

**NUTRITIONAL, SENSORY AND FUNCTIONAL PROPERTIES OF A BAMBARA
GROUNDNUT COMPLEMENTARY FOOD**

BY

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PREFACE

The work described in this thesis was carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, from January 2015 to November 2016, under the supervision of Dr Kirthee Pillay and Dr Muthulisi Siwela.

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Dr Muthulisi Siwela (Co-supervisor)

DECLARATION

I, Adewumi Toyin Oyeyinka declare that:

1. The research reported in this thesis, except where otherwise stated, is my original research.
2. This thesis or any part of it has not been submitted for any degree or examination at any other university.
3. This thesis does not contain any other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons.
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ABSTRACT

Protein energy malnutrition (PEM) in children especially those at the weaning stage is a major public health concern in most countries in the predominantly resource-poor developing regions, especially sub-Saharan Africa. Plant protein sources, such as Bambara groundnut (BGN) are widely used in complementary feeding in developing regions mainly because they are affordable, compared to animal protein sources. BGN, contains appreciable levels of chemical substances possessing both antioxidant and antinutritional properties, suggesting that BGN may have positive and negative effects on human nutrition and health. In the southern African region, very few or no studies have been conducted to determine the effects of various traditional processing methods on the nutritional and functional properties of BGN. The acceptance of BGN for use in complementary foods by population groups vulnerable to PEM is essential, but currently it is not known whether or not BGN is acceptable to such population groups in Southern African countries, including South Africa, for that purpose. The overall objective of the current study was to evaluate the nutritional, sensory and functional properties of a BGN complementary food suggested by caregivers.

The objective of the first investigation was to determine the physico-chemical, functional and nutritional properties of two BGN landraces in comparison with a reference dry bean. The BGN landraces differed in their physical, nutritional and antioxidant properties. The brown bambara landrace and the reference dry bean had higher L* values (41.99 and 45.69 respectively) than the red landrace (26.64). This could be linked to the higher tannin and phenolic content observed in the red landrace as dark coloured grains are known to have higher tannin content. In addition, brown bambara landrace had higher resistance to cutting than the other grains. Protein content of the bambara grains (23.25 g/100 g and 19.22 g/100 g for brown and red bambara respectively) were significantly ($p \leq 0.05$) lower than the reference dry bean (27.36 g/100 g). Processing had varying effects on the nutrient composition of the grains. Dehulling increased protein concentration while roasting of the grains slightly reduced it. Processing methods significantly ($p \leq 0.05$) reduced the antinutrient content of all the samples with a subsequent increase in the *in vitro* protein digestibility. Dehulling of the grains resulted in an 83% and 18% decrease in total phenolics and antioxidant activities respectively while roasting increased the total phenolic content. Bambara groundnut had comparable nutrient quality with the reference dry bean and hence could serve as a good source of both macro- and micronutrients.

The second investigation was conducted to assess consumer awareness and acceptability of BGN as a protein source using subjects recruited from the uMshwathi municipality in KwaZulu-Natal province, South Africa. Focus group discussions were subsequently done after the consumer acceptability study. The survey participants (approx. 64%) were neither familiar with BGN nor its processing methods. Unavailability of the grains and insufficient knowledge on processing methods were some of the reasons stated for its underutilisation. However, the participants were willing to cultivate BGN if the seeds were available. They were also willing to include BGN in their family and infant diets and would use it to prepare a puree for complementary feeding. Purees were therefore prepared from BGN and the reference dry bean using standardized methods. Overall, the sensory attributes of the BGN purees compared well with those of the reference dry bean puree. The colour of the grains significantly ($p \leq 0.05$) influenced the overall acceptability of the purees. Overall acceptability of the BGN purees increased with the age of the participants. Educational programmes including awareness of bambara grains, its health benefits and sensitisation on possible methods of processing may be important to facilitate utilisation.

