changes cause significant modification to the food. Fermented foods constitute a major portion of human diets all over the world and provide 20-40% of the total food supply (Abdel *et al*., 2009). Fermented foods, unlike non-fermented foods, have a longer shelf-life, making fermentation a key factor in the preservation of such foods (Nyanzi and Jooste, 2012). The most common organisms responsible for fermentation of foods are acid-forming bacteria such as lactic acid bacteria (LAB) as, for example, *Lactobacillus*, *Lactococcus*, *Leuconostoc, Enterococcus, Streptococcus, Aerococcus* and *Pediococcus* (Chelule *et al.,* 2010, Agarry *et al.,* 2010).
Most pathogenic micro-organisms found in food cannot survive the low pH of fermented foods. The fermentation of food has been found to reduce the risk of pathogenic micro-organisms growing in the food (Abdel *et al.,* 2009). However, there have been reported cases of persistent pathogens in fermented foods (Colak and Hampikyan, 2007; Ijabadeniyi, 2007; Dineen *et al.,* 1998). Fermentation leads to the significant lowering of anti-nutrients of cereal products (Oyewole and Isah, 2012a). Although fermentation improved, to some extent, the nutritional quality and safety of maize-based food products, some including those fermented, are still deficient in micronutrients such as amino acids and vitamins, which calls for the need for fortification.

2.8 Effects of compositing on sensory acceptance of cereal based food products

Addition of nutritionally richer foods to cereal-based foods can affect the sensory properties of a food. There is need therefore to conduct sensory evaluations whenever addition is done. Although no sensory evaluations on fortified amahewu could be found, literature will be reviewed on effect of compositing on other cereal-based foods.

Onyango (2011) added cowpea to a sorghum prepared dish (injera) and found out that the viscosity of the porridge had decreased. This however did not affect the acceptance of the product by consumers. Ayo, (2014) conducted a sensory analysis after adding bambara groundnut flour to acha-based fura which is a fermented product made from a native rice acha (*Digitaria exilis).* The sensory evaluation, in comparison to the ordinary product indicated that the acceptance mean score for taste, flavour and colour had increased by 1.1. However, the acceptance decreased upon further addition of the bambara groundnut flour. Ayo (2014), concluded that the general acceptability of the product increased at 10% addition of bambara but decreased beyond that. A study by (Sanni *et al*., 1999) on the sensory acceptability of compositing soybean and cereal indicated that the composite was rated above average and was more preferred to the cereal counterpart. This was attributed to the flavour conferred by the soybean. Compositing can therefore have different effects on the sensory acceptance of a product. This mainly depends on the nature and type of raw materials used as well as the processing methods used.

2.8.1 Effects of fermentation on the sensory attributes of food products

Sensory acceptance of a product is mainly based on the physical attributes of a product. The way in which products are produced have a bearing on the appearance of the final product. Fermentation processes are able to give completely new structures, sensory characteristics as well as rheological properties to foods. The factors that determine this are microbiological purity of the strain used, composition and nature of the raw material as well as the processing parameters (Mehta *et al*., 2012). There are four different types of fermentation depending on the products produced and these are: lactic acid fermentation which is well known for producing sauerkrauts, yoghurts and fermented milks, alcoholic fermentation which is responsible for the production of wines, acetic fermentation which produces ciders and vinegars. Of major importance is lactic acid fermentation because it is the one used for production of important foods (Steinkraus, 1997, Mensah, 1997).

The flavour and aroma compounds of fermented foods come from the degradative processes undergone by the main components of the raw materials, proteins, lipids and carbohydrates. Proteolytic, lipolitic and glycolytic processes are therefore the main sources of the flavour and aroma compounds of fermented foods (Mugocha *et al*., 2000). Fermentative bacteria cause a lot of changes in the fermented product. The production of lactic acid by lactic acid bacteria (LAB) which then results in the desirable sour taste of fermented foods. LAB can also produce compounds such as acetyl, diacetyl as well as acetaldehyde (Mehta *et al*., 2012). Esters come from the esterification reactions which occur between short-to-medium chain fatty acids and alcohols. Ester production results in the formation of aromas that can be classified as fruity, rose, floral, honey, and apricot. Pyrazines, also produced through fermentation also contribute to nutty, roasty and toasty flavours in foods (Mehta *et al.,* 2012).

Fermentation is also responsible for the rheological changes in food. The rheological changes of fermented foods are also determined by the appropriate treatment of raw materials before and during fermentation. Lactic acid bacteria are able to produce polysaccharides called exo-polysaccharides which can act as thickening agents in foods. Most fermented products have desirable sensory attributes as compared to their unfermented counterparts (Svanberg and Lorri, 1997). Although the protein enrichment of maize-based foods has been done, its main focus has been on other legumes such as cowpeas and soybean. Bambara groundnut is under-utilised; it has been ranked the third most important legume. This is due to the legume’s ability to adapt to stressful conditions as well as its availability in the marginalised areas. Bambara groundnut can therefore be added to amahewu and hence aid in delivering proteins into the lives of those that cannot afford meat protein.

The figures associated with protein energy malnutrition all over the world are overwhelming. According to WHO (2007), protein energy malnutrition plays a role in approximately 5.4 million child deaths each year. Protein energy malnutrition is also responsible for magnifying the effect of every disease. According to Black *et al*., (2003) geographically, more than 70 percent of protein energy malnourished children live in Asia, 26 percent in Africa and 4 percent in Latin America and the Caribbean. In many cases, their plight began even before birth with a malnourished mother. Therefore, it can be concluded that protein energy malnutrition affects people of all age groups and therefore needs more attention to curb its effects. The protein content and quality of maize can be improved by using adding protein-rich legumes such as bambara, to alleviate PEM. There is a need to determine the effect of such improvement on protein nutrition. Therefore, this study investigated the effect of fortifying maize with bambara groundnut to improve the nutritional composition, *in vitro* protein digestibility, sensory characteristics and consumer acceptability of the product.

CHAPTER 3
METHODOLOGY

3.1 Introduction

Chapter 3 describes the methods used in this study. It covers the background information, study design, materials and methods, statistical analysis and ethical considerations.

3.2 Study design

3.2.1 Plant materials

Two maize types was used in this study: Provitamin A-biofortified maize and White maize (the control). Bambara groundnuts were obtained from Makhatini farm station, KwaZulu-Natal province of South Africa. Provitamin A-biofortified maize and white maize was obtained from UKZN. Bambara groundnut served as the protein source which was used to supplement amahewu.

3.2.2 Experimental design

The following parameters was considered: Solid/ Solvent ratio: 1 part of maize meal in 7 parts of water for the production of amahewu and 0.5% wheat bran as inoculum. This method was used based on the previous study done by Awobusuyi *et al.,* (2016). Different concentrations (10%, 20%, 30% and 50% of bambara flour were tested on amahewu, However for this research 30% defatted bambara flour was used as it was the most acceptable from the preliminary experiment carried out. Method for defatting bambara flour is shown in fig 3.1. The Bambara groundnuts were subjected to two traditional processing methods; roasting and germination (Fig 3.2).

3.3 Preparation of the bambara groundnut flours

The Bambara groundnuts (BGN) were partitioned into 3 batches, which are raw BGN, roasted BGN and germinated BGN (Fig 3.2).

3.3.1 Roasted bambara groundnut flour

Bambara groundnuts were graded, cleaned and soaked in warm water for 24 hours to allow easy de-hulling. The water was drained and the seeds de-hulled manually and allowed to dry in a hot air oven at 45^{o}C for 24 hours. The seeds were then roasted in an oven at 180^{o}C for 15 minutes. Thereafter the seeds were ground into flour and sieved using a 0.4 mm wire mesh screen.

3.3.2 Germinated bambara groundnut flour

Seeds were sorted, cleaned, graded and soaked in cold water for 24 hours. The seeds were germinated for 72 hours inside a jute bag with watering at 12 hours intervals to prevent mould growth. After germination, the seeds were thoroughly washed, drained and dried at 45^{o}C in an oven. Thereafter the seeds were ground into flour and sieved through a 0.4 mm wire mesh screen.

3.3.3 Raw Bambara groundnut flour

To produce raw flour, bambara groundnuts were manually dehulled and ground into flour. The dried seeds were then milled and sieved through a 0.4 mm wire mesh screen.

3.3.4 Defatting the flours

Bambara groundnut was defatted to bulk up the protein content in the resulting flours. Defatting of the three flours were done using hexane as the extraction reagent and stirring using the magnetic stirrer. The extractions were repeated twice in order to obtain a better yield. The extraction was done using 10 g/100 g w/v hexane. The defatted flours were air-dried at room temperature and the hexane evaporated for 24 hours overnight. The defatted flours were subsequently kept separately in air tight containers at 4^{o}C.

Mix sample with hexane in conical flask

↓

Stir for 1 hour, allow to set for30 minutes

↓

Decant hexane, repeat procedure twice

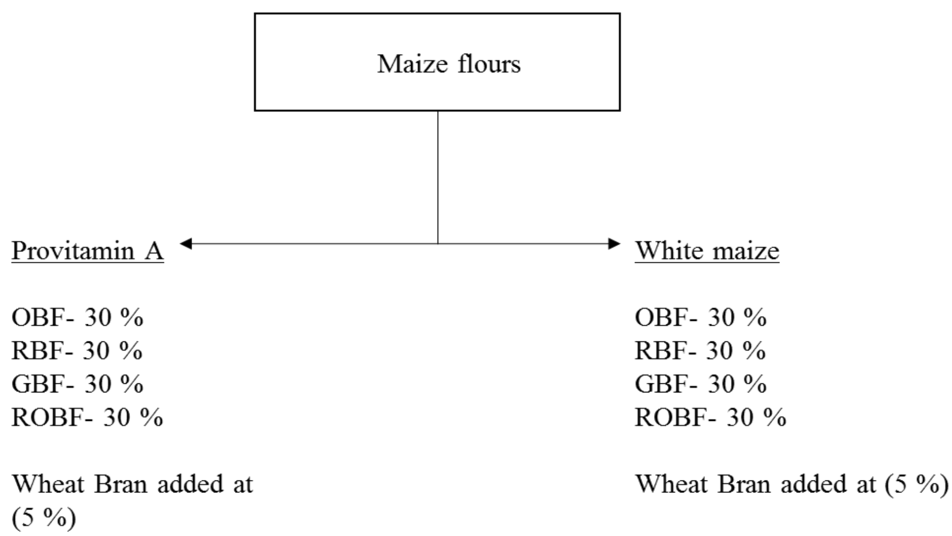
↓

Evaporate hexane for 24hours at room temperature

Fig 3.1: Process flow diagram for defatting flours.

3.3.5 Preparation of white and provitamin A maize flour

The white and provitamin A maize were ground and separately subjected to the same conditions in order to obtain a thin gruel of the maize. Amahewu samples were prepared according to a traditional method, which was described by regular consumers of amahewu in rural KwaZulu-Natal. The method involved adding one part of maize meal to seven parts of water and then boiling at 90°C, with occasional stirring, for 15 minutes. The resulting porridge was left to cool to approximately 40°C. The porridges were then sub-divided into 4 batches of each provitamin A-biofortified and white maize samples. To three samples of each maize type, the defatted bambara flours was added at 30% substitution as illustrated in Fig 3.2. To one of each maize type, no defatted bambara flour was added and this was fermented to act as controls for each maize type.



OBF- Ordinary bambara flour
RBF- Raw bambara flour
GBF- Germinated bambara flour
ROBF- Roasted bambara flour

Fig 3.2: Flow diagram showing partitioning of maize samples.

3.3.6 Fermentation of maize samples

After the partitioning, four samples of provitamin A-biofortified maize and four of white maize were obtained. Each sample was divided into two. Addition of wheat bran (W) at 0.5% was done to all the samples, including provitamin A maize porridges, as illustrated above (Fig 3.2). The inoculum was added based on percentage of solids present. The samples with wheat bran were fermented at 30ºC for 20 hours. The flasks were periodically shaken and pH was taken at 6 hours intervals.

3.4 Nutritional analysis

Amahewu samples was pasteurized at 63°C for 30 minutes to stop the fermentation process and thereafter, allowed to cool before analysis. The nutritional composition of all amahewu samples were determined using standard methods.

3.4.1 Ash

Ash was determined by combusting the samples in a furnace set at 550ºC for four hours, following the AOAC Official Method 923.03 (AOAC, 2005). The crucibles were accurately weighed and their mass recorded. Three to five-grammes of the sample was weighed, and a 7ml glycerol/methanol mixture was added to each dish and allowed to wet all the particles. The crucibles were placed on a hot plate, under a fume hood and the temperature was slowly increased. The matter in the crucibles was ignited and burnt until all the organic material has volatilised. The sample was ashed in a muffle furnace at 550 ºC for four hours. The sample was placed in a desiccator and allowed to cool for 1 hour. The sample was then weighed and the mass of the residue determined and expressed as a percentage of the whole sample. The following equation was used to calculate the percentage of ash in the sample.

$$\% \text{ ash} = \frac{\text{mass of ash (g)}}{\text{mass of sample (g)}} * 100$$

3.4.2 Protein

In a clean, dry digestion tube, 3-5 grammes of sample was weighed. Accurately, 4 grammes of a catalyst was also added together with 25 ml concentrated H_2SO_4. The digestion tubes were connected to NaOH trap, which is connected to a suction tube. The vacuum was used to draw the fumes into the NaOH trap to absorb the noxious fumes. The unused opening was closed using a cotton plug and heating commence and maintained as the sample boiled. The digestion was allowed to proceed for approximately 1-2 hours and digestion completed when the solution turned light clear green.

3.4.2.1 Procedure

The Buchi 321 distillation unit was switched on and allowed to pre-heat. The digested sample was put into the digesting vessel. The holder was pressed downwards and released when the tube was in place. The sample was diluted in water in ratio 1:3. In an Erlenmeyer flask, 25 ml of 2% boric acid and 6 drops of methyl red indicator was added. The Erlenmeyer receiving flask was placed under long tubes. Sodium hydroxide (32%) was added to the sample until it turned dark brown. The distillation time was set between 2.5 to 3 minutes as the distillation proceeded. The residue aspirations switch was switched on so that at the end of distillation, the residues was distilled into the sink. The distillate was titrated with standard 0.1 N sulphuric acid. The end point was reached when the light blue solution turned colourless to pale pink.

The protein content is calculated by: $\% \text{ N} = \dfrac{\text{titration in ml} - \text{blank in ml} * 1.4801}{\text{Mass of sample}}$

$$\% \text{ Protein} = 6.25 * \% \text{ N}$$

3.4.3 Total carbohydrate content

Carbohydrates were analysed based on the difference.

Carbohydrates % = (moisture % + fat % + protein %) *100

3.4.4 Fat

The fat content of the samples was determined according to the Soxhlet procedure, using a Büchi 810 Soxhlet Fat Extractor (Büchi, Flawil, Switzerland), according to the AOAC Official Method 920.39C (AOAC 2002). A 250ml round bottom flask was weighed, cooled in a desiccator and the mass was recorded, 5 grams of sample was also weighed and the mass was recorded. The sample was transferred to an extraction thimble and the thimble was placed in an extraction chamber. The condenser was connected to the chamber and the tap opened to allow for steady flow through the condenser. The heating mantle was turned on to a low or medium temperature setting. The fume cupboard extractor fan was turned on. The sample was extracted by refluxing the solvent for at least 5 minutes then the solvent was stopped via the condenser chamber as soon as the solvent level drops to below the reflux chamber level. The heating mantle was switched off just before all of the solvent evaporates out of the round bottom flask. The round bottom flask was not allowed to dry up as this would cause the fat extract to char at the bottom and thus affect the final recording. The flask containing the extract was allowed to cool in the desiccator for 3 to 4 hours and the mass was recorded. The percentage of fat in the sample was calculated using the formula:

$$\% \text{ fat} = \frac{\text{weight of residue (g)}}{\text{weight of sample (g)}} * 100$$

Note: weight of residue= original sample mass – mass of fat extract

3.4.5 Moisture content

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

3.4.6 Individual mineral elements

Mineral content was determined according to the AOAC method no. 6.1.2 (AOAC 1984), using the Inductively Coupled Plasma (ICP) Spectroscopy. Ground samples of each amahewu sample were acid-digested by addition of 1 mL of 55% (v/v) HNO3.

3.4.7 Amino acid analysis

The amino acid profile of the samples was analysed by the Waters API Quattro Micro Method, which consists of a column C18, 1.7 µm, 2.1 x 100 mm and a binary solvent manager. Samples (400 mg) were subjected to Waters AccQ Tag Ultra Derivatization kit; 10 µL of the undiluted sample were added to the Waters AccQ Tag kit constituents and placed in a heating block at a temperature of 55^{O}C for 10 min. Injection volume was 1µL.

3.4.8 *In-Vitro* protein digestibility

According to the method of Bruce Hamaker (1987). Amahewu sample, 0.2 g was weighed; 35 mL of 0.1 M phosphate buffer; pH 2 containing 1.5 mg pepsin/mL was added. Pepsin-sample mixture was incubated at 37^{O}C for two hours, with continuous shaking. Digestion was stopped by adding 2 mL of 2 M NaOH. The suspension was centrifuged at 4800 rpm at 4^{O}C for 20 minutes and the supernatant was discarded. The residue was washed with 15 ml of 0.1 M phosphate buffer, pH 7, and centrifuged. Again the supernatant was discarded and the residue washed on Whatman's No 3 filter paper. The filter paper containing the undigested protein residue was folded and placed in a digestion tube and dried for two hours at 80^{O}C. The dried sample was analysed for protein, using the micro kjeldahl method.

3.5 Sensory analysis

3.5.1 Preparation of amahewu samples

Amahewu samples were prepared according to a traditional method, which was described by regular consumers of amahewu living in rural KwaZulu-Natal (section 3.3.5). The method involved adding one part of maize meal to seven parts of water and then boiling at 90^{O}C, with occasional stirring, for 15 minutes. The resulting porridge was left to cool to approximately 40^{O}C. To three of each of the maize samples (both provitamin A and white maize samples), was added the pre-treated, defatted bambara flours at 30% substitution as illustrated in Fig 3.2. To one of each maize sample, no defatted bambara flour was added and this was fermented to act as controls for each. Wheat bran (W) was added at 0.5% concentration to porridges and these were allowed to ferment at 37^{O}C. The provitamin A biofortified maize meal was used to prepare the test amahewu samples, while the white maize meal was used to prepare the reference amahewu samples.

3.5.2 Consumer acceptability evaluation

Consumer acceptability was carried out among regular consumers of amahewu, between the ages of 18 and 45. They were recruited from the University of Kwazulu Natal, Howard college campus. To ensure reliable data, before sensory evaluation, a session was held to explain to panellists the importance of the study and the evaluation procedure, including how the sensory attributes of amahewu were to be evaluated. Individual consumers (panellists) evaluated the products based on the following acceptability attributes: aroma, mouth feel, taste, colour and overall acceptability. The amahewu samples were evaluated using a nine-point hedonic rating scale (1=dislike extremely; 9=liked extremely). Refrigerated Amahewu samples were served in polystyrene cups. The samples were labelled with three-digit codes obtained from a table of random numbers and were served in a random order, which was determined using a table of random permutations of nine. Each panellist was provided with water to cleanse the palate between samples.

3.5.3 Statistical analysis

Mean acceptability scores were computed. One-way analysis of variance (ANOVA) was done; and the mean separation was by Fisher Least Significance Difference (LSD) test

($p < 0.05$). Principal component analysis (PCA) was used to determine the similarity and difference in the acceptability of amahewu products.