The third investigation assessed the effect of processing on nutritional quality of BGN grain puree. Red bambara landrace had a shorter cooking time (127-240 min) compared to the brown landrace (132-260 min). Soaking of the grains in both hot and cold water before cooking further reduced the cooking time (by approx. 13-55%) of the grains. The protein content of the red bambara puree (19.48 g/100 g) was significantly ($p \leq 0.05$) lower than the reference dry bean (23.23 g/100 g) and brown bambara (23.36 g/100 g) puree. Potassium, phosphorus and calcium were the major minerals present in the puree. The amino acid content was higher in the brown bambara puree than the red bambara puree. Cooking reduced the amino acid content of the reference dry bean but resulted in an increase in the concentration of most of the amino acids in the bambara samples. However, a 100, 80 and 20% decrease in methionine content was observed in the brown, red bambara and reference dry bean respectively. Most of the macronutrients were well retained during processing into a puree. Increased *in vitro* starch and protein digestibility were observed in all the purees and this could be attributed to a decrease in the antinutrient content. FTIR spectra indicated that there were changes in protein conformation after thermal processing which may also have influenced *in vitro* digestibility. Overall, the investigation demonstrates that soaking BGN grain followed by wet cooking reduces processing time and improves the nutritional quality of the bambara puree.

Arguably, this study is the first to show willingness of a nutritionally vulnerable South African rural community to use BGN in complementary feeding. Thus, this study has demonstrated that BGN has the potential for utilisation as an additional tool for addressing PEM, which is prevalent particularly among children in resource poor communities of South Africa and sub-Saharan Africa (SSA) as a whole. However, challenges with grain availability and limited food properties of the grain, including limitations in processing and nutritional quality attributes should be addressed through several integrated processes, including development of suitable BGN varieties, commercialisation of BGN and a policy environment that supports the adoption of increased utilisation of BGN as a food source.

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DEDICATION

This thesis is dedicated to God Almighty whose grace helped me this far.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ADF	Acid Detergent Fibre
AOAC	Association of Official Analytical Chemists
BGN	Bambara groundnut
DALYS	Disability-Adjusted Life Years
DAFF	Department of Agriculture, Forestry and Fisheries
DoH	Department of Health
DW	Dry Weight
FAO	Food and Agricultural Organization
FTIR	Fourier Transform Infrared Spectroscopy
HFIS	Household Food Insecurity
HFS	Household Food Security
HIV	Human Immunodeficiency Syndrome
INP	Integrated Nutrition Programme
INS	Integrated Nutrition Strategy
IVPD	<i>In Vitro</i> Protein Digestibility
KZN	KwaZulu-Natal
MAM	Moderate Acute Malnutrition
MDG1	Millennium development Goal One
MUAC	Mid-Upper Arm Circumference
NDF	Neutral Detergent Fibre
NFCS	National Food Consumption Survey
PEM	Protein Energy Malnutrition
SAM	Severe Acute Malnutrition
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
TPC	Total Phenolic Content
UN	United Nations
UNICEF	United Nations Children's Fund
WHO	World Health Organization

CHAPTER 1

INTRODUCTION, THE PROBLEM AND ITS SETTING

1.1 Importance of the study

In many developing countries worldwide, childhood malnutrition in the form of protein energy malnutrition (PEM) is the most common deficiency condition (Müller & Krawinkel 2005; de Onis, Monteiro, Akré & Clugston 1993) leading to more than 33% of deaths globally [World Health Organization (WHO) 2013)]. Reports by the United Nations Children's Fund (UNICEF) (2007) revealed that over 25% of children under 5 years of age were underweight and 10% wasted. An increase of 1.8% in the number of underweight South African children has also been reported over a period of 20 years between 1990 and 2010 (Lutter, Daelmans, Onis, Kothari, Ruel, Arimond, Deitchler, Dewey, Blössner & Borghi 2011). PEM can manifest in various forms, such as acute or moderate malnutrition (WHO 2003a). This type of malnutrition results from a deficiency of protein and energy in the diet. Acute malnutrition as described by the Department of Health (DoH) (2014) in KwaZulu-Natal province is caused by a decrease in food consumption and/or illness resulting in bilateral pitting pedal oedema and/or a sudden weight loss (DoH 2014). Anorexia or poor appetite and medical complications are clinical symptoms indicating the severity of these deficiencies. Acute malnutrition could manifest as severe acute malnutrition (SAM) which is defined by the presence of bilateral pitting pedal oedema or severe wasting or moderate acute malnutrition (MAM) which is characterised by moderate wasting (DoH 2014).