CHAPTER 4

RESULTS

Chapter 4 describes the results of the study, in which provitamin A-biofortified maize and white maize complemented with 30% defatted, germinated, roasted and raw bambara were tested and analysed for their nutritional, In-vitro protein digestibility and consumer acceptance.

4.1 Nutritional composition of bambara added amahewu

The protein content increased substantially in amahewu samples composited with bambara flours (Table 4.1). The protein content of amahewu almost doubled with the inclusion of bambara at the 30% level of substitution. Pre-treating bambara by germination and roasting also significantly influenced the protein contents of the resulting amahewu samples. Amahewu composited with germinated bambara flour (AGBF) showed the highest increase in protein for both provitamin A and white maize amahewu samples. Maize type did not have any major influence on protein levels of amahewu. However, as expected, amahewu without bambara had the lowest protein content for both provitamin A and white maize amahewu samples.

Carbohydrate was the major nutrient of amahewu samples ranging from 63-83%. The inclusion of bambara slightly decreased the carbohydrate content of amahewu samples. The carbohydrate content did not vary with the type of maize used in the preparation of amahewu. Both provitamin A and white maize amahewu recorded the highest carbohydrates content. The fat and ash contents in amahewu samples were generally low without the addition of bambara. Pre-treating bambara by germinating and roasting had no significant effect on the ash and fat content of the Bambara-containing amahewu samples.

Table 4.1: Proximate composition of bambara added amahewu (g/100 g, db) [1]

Samples Provitamin A	*CHO	Protein	Fat	Ash	Moisture
AROBF-Y	$73^{c} \pm 0.79$	$29.7^{b} \pm 0.25$	$0.06^{c} \pm 0.01$	$0.03^{a} \pm 0.01$	$3.16^{b} \pm 0.03$
AGBF-Y	$63^{a} \pm 1.12$	$34.3^{d} \pm 0.23$	$0.08^{a} \pm 0.01$	$0.04^{a} \pm 0.1$	$3.14^{a} \pm 0.03$
ARBF-Y	$69.6^{b} \pm 0.85$	$31^{c} \pm 0.31$	$0.06^{b} \pm 0.03$	$0.03^{b} \pm 0.05$	$3.17^{b} \pm 0.01$
AWB-Y	$83.3^{d} \pm 0.32$	$13.6^{a} \pm 0.4$	$0.05^{b} \pm 0.04$	$0.02^{a} \pm 0.01$	$3.2^{c} \pm 0.02$
White maize products					
AROBF-W	$68^{b} \pm 0.69$	$24^{b} \pm 0.14$	$0.05^{c} \pm 0.01$	$0.02^{a} \pm 0.01$	$3.16^{b} \pm 0.03$
AGBF-W	$63.5^{a} \pm 1.12$	$32.3^{c} \pm 0.23$	$0.06^{a} \pm 0.01$	$0.03^{a} \pm 0.1$	$3.21^{c} \pm 0.02$
ARBF-W	$68.6^{b} \pm 1.09$	$28.6^{b} \pm 0.31$	$0.06^{bc} \pm 0.01$	$0.03^{b} \pm 0.01$	$3.15^{a} \pm 0.01$
AWB-W	$82.8^{c} \pm 1.45$	$14^{a} \pm 0.14$	$0.05^{b} \pm 0.04$	$0.02^{a} \pm 0.01$	$3.18^{b} \pm 0.02$

*CHO- Carbohydrate calculated by difference
Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test.
AROBF-amahewu +roasted bambara flour
AGBF-amahewu +germinated bambara flour
ARBF-amahewu +raw bambara flour
AWB- amahewu without bambara. Y means (Yellow provitamin A biofortified maize) W means (White maize)

4.1.2 Mineral composition of bambara added amahewu

The levels of individual mineral elements in the amahewu samples composited with 30% bambara flour are presented in Table 4.2. Major minerals in amahewu samples were potassium followed by magnesium. The addition of bambara in the preparation of amahewu increased some minerals, especially the zinc and iron contents. The iron content of composited amahewu (34-24 mg/ 100 grammes samples) was slightly higher compared to unfortified Provitamin A-biofortified maize and white maize amahewu and so was the zinc level. Some differences were observed with the different pre-treatments. Amahewu samples containing the germinated bambara had a slightly high level of these micronutrients compared those of roasted bambara, which suggests that germination and roasting of bambara may be effective in boosting the mineral profile of amahewu. When comparing the two types of maize, amahewu made with provitamin A maize had a slightly higher content of minerals, including iron, zinc, potassium and magnesium.

Table 4.2: Mineral composition of bambara added amahewu (mg/100 g, db)

Selected Minerals	Provitamin A Products				White Maize products			
	AROBF (Y)	**ARBF (Y)**	**AGBF (Y)**	**AWB (Y)**	**AROBF (W)**	**ARBF (W)**	**AGBF (W)**	**AWB (W)**
Fe	34^{a}	32^{a}	38^{a}	20^{b}	27^{b}	24^{b}	31^{a}	20^{b}
Zn	32^{a}	33^{a}	36^{a}	20^{b}	29^{a}	23^{b}	28^{a}	18^{b}
K	6960^{c}	6060^{c}	8710^{a}	4055^{d}	7660^{b}	6100^{c}	7720^{b}	4950^{d}
Mg	5520^{b}	4310^{c}	6640^{a}	4630^{c}	4590^{c}	4300^{c}	5250^{b}	4960^{c}
Na	55^{d}	80^{a}	77^{a}	72^{b}	59^{d}	46^{e}	67^{c}	74^{b}
P	210^{b}	187^{c}	254^{a}	182^{c}	205^{b}	180^{c}	212^{b}	212^{b}

AROBF-amahewu +roasted bambara flour
AGBF-amahewu +germinated bambara flour
ARBF-amahewu +raw bambara flour
AWB- amahewu without bambara
Y means (Yellow provitamin A biofortified maize)
W means (White maize)

4.1.3 Amino acid composition of bambara added amahewu

The amino acid profile of amahewu samples composited with 30% bambara flour is presented in Table 4.3. The major amino acids in all amahewu samples were glutamic and aspartic acid, which may include glutamine and asparagine. The addition of bambara significantly influenced the amino acid profile of the resulting amahewu samples. The major effect of adding bambara was noted on lysine, which almost doubled with the addition of bambara. Lysine is an essential amino acid that is generally known to be deficient in cereals. When comparing the lysine content to the WHO requirement for infant, lysine accounted for 60% of the recommended intake for children compared to 50% with no bambara added which indicates about 10% increase at 30% level of inclusion of bambara. The lysine content of composited amahewu accounted for 100% requirements for adults. The effect of pre-treating bambara on lysine contents was also noted. Amahewu composited with germinated bambara flour recorded the highest increase as germination has been reported to improve amino acid content of cereals. Further, the types of maize used in the preparation amahewu had a major effect on the amino acid profile. In general, provitamin A-biofortified maize samples prepared with added bambara flour showed the highest increase when compared to their white maize counterparts. Lysine, an essential amino acid which is generally known to be deficient in cereals increased considerably. This apparent increase of lysine could be attributed to the addition of bambara flour. .

Table 4.3: Amino acid profile of bambara added amahewu (g/100 g protein)

Amino Acid g/100g	AWB-Y	ARBF-Y	AROBF-Y	AGBF-Y	AWB-W	ARBF-W	AROBF-W	AGBF-W	FAO/WHO recommended pattern Preschool 2-5 yrs	Adults
Histidine	1.5	2.1	1.7	2.9	0.8	1.3	1.5	2.2	1.9	1.6
Serine	2.7	3.9	3.0	5.5	1.6	2.3	2.9	3.7		
Arginine	2.5	4.1	2.8	5.0	1.3	2.2	2.7	3.2		
Glycine	1.9	2.8	2.2	3.8	1.2	1.7	2.1	2.8		
Aspartic Acid	3.9	4.9	4.1	7.7	1.9	3.3	4.5	4.5		
Glutamic A	8.9	11	9.9	19	5.8	7.8	10.6	14		
Threonine	1.8	2.6	2.0	3.7	1.1	1.6	1.9	2.7	3.4	0.9
Alanine	3.2	4.4	3.6	6.9	2.4	2.8	3.8	5.7		
Proline	3.6	5.2	4.2	8.4	2.7	3.3	4.2	6.8		
Lysine	2.0	2.6	2.8	3.2	0.8	1.7	1.9	1.9	5.8	1.6
Tyrosine	1.5	2.9	1.8	3.3	1.0	1.4	1.7	2.2		
Valine	2.4	3.3	2.7	4.7	1.4	2.0	2.5	3.4	3.5	1.3
Isoleucine	1.9	2.7	2.1	3.8	1.0	1.5	2.0	2.5	2.8	1.3
Leucine	5.5	8.1	6.3	12	3.9	4.8	6.4	9.2	6.6	1.9
Phenylalanine	2.7	4.2	3.1	5.7	1.5	2.3	3.0	3.6	6.3	1.9

Where: AROBF-amahewu +roasted bambara flour
AGBF-amahewu +germinated bambara flour
ARBF-amahewu +raw bambara flour
AWB- amahewu without bambara flour
Y means (Yellow provitamin A biofortified maize) W means (White maize)
FAO/WHO (1989) recommended pattern (pre-school children aged 2-5 years; adults)

4.2 *In-vitro* protein digestibility

The in-vitro protein digestibility data are presented in Figure 4.1. In general, the addition of bambara significantly increased the protein digestibility of amahewu. The protein digestibility of amahewu (65%) increased by almost 45% with the inclusion of bambara. Pre-treating bambara seemed to have a major effect on protein digestibility of amahewu. Amahewu composited with germinated bambara flour recorded the highest IVPD increase. The type of maize used also seemed to influence the protein digestibility. Amahewu made with provitamin A maize generally showed a high percent digestibility compared to their white maize counterparts. This was followed by the roasted amahewu. Amahewu without bambara flour recorded the least increase in both provitamin A and white maize samples.

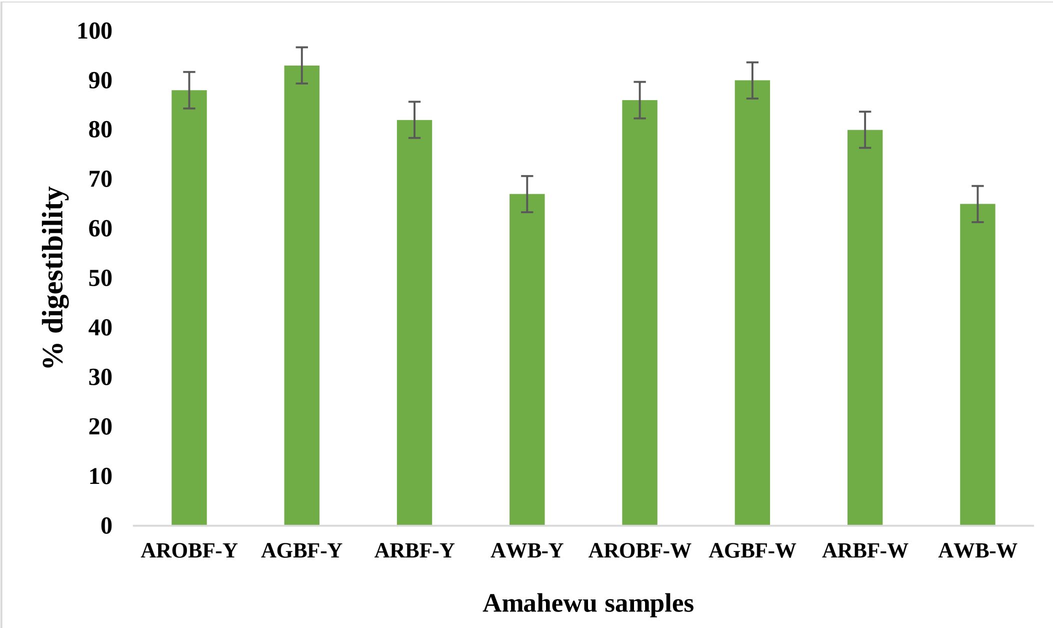


Fig 4.1: Protein digestibility of bambara added amahewu samples

AROBF-amahewu +roasted bambara flour

AGBF-amahewu +germinated bambara flour

ARBF-amahewu +raw bambara flour

AWB- amahewu without bambara

Y means (Yellow provitamin A biofortified maize)

W means (White maize)

4.3 Consumer acceptability of bambara added amahewu

The consumer acceptability data are shown in Table 4.4. Generally, the addition of bambara did not negatively affect the sensory acceptability of amahewu. The type of maize used in the preparation of amahewu had a major effect on the acceptability of the products. In general, provitamin A-biofortified amahewu samples composited with bambara flour were all /or very much more liked when compared to their white maize counterparts. Provitamin A amahewu composited with germinated bambara flour (AGBF) was the most liked for its colour, whilst amahewu with roasted bambara flour (AROBF) was the most liked for taste, aroma and overall acceptability. There was no significant difference in the mouthfeel of all the samples and the products were equally rated (Table 4.4). Provitamin A and white maize amahewu samples composited with raw bambara (ARBF) was the least liked for its taste. This could be attributed to the beany flavour associated with bambara. White maize amahewu samples composited with germinated and roasted bambara flour were also more acceptable compared to their counterparts. Amahewu without bambara (control) was the least liked in terms of aroma. Overall, the addition of pre-treated bambara flour to amahewu improved the sensory qualities of amahewu.

Principal component analysis (PCA) data are shown in Figure 4.2a,b. The two components accounted for 98% of the total variation in the sensory attributes data (Fig 4.2a,b). The first component explained 96% of the variation and provitamin A amahewu samples on the left were clearly differentiated from white maize amahewu samples on right side of the loading plot. Yellow colour and taste of roasted and germinated provitamin A amahewu samples were somehow strongly perceived compared to those of white maize amahewu samples. As described above, the taste and colour of amahewu made with provitamin A biofortified maize were extremely liked compared to their white maize counterpart.

Table 4.4: Consumer acceptability of bambara added amahewu

Samples	Colour	Taste	Aroma	Mouthfeel	Overall Acceptability
AROBF-Y	7.7 ± 0.6^{b}	8.1 ± 0.4^{b}	8.2 ± 0.5^{b}	6.8 ± 0.5^{a}	$8.6 \pm 0.6^{a,b}$
AGBF-Y	8.1 ± 0.7^{a}	7.7 ± 0.7^{a}	8.0 ± 0.6^{a}	6.9 ± 0.6^{a}	8.4 ± 0.6^{a}
ARBF-Y	7.7 ± 0.6^{b}	7.2 ± 0.7^{b}	7.5 ± 0.5^{c}	6.9 ± 0.4^{a}	8.3 ± 0.4^{b}
AWB-Y	7.3 ± 0.6^{c}	7.5 ± 0.7^{c}	7.0 ± 0.5^{d}	6.4 ± 0.4^{b}	8.0 ± 0.4^{c}
White Maize products					
AROBF-W	6.3 ± 0.4^{d}	6.3 ± 0.4^{d}	6.0 ± 0.4^{g}	6.2 ± 0.8^{d}	6.9 ± 0.5^{e}
AGBF-W	6.2 ± 0.6^{d}	6.1 ± 0.5^{d}	6.5 ± 0.5^{e}	6.1 ± 0.6^{d}	6.7 ± 0.4^{d}
ARBF-W	6.3 ± 0.5^{d}	5.8 ± 0.6^{e}	6.3 ± 0.6^{f}	6.6 ± 0.4^{c}	$6.6 \pm 0.5^{d,e}$
AWB-W	6.2 ± 0.4^{d}	6.3 ± 0.5^{d}	5.9 ± 0.6^{g}	6.4 ± 0.5^{c}	$6.5 \pm 0.8^{d,e}$

Mean ± SD (n=70)
Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test.
AROBF-amahewu +roasted bambara flour,
AGBF-amahewu +germinated bambara flour,
ARBF-amahewu +raw bambara flour
AWB- amahewu without bambara.
Y means (Yellow provitamin A biofortified maize), W means (White maize)

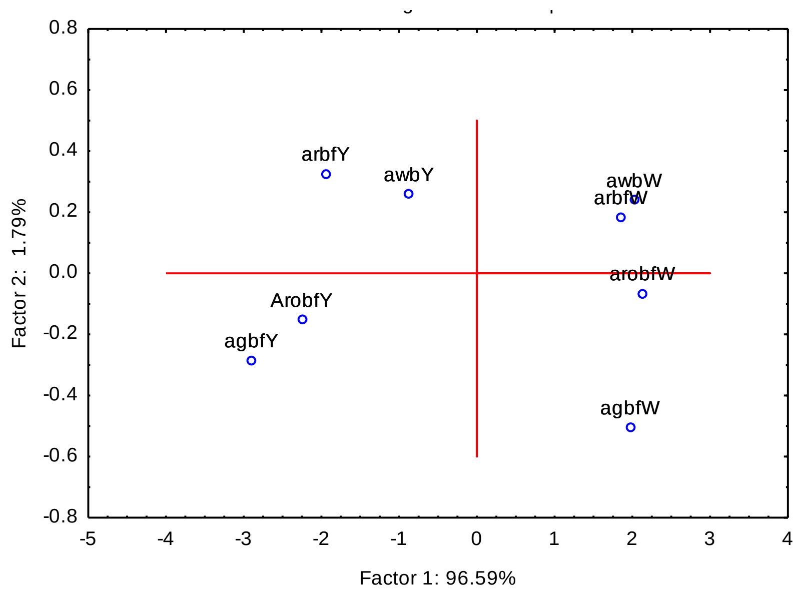


Fig 4.2a: Principal component analysis (PCA 1) of bambara added amahewu samples

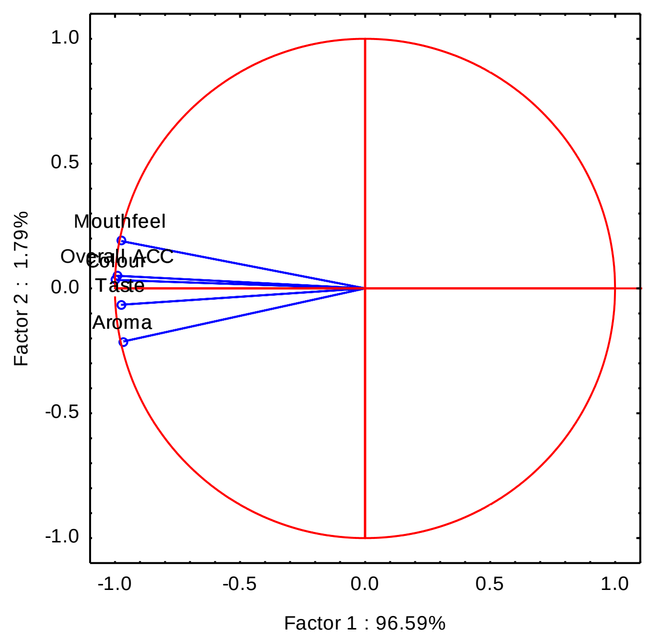


Fig 4.2b: Principal component analysis (PCA 2) of bambara added amahewu samples

CHAPTER 5

DISCUSSION

This section interprets the findings of this study and the results shown in chapter 4.

5.1 Nutritional composition of bambara added amahewu

Protein deficiency is a major problem in developing regions, particularly in Sub-Saharan Africa (Muller and Krawinkel 2005). Protein vital to human life, its deficiency can cause very serious health conditions, and even death.