Infants grow rapidly in their early years and breast milk alone is adequate for the first six months of life (Sanni, Onilude & Ibidapo 1999; Malleshi, Daodu & Chandrasekhar 1989). However, due to the rapid growth beyond six months of age there is a need for solid foods with adequate essential nutrients as breast milk alone can no longer meet complete nutritional requirements (Heckman & Masterov 2007). More often than not, infants are weaned hurriedly onto starch-based foods with low energy and nutrient densities (Nnam 2001).

According to the WHO (2011), any foods or liquids other than breast milk given to young children during the period of complementary feeding are regarded as complementary foods. The complementary feeding period is the period during which infants are fed with other foods alongside breast milk as recommended by the WHO and should commence at about 6 months of age. In developing regions, especially sub-Saharan Africa (SSA), complementary foods are mostly local staple foods, usually in the form of gruels and porridges. These are mostly

cereal-based and do not meet the infant's nutrient needs (Sanni *et al* 1999). For example, infants in South African rural communities are usually fed with soft white maize porridge as the first complementary food (Faber & Kruger 2005). Although, cereal grains are good sources of carbohydrates, they lack lysine, a major essential amino acid (Friedman 1996).

It is pertinent to note that the quality of protein in the human diet is critically important, especially in developing countries where diets are mainly cereal-based (Adenike 2013). In cereal grains, both the content and quality of protein are limited. Several efforts have been made to improve the quality of infant foods through research (Adebayo-Oyetero, Olatidoye, Ogundipe, Akande & Isaiah 2012; Ijarotimi, Oyewo & Oladeji 2009; Wadud, Abid, Ara, Kosar & Shah 2004; Egounlety 2002; Egounlety, Aworh, Akingbala, Houben & Nago 2002). Many of these studies focused on the use of legumes in fortifying complementary foods for improved protein and energy contents. Egounlety (2002) reported a higher protein and energy content of a cereal-based weaning food fortified with soybean, cowpea and bambara groundnuts compared to a plain cereal food. The report by Nnam (2001) also showed an appreciable nutrient density in a composite blend of germinated sorghum and bambara groundnut (BGN) flour with fermented sweet potato flour. Also, Ijarotimi *et al* (2009) reported a simultaneous increase in energy, protein, fat and fibre content of a banana-based complementary upon addition of BGN flour. A better protein quality was reported for a maize based complementary food fortified with tempe, a fermented soybean product (Egounlety *et al* 2002).

Legumes serve as a good source of protein, carbohydrates, minerals (such as calcium, potassium and phosphorus), dietary fibre and water-soluble vitamins (Fasoyiro, Widodo & Kehinde 2012). Cowpea, soybean, pigeon pea, groundnut and BGN are typical legumes used as human foods in Africa (Fasoyiro *et al* 2012). Grain legumes are the largest single source of vegetable protein in human diets and have historically been part of meals throughout the world (Devi & Saxena 2014). They are inexpensive sources of protein compared to cheese, milk, meat and fish and are considerably richer in calcium than most cereal grains (contain about 100 to 200 mg of calcium per 100 g grain) and are thus of great importance to developing countries (Jain, Kumar & Panwar 2009).