The protein content of amahewu composited with bambara flour increased substantially after fermentation. Both provitamin A-biofortified and white maize amahewu composited with germinated bambara (AGBF) showed a higher protein increase. Previous studies reported that germination leads to the synthesis of enzymatic proteins (Sanni *et al.,* 1999). Proteins contribute to cell growth, repair and maintenance, act as enzymes and hormones, maintain fluid, electrolyte and acid base balance and also maintain a strong immune system by supporting the increased production of antibodies in response to common infections such as colds, flu or allergic reactions (Thompson *et al*., 2008). Amahewu samples with added bambara flour significantly increased when compared to samples without bambara. Bambara groundnut has been reported to contain a substantial amount (29-30 %) of protein (Adebowale *et al.*, 2011, Yusuf *et al*., 2008). These results suggested that bambara could effectively be used in complementation with cereals for improved protein. Hence could be applied to combat protein energy malnutrition.

An increase in protein content was accompanied by a decrease in carbohydrate content of amahewu samples. Micro-organisms utilise carbohydrates as an energy source during fermentation and produce carbon dioxide as a by-product. This causes the nitrogen in the fermented product to be concentrated and the proportion of protein in the total mass increases (Onyango, 2004). Although, there was a slight increase in the ash and fat content of amahewu samples, the values were still low. The low fat content could have been due to the oxidation of fat for energy production by inherent organisms. Furthermore, low fat and moisture contents are necessary for the storage quality of the product.

5.1.2 Mineral composition of bambara added amahewu

Results obtained showed that the addition of 30% bambara improved the mineral content of amahewu. Provitamin A biofortified amahewu samples recorded the highest increase in mineral content among all samples. Amahewu composited with germinated bambara flour (AGBF) had the highest mineral content for both provitamin A and white maize samples when compared to other samples. The zinc and iron contents increased considerably after fermentation. Micronutrient deficiencies affect about two billion people in the world. Globally 740 million people are micronutrient deficient, about two billion people are deficient in zinc and one billion have iron deficiency anaemia (Muller and Krawinkel, 2005). Apart from being responsible for how insulin is stored and released, zinc is also directly involved in the way it works. Zinc is essential for wound healing and for many other basic metabolic processes, forming an integral part of a number of enzymes as well as playing an integral role in many other essential functions within the body (Evans, 2010). The effect of pre-treatments such as the roasting and germination process of bambara could also have contributed to the increase. This result agrees with the work done on fermented maize composited with bambara (Mbata *et al.*, 2009) and fermented porridges such as uji and ugali composited with cowpea (Anyango *et al.,* 2011b). Processing methods such as germination and fermentation have been shown to destroy anti-nutritional factors such as phytates which are responsible for binding the minerals (Thorpe *et al.,* 2001). Heat treatments such as roasting and frying have also been shown to destroy anti-nutritional factors in legumes (Huisman *et al.,* 2001), this may explain why amahewu composited with roasted bambara flour (AROBF) also recorded a high mineral content after amahewu composited with germinated bambara flour. The minerals become more assayable as the level of anti-nutritional factors decreases.

5.1.3 Amino acid composition of bambara added amahewu

The results showed an appreciable improvement in the amino acid profile of amahewu samples with the addition of bambara flour when compared to those without bambara flour.

Provitamin A biofortified amahewu samples with bambara flour showed the highest improvement in amino acid profile than their white maize counterparts (Table 4.3). There was a substantial increase in the lysine content of all composited amahewu samples. This is because bambara groundnut is very rich in lysine. Amahewu composited with germinated bambara flour (AGBF) recorded the highest increase for most essential amino acids including Lysine. Earlier studies have documented increased lysine after germination (Tsai *et al.,* 1975). This agrees with the finding of Wu and Wall (1980), who showed that germination of cereals followed by other processing techniques is essential to improving lysine content. Lysine is an essential amino acids which is vital for growth and maintenance of the body and are often limiting in some cereals (FAO, 1985; Asiedu *et al.,* 1993). The biological functions of lysine include; synthesis of connective tissues such as bone, skin, collagen, and elastin; synthesis of carnitine and resultant conversion of fatty acids to energy; support for healthy growth and development as well as the maintenance of healthy immune function, particularly with regard to antiviral activity This results showed that 30% bambara groundnut in addition to germination significantly improved the protein quality by elevating the levels of amino acids in amahewu samples and is in agreement with the report of Mbata *et al.* (2009), Chelule *et al.* (2010). As compared to FAO/WHO standard the concentrations of all the essential amino acids in all the provitamin A biofortified amahewu samples were generally higher than the pattern of amino acid requirements for adults and accounted for up to 60% for pre-school children.

5.2 *In-vitro* protein digestibility

The protein digestibility of amahewu with the addition of bambara groundnut was higher (approx. 93 %), when compared to raw amahewu (amahewu without bambara) (Figure 4.1). Similar improvement in protein digestibility, following the fermentation of maize gruel, has previously been reported (Awobusuyi *et al.,* 2016; Mohiedeen *et al*., 2010; Mardia *et al.*, 2002; Monawar 1983; Hasseltine, 1983). Amahewu composited with germinated and roasted bambara flour had higher in-vitro protein digestibility compared to other amahewu samples. The higher IVPD of amahewu composited with germinated bambara flour may be attributed to the effect of germination anti-nutrient factors. Previous studies have shown that germination could reduce the level of anti-nutritional factors in fermented foods thus increasing the bioavailability of proteins through the release of proteolytic enzymes and break down of polyphenols (Oluwole and Taiwo, 2009). Similar observations have been made on porridges composited with cowpea (Anyango *et al*., 2011b). Also, heat treatments such as roasting have also been reported to have an effect of reducing the anti-nutritional factors in legumes which could have enhanced the IVPD of amahewu (Oluwole and Taiwo, 2009). Partial degradation of storage proteins into more simple and soluble products could have also contributed to the increased IVPD (Mohiedeen *et al.* (2010). Monawar (1983) found that the reduction in pH during fermentation also enhances the activity of native proteolytic enzymes and consequently promotes the breakdown of proteins to smaller polypeptides which are easily digested by enzymes. Overall, an improvement on digestibility will lead to better protein absorption and retention in humans following the consumption of protein fortified amahewu with either roasted or germinated bambara flour.

5.3 Consumer acceptability of bambara added amahewu

The colour of provitamin A biofortified maize amahewu samples with added bambara flour were significantly liked than their white maize counterparts. Provitamin A amahewu composited with roasted bambara (AROBF) and germinated bambara (AGBF) were more acceptable than amahewu samples prepared without bambara (AWB). There was no significant difference between fortified and unfortified amahewu samples made with white maize (Table 4.4). Previous research found the colour acceptability of biofortified maize food products lower than their white maize counterparts (Pillay *et al*., 2011). The use of the provitamin A biofortified maize seems to have influenced the colour acceptability of the product in a good way. These are very promising findings, because previous research indicates that the unfamiliar colour was a major cause of low consumer preference for yellow maize compared to white maize (Pillay 2011; De Groote 2010; Kimenju 2008; Tshirely and Santos 1995; Stevens and Winter-Nelson 2008).

The taste acceptability of amahewu prepared using provitamin A-biofortified maize was more acceptable than that of white maize amahewu (Table 4.4). However, the taste of provitamin A biofortified amahewu sample with added roasted bambara (AROBF) was more preferred while amahewu composited with raw bambara (ARBF) was the least liked. Similar observations were made for white maize samples. However, panellists did not find any differences in the mouthfeel of all the samples and the products were rated similarly. General improvement of product quality after fermentation has been reported widely in the literature by Awobusuyi *et al* (2016); Holzapfel (1997); Chelule *et al.*, (2010); Larry *et al*., (1990). Amahewu made with provitamin A had higher acceptability scores for aroma compared to their white maize counterparts. However, amahewu composited with roasted bambara was more liked across all samples. Roasting has been reported to produce flavour and aroma compounds. Annan *et al.* (2003) and Leroy (2004) reported that fermentation improves the aroma of fermented maize products due to the release of aromatic compounds. Overall, provitamin A amahewu samples were more acceptable to consumers than their white maize counterparts.

Principal Component Analysis (PCA) was used to summarise the variation in the sensory attributes of amahewu samples. Figure 4.2 shows the projection of scores of amahewu and Figure 4.3 illustrates loading projections of sensory attributes. The two PCAs described 98% of the total variation in the sensory attributes data. The first principal component (PCA 1) accounted for 96% of the total variation. Provitamin A biofortified amahewu samples were differentiated from their white maize counterparts. PCA indicates that the sensory attributes mainly influencing the overall acceptability of amahewu were colour and aroma. It appears that these two sensory attributes largely influenced the overall acceptability of amahewu because they were intense, and at the same time, highly acceptable, which is the characteristic of fermented foods.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Overall, the hypotheses in this study was accepted. The results show that provitamin A-biofortified maize amahewu containing 30 % bambara flour is just as acceptable as white maize to consumers who regularly consume white maize amahewu.

• One of the objective of this study was to determine the effect that the addition of bambara groundnut flour will have on the nutritional quality of provitamin A-biofortified amahewu. The findings shows that there was an improvement in amino profile, especially an increase in the essential amino acid lysine content, and minerals, such as iron and zinc. This further suggests that amahewu containing bambara flour is much better, nutritionally, than amahewu without bambara. The lysine content of composited bambara/maize amahewu is nutritionally adequate for adults and fairly adequate for age groups lower than five years.

• This study also set out to determine the effect that the addition of bambara groundnut flour will have on the consumer acceptability of provitamin A-biofortified amahewu. The results indicate that consumers prefer provitamin A biofortified maize amahewu over white maize amahewu and, further, they prefer amahewu with the addition of germinated and roasted bambara over those without. Roasting of bambara improved the taste, aroma and overall acceptability of amahewu. The consumers used in this study have grown up in a cultural environment where white maize is accepted as the traditional food. The findings of this study suggest that there is an opportunity to change the cultural mind-set of preference for white maize.

• The study has demonstrated that, the addition of bambara flour with provitamin A biofortified maize, in the form of amahewu, has the potential to contribute to the alleviation of protein malnutrition among the targeted communities, especially the poor rural communities who are highly vulnerable to PEM.

6.1.2 Limitations of the study

- Shelf life of amahewu with the addition of bambara was not determined.
- The sensory evaluation could have been done to include weaning mothers and children of school going age.
- Defatting bambara groundnut could have reduced its energy content.
- Non-protein nitrogen could account for the increase in total protein content as this was not analysed separately.