The protein content of legume grains is almost double that of cereals and is dependent on the type of the legume (Friedman 1996). However, legume grains are generally deficient in sulfur

containing amino acids such as methionine and cysteine though rich in other essential amino acids. Further, the chemical composition, including protein content, of legumes can vary with genetic and environmental factors (Fasoyiro *et al* 2012). On the other hand, cereal grains are generally deficient in the essential amino acids lysine and tryptophan. A combination of these two staple grains (legume and cereal grains) can improve the nutritional quality of foods (Fasoyiro *et al* 2012), including complementary foods.

Grain legumes contain anti-nutritional factors such as lectin, saponin, haemagglutinin, protease inhibitor, oxalate, goitrogen, phytate, trypsin inhibitor and tannin (Apata & Ologhobo 1997). These compounds reduce protein availability and digestibility though some of these anti-nutritional factors have been reported to have health-promoting effects, which are thought to be largely due to their antioxidant properties (Amarowicz & Pegg 2008). Tannin, a polyphenolic compound, has been reported to exhibit significantly high antioxidant activity (Morrow 1991). Raw legume grains have a higher content of anti-nutritional factors, but these can be eliminated or reduced by processing (Jain *et al* 2009).

Bambara groundnut (*Vigna subterranea*) is an underutilised grain legume grown in a number of countries in SSA (Fasoyiro *et al* 2012). It is cultivated by farmers as a “famine culture” crop because it has several natural agronomic advantages which includes a high nutritional value and drought tolerance (Azam-Ali, Sesay, Karikari, Massawe, Aguilar-Manjarrez, Bannayan & Hampson 2001). Bambara grain contains protein, carbohydrate, fat and ash content in the ranges of 15-20%, 58-67%, 4-7% and 3-4.4%, respectively (Abiodun & Adepeju 2011; Sirivongpaisal 2008). There are various cultivars of BGN, which have distinct colours ranging from cream, through brown, maroon to black and varied seed sizes and seed coat thickness (Nti 2009). BGN is consumed either in an immature green state or as matured seeds which are very hard and therefore require boiling before any specific preparation (Abiodun & Adepeju 2011). Thus, the underutilisation of bambara may be partly due to the hard to cook phenomenon, which is common in most legumes. Limited information on potential food uses and lack of sensitisation regarding the nutritional value of bambara grain (Fasoyiro *et al* 2012; Fasoyiro, Ajibade, Omole, Adeniyi & Farinde 2006) also contribute to its underutilisation. Previous studies on bambara groundnut grown in Southern Africa focused mainly on the characterisation of its major components such as starch (Oyeyinka, Singh & Amonsou 2016; Oyeyinka, Singh, Adebola, Gerrano & Amonsou 2015) and protein isolates (Arise, Ijabadeniyi & Amonsou 2015). These studies revealed that bambara starch

and proteins are potential ingredients for the food industry. Although there are efforts through breeding to improve the agronomic traits of bambara groundnut grown in Southern Africa (Shegro, van Rensburg & Adebola 2013; Madamba 1995), landraces cultivated by farmers still dominate the production areas in Southern Africa (Oyeyinka *et al* 2016). The use of bambara groundnut in the formulation of complementary foods has been widely studied in developing nations especially in Africa. However, most of these formulations are yet to be fully commercialised.

As with most developing countries, commercial complementary foods are available and convenient to use. However, most of them are expensive and generally only affordable to those living in the urban areas. In South Africa, complementary foods made from maize are deficient in essential amino acids (Faber & Wenhold 2007). Therefore, it is necessary to seek alternative sources of these nutrients to complement the limiting amino acids present in cereal grains. These shortcomings can be addressed by combining cereals and legumes in diets since legumes are known to be excellent sources of lysine and histidine, (essential amino acids in infants) which are deficient in cereals. Complementary foods based on locally grown BGN, would thus be a low-cost, nutrient dense food for children in rural communities. Complementary foods based on BGN could have the potential to prevent malnutrition usually seen during the complementary feeding phase of children from resource-poor communities, especially rural communities in sub-Saharan African countries, including South Africa. Currently, there are no published scientific data and information on the chemical, physical and functional properties of a complementary food based on BGN alone. The nutritional value of complementary foods must meet dietary requirements as well as be sensorially acceptable. This is because sensory attributes play a major role in the acceptance of new food products (Nti 2009). Therefore, there is a need to assess and evaluate the possibility of using BGN to produce sensorially acceptable and nutritious complementary foods targeted at children vulnerable to PEM. The antinutritional and antioxidant (potential health-promoting) properties should be investigated as they would influence the nutritional and health-promoting value of BGN.