6.2 Recommendations

Provitamin A biofortified maize with the addition of 30% bambara flour can be used to successfully produce amahewu. However, bambara groundnut needs to be pre-heated to inhibit anti nutrients present in legumes.

However, it is important that future research focus on:

•The effect of pre-treated bambara flour on the shelf life and microbial quality of amahewu over a period of time.

• Sensory acceptability should be carried out with a wider and more diverse range of consumer groups, to include infants, caregivers and weaning mothers. Consumer acceptability studies should be carried out using subjects from other provinces in South Africa and should include the popular maize foods consumed in the respective provinces.

REFERENCES

Adebowale, K. O. and Lawal, O. S. 2004. Comparative study of the functional properties of bambarra groundnut (Voandzeia subterranean), jack bean (Canavalia ensiformis) and mucuna bean (Mucuna pruriens) flours. *Food Research International*, 37**,** 355-365.

Adebowale, Y., Schwarzenbolz, U. and Henle, T. 2011. Protein Isolates from Bambara Groundnut (Voandzeia Subterranean L.) Chemical Characterization and Functional Properties *International Journal of Food Properties* 14**,** 758-775.

Adelekan, A. and Oyewole, O. 2010. Production of Ogi from germinated sorghum supplemented with soybeans. *African Journal of Biotechnology,* 9**,** 7114-7121.

Aletor, V. and Adeogun, O. 1995. Nutrient and anti-nutrient components of some tropical leafy vegetables. *Food Chemistry,* 53**,** 375-379.

Aloys, N. and Angeline, N. 2009. Traditional fermented foods and beverages in Burundi. *Food Research International,* 42**,** 588-594.

Alonso, R., A. Aguirre and F. Marzo, 2000. Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry,* 68: 159-165.

Amarteifio, J. O. and Moholo, D. 1998. The Chemical Composition of Four Legumes Consumed in Botswana. *Journal of Food Composition and Analysis,* 11**,** 329-332.

Aminigo, E. and Akingbala, J. 2004. Nutritive composition and sensory properties of ogi fortified with okra seed meal. *Journal of Applied Science and Environmental management*, 8,(2) 23-28.

Anyango, J. O., De Kock, H. L. and Taylor, J. 2011b. Impact of cowpea addition on the Protein Digestibility Corrected Amino Acid Score and other protein quality parameters of traditional African foods made from non-tannin and tannin sorghum. *Food chemistry*, 124**,** 775-780.

Asiedu M, Lied E, Nilsen R and K Sandnes. 1993. Effect of Processing (sprouting and/or fermentation on sorghum and maize II: vitamins and amino acid composition. Biological evaluation of maize protein. *Food Chemistry*; 48: 201 – 204.

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

Association of Official Analytical Chemists (AOAC) International 2005. Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Association of Official Analytical Chemists (AOAC) International 1984. Official Methods of Analysis, 14th edition. Association of Official Analytical Chemists. Arlington, Virginia, USA.

Awobusuyi, Temitope D., Amonsou, Eric O., Muthulisi Siwela and Unathi Kolanisi 2016. Provitamin A retention and sensory acceptability of amahewu, a non-alcoholic cereal based beverage made with provitamin A-biofortified maize. *Journal of the Science of Food and Agriculture*. 96:1356-1361.

Ayo, J. A., Popoola, C. A., Ayo, V. A. and Andrew, E. 2014. Effect of added Bambara groundnut flour on the quality of Acha based fura. *British Journal of Applied Science and Technology,* 4**,** 168-176.

Baker, A. A. B. A., El Tinay, A. H. and Yagoub, A. E. A. 2010. Protein content and digestibility of sorghum (Sorghum bicolor)-based fermented gruel: effect of supplementation with soybean protein. *Electronic Journal of Environmental, Agricultural & Food Chemistry,* 9.

Baryeh, E. A. 2001. Physical properties of bambara groundnuts. *Journal of food engineering,* 47**,** 321-326.

Bengtsson, A., Namutebi, A., Alminger, M. L. and Svanberg, U. 2008. Effects of various traditional processing methods on the all trans, β-carotene content of orange-fleshed sweet potato. *Journal of Food Composition and Analysis,* 21**,** 134-143.

Beta, T., Rooney, L., Marovatsanga, L. and Taylor, J. 2000. Effect of chemical treatments on polyphenols and malt quality in sorghum. *Journal of Cereal Science,* 31**,** 295-302.

Black, R. E., Morris, S. S. and Bryce, J. 2003. Where and why are 10 million children dying every year? *The Lancet,* 361**,** 2226-2234.

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D. and Webb, C. 2003. Cereal-based fermented foods and beverages. *Food Research International* 36**,** 527-543.

Boye, J.I. Aksay, S. Roufik, S. Ribéreau, S. Mondor, M. Farnworth, E. Rajamohamed, S.and Boye, H. 2010a. Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International.* 43, 537–546.

Boye, J.; Zare, F.; Pletch, A. 2010b. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research. International*. 43, 414-431.

Bulusu, S., Laviolette, L., Mannar, V., and Reddy, V. 2007. Cereal fortification programmes in developing countries. Pages 91-105 in: Issues in Complementary Feeding. C. Agostoni, and O. Brunser, eds. Nestlé Nutrition Workshop Series, Vol 60. Paediatric Programme, Nestec: Basel.

Bvochora, J. M., Reed, J. D., Read, J. S. and Zvauya, R. 1999. Effect of fermentation processes on proanthocyanidins in sorghum during preparation of Mahewu, a non-alcoholic beverage. *Process Biochemistry,* 35**,** 21-25.

Calvo, M. S. and Whiting, S. J. 2003. Prevalence of vitamin D insufficiency in Canada and the United States: importance to health status and efficacy of current food fortification and dietary supplement use. *Nutrition reviews,* 61**,** 107-113.

Chelule, P., Mokoena, M. and Gqaleni, N. 2010. Advantages of traditional lactic acid bacteria fermentation of food in Africa. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology,* 2**,** 1160-1167.

Colak, H. and Hampikyan, H. 2007. Presence of Listeria monocytogenes in Turkish style fermented sausage (sucuk). *Food Control* 18, 30-32

Dakora, F. D. and Keya, S. O. 1997. Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. *Soil Biology and Biochemistry,* 29**,** 809-817.

De Almeida Costa, G.E.; da Silva Queiroz-Monici, K.; Reis, M.; de Oliveira, S.M. 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cookedpea, commonbean, chickpea and lentil legumes. *Food Chemistry*, 94, 327–330.

Deol, J. K. & Bains, K. 2010. Effect of household cooking methods on nutritional and anti nutritional factors in green cowpea (Vigna unguiculata) pods. *Journal of Food Science and Technology,* 47**,** 579-581.

De Groote, H. Kimenju, S. C. and Morawetz, U. B. 2010. Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. *Agricultural Economics* 41: 116.

Dineen, Sean S., Takeuchi, Kazue., Soudah, Jane. 1998. Persistence of Escherichia coli O157:H7 in Dairy Fermentation Systems. *Journal of Food Protection* 12, 1597-1697.

Egounlety, M. 2002. Production of legume-fortified weaning foods. *Food Research International,* 35**,** 233-237.

Egounlety, M. and Aworh, O. C. 2003. Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna unguiculata L. Walp) and groundbean (Macrotyloma geocarpa Harms). *Journal of Food Engineering*, 56**,** 249-254.

Ezeji, C. and Ojimelukwe, P. 1993. Effect of fermentation on the nutritional quality and functional properties of infant food formulations prepared from bambarra-groundnut, fluted-pumkin and millet seeds. *Plant Foods for Human Nutrition,* 44**,** 267-276.

FAO/WHO/UNU, Expert consultation Energy and protein requirements.1985. WHO Technical Report Series, No 721, Geneva.

Faber, M. and Wenhold, F. 2007. Nutrition in contemporary South Africa. Water South Africa, 33(3):393-399. (Special Edition) 2007.

Food and Agriculture Organization (FAO) Of The United Nations (UN) and International Life Sciences Institute (ILSI). 1997. Preventing micronutrient malnutrition: a guide to food-based approaches. ILSI PRESS, Washington, USA. FAO CORPORATE DOCUMENT REPOSITORY

Food and Agriculture Organization (FAO) Of the United Nations (UN). 2009. Capacity Building for Nutrition Education. FAO. Available at: <http://www.fao.org/ag/humannutrition/nutritioneducation/49739/en/zmb/>.Date acessed 25/06/2016.

Friedman, M. 2004. Nutrition: Effects of food processing. Pages 328-340 in: Encyclopaedia of Grain Science. C. Wrigley, H. Corke, and C. E. Walker, eds. Elsevier: Oxford.

Furham M. P., Charney P., and Mueller C. M. 2004. Hepatic protein and nutrition assessment. *Journal of American Dietetic Association.* 104:1258-1264.

Gadaga, T. H., Mutukumira, A. N., Narvhus, J. A. and Feresu, S. B. 1999. A review of traditional fermented foods and beverages of Zimbabwe *International Journal of Food Microbiology* 53**,** 1-11.

García, M. C., Torre, M., Marina, L. M., Laborda, F. and Rodriquez, A. R. 1997. Composition and characterization of soyabean and related products. *Critical reviews in food science and nutrition,* 37**,** 361-391.

Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G. and Faria, J. A. 2010. Functional foods and nondairy probiotic food development: trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety,* 9**,** 292-302.

Ghavidel, R. A. and Prakash, J. 2007. The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *LWT-Food Science and Technology,* 40**,** 1292-1299.

Gibson, R. 2005. Principles of nutritional assessment. 2nd ed. New York: Oxford University Press.

Gibney, M. J., Vorster, H. H., and Kok, F. J. 2002. Human Nutrition. Blackwell Publishing: Oxford. Pp 46-62.

Grillenberger, M. 2006. Impact of animal source foods on growth, morbidity and iron bioavailability in Kenyan school children. PhD thesis, Wageningen University: Wageningen.

Guyot, J. P. 2012. Cereal-based fermented foods in developing countries: ancient foods for modern research. *International Journal of Food Science & Technology,* 47**,** 1109-1114.

Hamaker, B. R., Kirleis, A. W., Butler, L. G., Axtell, J. D. and Mertz E. T. 1987. Improving the invitro protein digestibility of sorghum with reducing agents. Proceedings of the National Academyof Science 84:626-628.

Hesseltine, C. 1983. The future of fermented foods. *Nutrition reviews,* 41**,** 293-301.

Holick, M. F., Shao, Q., Liu, W. W. and Chen, T. C. 1992. The vitamin D content of fortified milk and infant formula. *New England Journal of Medicine*, 326**,** 1178-1181.

Holzapfel, W., 1997. Use of starter culturesin fermentation on a household scale. *Food control*, 8, 241-258.

Huisman, J., Tolman, G., Garnsworthy, P. and Wiseman, J. 2001. Antinutritional factors in the plant proteins of diets for non-ruminants. *Recent Developments in Pig Nutrition 3***,** 261-322.

Hurrell, R. F. 1997. Preventing iron deficiency through food fortification. *Nutrition Reviews,* 55**,** 210-222.

Ibrahim, F. S., Babiker, E. E., Yousif, E. N. and El Tinay, A. H. 2005. Effect of fermentation on biochemical and sensory characteristics of sorghum flour supplemented with whey protein. *Food Chemistry,* 92**,** 285-292.

Ijarotimi, O. S. and Esho, T. R. 2009. Comparison of nutritional composition and anti-nutrient status of fermented, germinated and roasted bambara groundnut seeds (< IT> vigna subterranea</IT>). *British Food Journal,* 111**,** 376-386.

Jideani, V. A. and Diedericks, C. F. 2014. Nutritional, Therapeutic, and Prophylactic Properties of Vigna subterranea and Moringa oleifera. http://dx.doi.org/10.5772/57338. *Antioxidant-Antidiabetic Agents and Human Health*.

Jirapa, P., H. Normah, M.M. Zamaliah, R. and Asmah, K. Mohamad, 2001. Nutritional quality of germinated cowpea flour (Vigna unguiculata) and its application in home prepared weaning foods. *Plant foods for Human. Nutrition*., 56: 203-216.

Joint, Food and Additives, W. E. C. O. F. 2000. Evaluation of certain food additives and contaminants. *World Health Organization technical report series,* 896**,** 1.

Kar, B. R., Rao, S. L., and Chandramouli, B. A. 2008. Cognitive development in children with chronic Protein Energy Malnutrition. *Behavioural Brain Function.* 4:1-12.

Khokhar, S. and Chauhan, B. 1986. Antinutritional factors in moth bean (Vigna aconitifolia): varietal differences and effects of methods of domestic processing and cooking. *Journal of Food Science,* 51**,** 591-594.

Kristof, N. D. 2010. Biofortification. *International Herald Tribune*, 2010 Nov 26.

Kumar, R. 1992. Antinutritional factors. The potential risks of toxicity and the methods to alleviate them. *Legume trees and other fodder trees as protein source for livestock. FAO Animal Production and Health Paper***,** 145-160.

Labadarios, D. 2005. Malnutrition in the developing world: The triple burden. *South African Journal of Clinical Nutrition* (SAJCN), 18(2):119-121, September 2005.

Labadarios, D., Swart, T., Maunder, E.M.W., Kruger, H.S., Gericke, G.J., Kuzwayo, P.M.N., Ntsie, P.R., Steyn, N.P., Schloss, I., Dhansay, N.A., Jooste, P.L., Dannhauser, A., Nel, J.H., Molefe, D., and Kotze, T.J.V.W. 2008. National Food Consumption Survey: Fortification Baseline (NFCS-FB-1): South Africa, 2005. *South African Journal of Clinical Nutrition* (SAJCN), 21(3):254-267.

Lasztity, R. 1984. The Chemistry of Cereal Proteins. CRC Press: Boca Raton, FL. Pp 1-2.

Li, S., Nugroho, A., Rocheford, T and White, W. S. 2010. Vitamin A equivalence of the β-carotene in β-carotene–biofortified maize porridge consumed by women. *The American journal of clinical nutrition,* 92**,** 1105-1112.

Li, S., Tayie, F. A., Young, M. F., Rocheford, T and White, W. S. 2007. Retention of provitamin A carotenoids in high β-carotene maize (Zea mays) during traditional African household processing. *Journal of agricultural and food chemistry,* 55**,** 10744-10750.

Liener, I. E. 1962. Toxic factors in edible legumes and their elimination. *The American Journal of Clinical Nutrition,* 11**,** 281-298.

Mardia, E., Hag, E. I., Abdullahi, H., El Tinay, A . H. and Nabila E. Y. 2002. Effect of fermentation and dehulling on starch, total polyphenols, phytic acid content and invitro protein digestibility of pearl millet. *Food Chemistry* 77: 193–196.

Mariam, S. 2005. Nutritive value of three potential complementary foods based on cereals and legumes.

Marrero, L. M., Payumo, P. M. Aguinaldo, A. R., and Homma, S. 1988. Nutritional characteristics of weaning foods prepared from germinated cereals and legumes. *Journal of Food Science*. 53:1399-1402.

Mbata,T. I., Ikenebomeh, M. J and Ezeibe, S. 2009. Evaluation of mineral content and functional properties of fermented maize (Generic and specific) flour blended with bambara groundnut (Vigna subterranean L) .*African Journal of Food Science Vol. 3(4). pp.107-112*

Mehta, B. M., Kamal-Eldin, A. and Iwanski, R. Z. 2012. *Fermentation: Effects on Food Properties*, CRC Press.

Mensah, P. 1997. Fermentation- the key to food safety assurance in Africa. *Food Control,* 8**,** 271-278.

Mkandawire, C. H. 2007. Review of bambara groundnut (Vigna subterranea (L.) Verdc.) production in Sub-Sahara Africa. *Agricultural Journal,* 2**,** 464-470.

Mohiedeen, I. E., El Tinay, A. H., Elkhalifa, A. E. O., Babiker, E. E. and Mallasy, L. O. 2010. Effect of fermentation and cooking on protein quality of maize (Zea mays.) cultivars. *International journal of food science & technology,* 45(6), 1284-1290.

Monawar, L. Y. 1983. Food value of Sudanese Indigenous cereal grains. Ph.D. thesis. University of Khartoum, Sudan.

Mugocha, P. T., Taylor, J. R. N. and Bester, B. H. 2000. Fermentation of a composite finger millet dairy beverage. *World journal of microbiology and biotechnology,* 16**,** 341-344.

Mugode, L., Ha, B., Kaunda, A., Sikombe, T., Phiri, S., Mutale, R., Davis, C., Tanumihardjo, S. and De Moura, F. F. 2014. Carotenoid Retention of Biofortified Provitamin A Maize (Zea mays L.) after Zambian Traditional Methods of Milling, Cooking and Storage. *Journal of Agricultural and Food Chemistry,* 62**,** 6317-6325.

Müller, O. and Krawinkel, M. 2005. Malnutrition and health in developing countries. *Canadian Medical Association Journal,* 173**,** 279-286.

Muoki, P. N. 2013. *Nutritional, rheological and sensory properties of extruded cassava-soy complementary porridges.* University of Pretoria.

Murevanhema, Y. Y. and Jideani, V. A. 2013. Potential of Bambara Groundnut (Vigna subterranea (L.) Verdc) Milk as a Probiotic Beverage-A Review. *Critical Reviews in Food Science and Nutrition,* 53**,** 954-967.

Muzhingi, T., Lngyintuo, A. S., Malaba, C. L. and Banzinger, M. 2008. Consumer acceptability of yellow maize products in Zimbabwe. *Food policy,* 33**,** 352-361.

Nestel, P., Bouis, H. E., Meenakshi, J. and Pfeiffer, W. 2006. Biofortification of staple food crops. *The Journal of Nutrition,* 136**,** 1064-1067.

Nti, C. A. 2009a. Effects of bambara groundnut variety and processing on the quality and consumer appeal for its products. *International Journal of Food Science & Technology,* 44**,** 2234-2242.

Nti, C. A. 2009b. Effects of bambara groundnut (Vigna subterranea) variety and processing on the quality and consumer appeal for its products. *International Jjournal of Food Science & Technology,* 44**,** 2234-2242.

Nuss, E. T. and Tanumihardjo, S. A. 2010. Maize: A Paramount Staple Crop in the Context of Global Nutrition. *Comprehensive Reviews in Food Science and Food Safety,* 9**,** 417-436.

Oluwole S.I. and Taiwo R. E. 2009. Comparison of nutritional composition and anti-nutrient status of fermented, germinated and roasted bambara groundnut seeds (vigna subterranea). *British Food Journal,* 111**,** 376-386.

Onis, M., and Blossner, M. 1997. WHO database on child growth and malnutrition. Programme of Nutrition. World Health Organization: Geneva.

Onyango, C., Noetzold, H., Bley, T. and Henle, T. 2004. Proximate composition and digestibility of fermented and extruded uji from maize-finger millet blend. LWT - Food Science and Technology, 37 (8): 827-832.

Pandey, S. and Urquia, N. Alleviating hunger and poverty in Africa-Role of agricultural research and development. African Crop Science Conference Proceedings, 2007. 1843-1854.

Pillay, K., Siwela, M., Derera, J. & Veldman, F. J. 2014. Provitamin A carotenoids in biofortified maize and their retention during processing and preparation of South African maize foods. *Journal of Food Science and Technology***,** 1-11.

Plahar, W., Leung, H. and Coon, C. 1983. Effects of dehydration and soy fortification on physicochemical, nutritional and sensory properties of Ghanaian fermented maize meal. *Journal of Food Science,* 48**,** 1255-1259.