1.2 Purpose of the study

The purpose of this study was to evaluate the potential of BGN, an underutilised legume for utilisation in complementary food to improve the protein intake of children vulnerable to

PEM, especially in resource-poor communities in sub-Saharan countries, including South Africa.

1.3 Study objectives

The objectives of this research were:

- 1.3.1 To determine the physico-chemical, functional and nutritional properties of bambara landraces.
- 1.3.2 To assess the consumer awareness of BGN as a food source and to formulate a complementary food made from bambara grains as suggested by caregivers.
- 1.3.3 To determine the acceptance of the formulated complementary food made from BGN to caregivers in rural KwaZulu-Natal, South Africa.
- 1.3.4 To assess the functional and nutritional properties of the formulated complementary food made from BGN.

1.4 Hypotheses

The following hypotheses were tested in this study:

- 1.4.1 The nutritional composition of the BGN landraces varies due to genetic factors.
- 1.4.2 Processing affects the antioxidant (potential health promoting) and antinutritional properties of the BGN.
- 1.4.3 Rural communities in KwaZulu-Natal Province are not aware of BGN or its processing techniques.
- 1.4.4 The consumer acceptance of the complementary food made with BGN is low due to its unacceptable sensory properties and the unfamiliarity of BGN amongst subjects.
- 1.4.5 The complementary food made from BGN has a superior nutritional composition compared to the complementary food made from regular dried beans.

1.5 Study parameters and general assumptions

The consumer awareness studies and the consumer acceptability studies of the formulated bambara complementary food were carried out in the Umgungundlovu district Municipality, KwaZulu-Natal, South Africa. The subjects were assumed to be of low socio-economic status as they were drawn from a rural area in KwaZulu-Natal. The subjects were black African females caring for children between the ages of six months and three years at the time of the study. The study was limited to two varieties of BGN and one variety of dried beans

(reference) which were obtained from conventional breeding performed at agricultural stations in the southern Africa region. Selected nutrients were analysed for in the raw and processed BGN and reference dry bean due to cost constraints.

1.6 Outline of the thesis

The structure of the thesis is as follows:

- Chapter 1: Introduction, the problem and its setting
- Chapter 2: Literature review
- Chapter 3: Background to study design and the consumer acceptability study site
- Chapter 4: Physical, nutritional and functional properties of grains of bambara groundnut landraces
- Chapter 5: Consumer awareness and acceptability of a bambara groundnut complementary food in rural KwaZulu-Natal, South Africa
- Chapter 6: Functional and nutritional properties of a bambara complementary food (puree)
- Chapter 7: Conclusions and recommendations

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

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter reviews malnutrition in children, with emphasis on the causes and effects, both on a long and short-term basis as well as the influence of complementary feeding on the nutritional status of South African children. Existing strategies to combat malnutrition and their effectiveness are reviewed. Further, the potential of utilising BGN to enhance the nutritional composition of complementary foods is reviewed.

2.2 Childhood malnutrition in sub-Saharan Africa and South Africa

According to the WHO, malnutrition refers to a “cellular imbalance between the supply of nutrients and energy and the body’s demand for them to ensure growth, maintenance, and specific functions” (de Onis, Monteiro, Akré & Clugston 1993). Malnutrition results from the inadequate consumption of macro- and micro nutrients required for metabolic activities of the body (Torpy, Lynn & Glass 2004). It is a global risk factor for illness and death especially among young children in developing countries (Müller & Krawinkel 2005). Malnutrition often results in irreversible stunted growth and may impede economic growth and poverty alleviation (Bain, Awah, Geraldine, Kindong, Siga, Bernard & Tanjeko 2013). Susceptibility to infectious diseases is more pronounced in children who suffer from growth retardation due to malnutrition (de Onis & Blössner 2003). Child growth is a globally acceptable phenomenon for assessing the nutritional status and health in populations (de Onis & Blössner 2003).