Rahma, E.H., 1988. Functional and electrophretic charcteristics of faba bean (Vicia faba) flour proteins as affected by germination. Nahrung, 32: 577-583.

Richardson, D. P. 1990. Food Fortification. *Proceedings of the Nutrition Society,* 49**,** 39-50.

Rodriguez- Amaya, D. 2000. Studies on various aspects of carotenoids. Sight for Life. Newsletter 3/2000, pp. 30.

Sanni, A. I., Onilude, A. A. and Ibidapo, O. T. 1999. Biochemical composition of infant weaning food fabricated from fermented blends of cereal and soybean. *Food Chemistry*, 65**,** 35-39.

Shewry, P. R., Napler, A. J. and Tatham, A. S. 1995. Seed storage proteins: Structure and biosynthesis. *The plant cell*, 7**,** 945-956.

Shetty P. 2006. Malnutrition and undernutrition. Medicine 34:524-529

Simango, C. 1997. Potential Use Of Traditional Fermented Foods For Weaning In Zimbabwe *Social Science,* 44**,** 1065-1068.

Simpson, H.C.R.; Lousley, S.; Geekie, M.; Simpson, R.W.; Carter, R.D.; Hockaday, T.D.R. and Mann, J.I. 1981. A high carbohydrate leguminous fibre diet improves all aspects of diabetic control. Lancet 317, 1–5.

Singh, V. and West, K. 2004. Vitamin A deficiency and xerophthalmia among school-aged children in Southeastern Asia. *European Journal of Clinical Nutrition,* 58**,** 1342-1349.

Soetan, K. and Oyewole, O. 2009. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science,* 3**,** 223-232.

Steinkraus, K. H. 1997. Classification of fermented foods: worldwide review of household fermentation techniques. *Food Control,* 8**,** 311-317.

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

Stone, H., Bleibaum, R. and Thomas, H. A. 2012. *Sensory evaluation practices*, Academic press.

Stipanuk, M. H. 2006. Protein and amino acid requirements. Pages 419-448 in: Biochemical Physiological, Molecular Aspects of Human Nutrition. M. H. Stipanuk, eds. Saunders Elsevier: St. Louis, MO.

Svanberg, U and Lorri, W. 1997. Fermentation and nutrient availability. *Food Control,* 8**,** 319-327.

Swanevelder, C. 1998. Bambara–food for Africa. *National Department of Agriculture, Government Printer, Republic of South Africa*.

Swapnil S. Patil, Margaret A. Brennan, Susan L. Mason and Charles S. Brennan.2016. The Effects of Fortification of Legumes and Extrusion on the Protein Digestibility of Wheat Based Snack.

Taylor, J. and Taylor, J. R. 2011. Protein biofortified sorghum: effect of processing into traditional African foods on their protein quality. *Journal of Agricultural and Food Chemistry,* 59**,** 2386-2392.

Thorpe, J., Beal, J., Bedford, M. & Partridge, G. 2001. Vegetable protein meals and the effects of enzymes. *Enzymes in Farm Animal Nutrition***,** 125-143.

Thompson, J. L., Manore, M. M., and Vaughan, A. V. 2008. The Science of Nutrition. Pearson Education Publishing: San Francisco, CA. Pp 220-259.

Towo, E., Matuschek, E. and Svanberg, U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds and in vitro accessible iron. *Food Chemistry,* 94**,** 369-376.

Tromp, C., Raghavan, G. & Alikhani, Z. 1995. Nutritive quality of whole soybeans heat-treated in a continuous flow processor. *Journal of Agricultural Engineering Research,* 61**,** 49-55.

Tsai CY, Dalby A and R. A Jones. Lysine and tryptophan increases during germination of maize seed. *Cereal Chemistry.* 1975; 52: 356 – 360.

United Nations Children's Fund (UNICEF). 2007a. Progress for Children: A world fit for children statistical review. New York: United Nations Children‟s Fund. Available at: <http: //www.unicef.org/progressforchildren/2007n6/index_41505.htm.>.

United Nations Children's Fund (UNICEF). 2007b. The State of the World’s Children 2008. New York: United Nations Children's Fund. Date accessed 15/07/2016.

United Nations Children's Fund (UNICEF). 2009. CHILDINFO: Monitoring the Situation of Children and Women. United Nations Children's Fund.

USDA. 2008. National Nutrient Database for Standard Reference, Release 20. www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl. Date accessed 15/07/2016.

Vitali, D., Dragojevic, I. V., and Sebecic, B. 2008. Bioaccessibility of Ca, Mg, Mn and Cu from whole grain tea biscuits: Impact of proteins, phytic acid and polyphenols. *Food Chemistry*. 110: 62-68.

Wang, N., Lewis, M., Brennan, J. and Westby, A. 1997. Effect of processing methods on nutrients and anti-nutritional factors in cowpea. *Food Chemistry,* 58**,** 59-68.

World Health Organization. 2000. Turning the tide of malnutrition responding to the challenge of the 21st century. [Internet] Geneva: WHO. http://www.who.org. Date accessed 15/07/2016.

World Health Organization, 2006. Guidelines on food fortification with micronutrients for the control of micronutrient malnutrition. *Geneva: WHO*.

World Health Organization, 2010. *World health statistics 2010*.World Food Programme (WFP). 2008. Facts and Figures: Hunger Facts. www.wfp.org.org/about wfp.

Wu YV, Wall JS (1980). Lysine and tryptophan increased during germination of cereal grains. *Cereal Chemistry*. 53: 222-226.

Yaday, S., and N. Khetarpaul, 1994. Indigenous legume fernentation: Effect of some antinutritionals and in vitro digestibility of starch and protein. *Food Chemistry*., 50: 403-406.

Yagoub, A. and Abdalla, A. A. 2007. Effect of domestic processing methods on chemical composition, in vitro digestibility of protein and starch and functional properties of bambara groundnut (Voandzeia subterranea) seed. *Research Journal of Agriculture and Biological Science,* 3**,** 24-34.

Yusuf, A. A., Ayedun, H. and Sanni, L. O. 2008. Chemical composition and functional properties of raw and roasted Nigerian benniseed (Sesamum indicum) and bambara groundnut (Vigna subterranean). *Food Chemistry,* 111**,** 277-282.

Zvauya, R., Mygochi, T and Parawira, W. 1997. Microbial and biochemical changes occuring during production of masvusvu and mangisi, traditional Zimbabwean beverages. *Plant foods for human nutrition,* 51**,** 43-51.

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

7 June 2016

Ms Temitope Deborah Awobusuyi 215082655
School of Agricultural, Earth and Environmental Sciences
Pietermaritzburg Campus

Dear Ms Awobusuyi

Protocol reference number: HSS/0670/016M (Linked to HSS/0217016CA)
Project title: Nutritional qualities and consumers acceptance of provitamin A biofortified amahewu complemented with defatted bambara flour

FULL APPROVAL-NO RISK

In response to your application received 30 May 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 Muthulisi Siwela
Cc. Academic Leader: Professor Onisimo Mutanga
Cc. School Administrator: Ms Marsha Manjoo

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

9 March 2016

Ms Temitope Deborah Awobusuyi (SN 215082655)
School of Agricultural, Earth & Environmental Sciences
College of Agriculture, Engineering & Science
UKZN
Email: siwelam@ukzn.ac.za

Dear

RE: PERMISSION TO CONDUCT RESEARCH

Gatekeeper's permission is hereby granted for you to conduct research at the University of KwaZulu-Natal (UKZN), towards your postgraduate studies, provided Ethical clearance has been obtained. We note the title of your research project is:

"Nutritional qualities and consumers acceptance of provitamin a biofortified amahewu complemented with defatted bambara flour".

It is noted that you will be constituting your sample by placing posters around the Pietermaritzburg campus, verbal invitations, announcements in lecture venues inviting students and lecturers to participate in the study.

Please ensure that the following appears on your questionnaire/attached to your notice:

- Ethical clearance number;
- Research title and details of the research, the researcher and the supervisor;
- Consent form is attached to the notice/questionnaire and to be signed by user before he/she fills in questionnaire;
- gatekeepers approval by the Registrar.

Data collected must be treated with due confidentiality and anonymity.

Yours sincerely

Mokoena

MR SS MOKOENA
REGISTRAR

Consent Form Set No ....

RESEARCH PARTICIPATION CONSENT FORM

School of Agricultural, Earth and Environmental Sciences/University of Kwazulu Natal

Sensory evaluation of provitamin A-biofortified amahewu complemented with defatted bambara

Project background: This project involves tasting and evaluating provitamin- A biofortified maize porridge. Maize is a cereal.

The data will be collected for analysis. You must be at least 18 years to participate.

Purpose of the research: To determine the Sensory evaluation of provitamin A biofortified amahewu complemented with defatted bambara

Specific procedures to be used: All participants will attend the training and evaluation sessions.

Benefit to the individual: Participants will have satisfaction in knowing they have assisted with the research and will receive a small reward.

Risk to the individuals: The expected risks are not known other than those encountered in normal daily food consumption. **Please do not participate in this research if you have a known allergic reaction to any food products.**

Medical liability: I understand that no financial compensation will be paid to me in connection with any physical injury or illness in the unlikely event of physical injury or illness as a direct or indirect result of my participation in this sensory project.

Confidentiality: Participants are not required to divulge any confidential information. Responses to sensory question via computer or paper ballot are tacked using number only. These numbers are not in any way related to participant's name.

Voluntary nature of participation: You do not have to participate in this research project. If you do agree to participate you can withdraw your participation at any time with no penalty.

Human subject statement: If you have any questions about this research project, contact Dr Muthulisi Siwela, Tel: 033-260-5459

I HAD THE OPPORTUNITY TO READ THIS CONSENT FORM, ASK QUESTIONS ABOUT THE RESEARCH PROJECT AND PREPARED TO PARTICIPATE IN THIS PROJECT.

____________________ ____________________

Signature **Date**

____

Participant name (Print)

____________________ ____________________

Researcher's Signature **Date**