The United Nations (UN) incorporated the key requirements for improving child health and nutrition announced by the 1990 World Summit for Children into its first Millennium Development Goal (MDG1) (Grover & Ee 2009). MDG1 was to halve the proportion of people suffering from hunger between 1990 and 2015. However, considering the current increase in hunger rate, achieving the said target has been quite challenging. The number of underweight children in Africa is said to be on the increase due to political and social instability and also the Acquired Immune Deficiency Syndrome (AIDS) epidemic (de Onis, Blössner, Borghi, Frongillo & Morris 2004).

Most of the world's malnourished children under the age of five years reside in Africa and Asia (Allen 2012). About five million of these children die annually as a result of malnutrition (Black, Morris & Bryce 2003). Death resulting from malnutrition has been found to be more pronounced in developing countries compared to developed nations. For instance, in 1990, 180 deaths per 1000 live births were recorded in SSA and only nine deaths per 1000 were recorded in developed countries. This death rate decreased to 175 and six deaths per 1000 live births in SSA and developed countries, respectively (UNICEF 2001). Tollman, Kahn, Sartorius, Collinson, Clark & Garenne (2008) reported that, in South Africa, between the years 2002 and 2005, 32 out of 417 deaths in children under four years was caused by malnutrition. Malnutrition may take several forms including under nutrition, which is further classified as protein-energy malnutrition (PEM) and micronutrient malnutrition (Bain *et al* 2013). This is discussed in the next section.

2.2.1 Protein-energy malnutrition

The term PEM has been used to describe a range of disorders characterised by growth failure or retardation in children (FAO/UN/WHO 1992). It results from insufficient dietary intake and infectious diseases (Kulkarni & Metgud 2014). Children are highly susceptible to this form of malnutrition due to their high energy and protein needs relative to body weight and their vulnerability to infection (Kulkarni & Metgud 2014). Manary & Sandige (2008) describe PEM as a major form of malnutrition observed in children in developing countries. It results from a lack of one or more macronutrients required by the body to sustain proper metabolic functions of the human body (Manary & Sandige 2008). It is usually accompanied by lower respiratory tract infections, diarrhoea and malaria (Bain *et al* 2013). PEM manifests as either moderate acute malnutrition (MAM) or severe acute malnutrition (SAM). MAM is said to exist when there is moderate wasting with a height/length Z-score between -2 and -3 standard deviation (SD) and a mid-upper arm circumference (MUAC) of between 11.5 and 12.4 cm [Department of Health (DoH) 2014]. SAM, an extreme case of MAM is characterised by the presence of severe wasting which implies a length/height Z-score of <-3 SD or MUAC < 11.5 cm in children between 6 and 59 months (DoH 2014). PEM in children appears most frequently during the weaning period, when solids foods are introduced, which is usually between 4-6 months of age (Prasad & Kochhar 2015; UNICEF 1990).

Wasting (weight for height Z score <-2 SD) has been estimated globally to affect about 55 million children (Black *et al* 2008). Twenty-five percent of the world's underweight children

below the age of 5 years reside in SSA with Congo, Ethiopia and Nigeria being mostly affected (Grover & Ee 2009). In 1992, about 192 million children were said to be suffering from PEM in the developing world and about 178 million of these children below 5 years of age were estimated to be stunted (height for age Z score <-2 SD). The highest prevalence of stunting was found in central Africa and south-central Asia (Black *et al* 2008; FAO/UN/WHO 1992).

2.2.2 Micronutrient malnutrition

Iron, zinc, vitamin A and iodine are the most prevalent micronutrient deficiencies worldwide (Ramakrishnan 2002). These micronutrients act as immunomodulators and determines an individuals' resistance to infections (Smuts, Dhansay, Faber, van Stuijvenberg, Swanevelder, Gross & Benadé 2005). Micronutrient deficiency is aggravated by infections and interferes with the utilisation of nutrients by altering metabolic pathways. This condition is particularly common in children with marginal micronutrient status and accounts for a high burden of disease in poor communities (Bhaskaram 2002). A joint report of the Food and Agriculture Organization (FAO) and WHO stated that micronutrient deficiency was seen in over 2 billion children worldwide (FAO/UN/WHO 1992). According to Black, Allen, Bhutta, Caulfield, de Onis, Ezzati, Mathers & Rivera (2008) about 0.6 and 0.4 million deaths worldwide were attributed to vitamin A and zinc deficiencies respectively. Also, iron and iodine deficiencies resulted in about 0.2% of childhood disability-adjusted life-years (DALYs) (Black *et al* 2008). In South Africa, vitamin A deficiency prevalence was reported to be approximately 44% for children under the age of five years. Among these children, black African children had slightly lower mean retinol concentration of 0.74 $\mu\text{mol/L}$ compared to coloured children (0.81 $\mu\text{mol/L}$) (Shisana, Labadarios, Rehle, Simbayi, Zuma, Dhansay, Reddy, Parker, Hoosain, Naidoo, Hongoro, Mchiza, Steyn, Dwane, Makoae, Maluleke, Ramlagan, Zungu, Evans, Jacobs, Faber & Team 2013). From the 1999 National Food Consumption Survey (NFCS), conducted in South Africa, one out of two children had an intake of approximately less than half the recommended dietary allowance (RDA) for important micronutrients (Labadarios, Steyn, Maunder, MacIntyre, Gericke, Swart, Huskisson, Dannhauser, Vorster, Nesmvuni & Nel 2005). Understanding the chain of events that result in malnutrition is important in order to improve its management. The causes of malnutrition are discussed in the next section.

2.2.3 Causes of malnutrition

Malnutrition in children is a consequence of a sequence of interlinked events that is best described by the UNICEF conceptual framework (Figure 2.1).

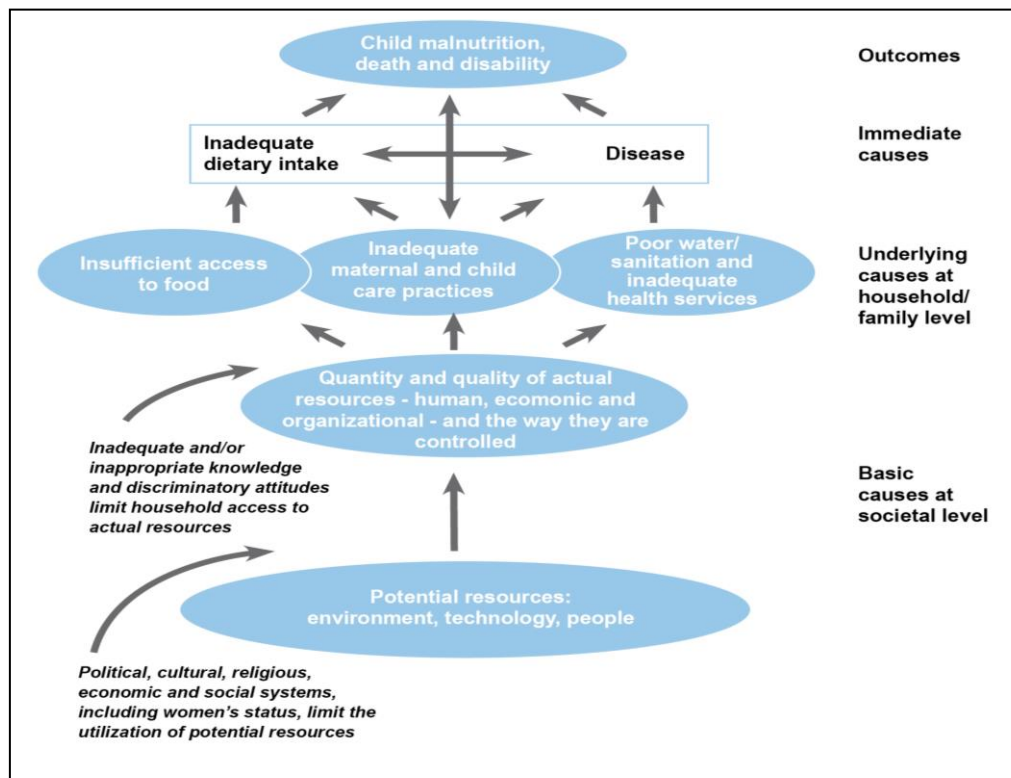


Figure 2.1 The UNICEF conceptual framework for causes of malnutrition in children (UNICEF 1998;1990)

This framework gives a detailed illustration of the complex interwoven factors causing malnutrition and the interrelationship between them. The causes of malnutrition are classified as immediate, underlying and basic. These embrace food, health and caring practices UNICEF (1990).

2.2.3.1 Immediate causes

Inadequate dietary intake and diseases which are the immediate causes of malnutrition as classified by UNICEF (1990) tends to create a vicious circle (Figure 2.2). When a malnourished child with compromised immunity becomes ill it worsens malnutrition due to the loss of appetite, malabsorption and metabolic changes which increase the body's requirement for nutrients (UNICEF 1998). The functioning of the body's immune-response

mechanisms is impaired and there is a reduction in the body's resistance to infection (UNICEF 1998).

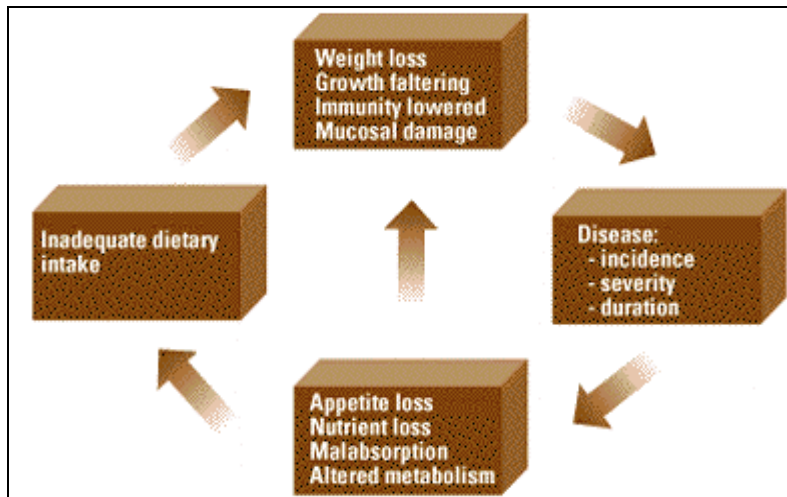


Figure 2.2 Malnutrition infection cycle (Tomkins & Watson 1989)

2.2.3.2 Underlying causes at household and family level

Inadequate access to food in a household [household food insecurity (HFIS)], maternal and child care, health services and poor water/sanitation are the main underlying causes of malnutrition at the household level (Martorell 1999; UNICEF 1998). These lead to the immediate causes of malnutrition.

Household food security (HFS) is said to exist if there is access to safe food of sufficient quality and quantity to ensure adequate intake and a healthy life for all members of the family at all times (Black *et al* 2008; Pinstrup-Andersen 2004). It depends on access to food financially, physically and socially as distinct from its availability (Bain *et al* 2013; UNICEF 1998). For instance, a family who is food insecure does not have financial access to sufficient food despite its availability. HFS in rural areas may be dependent on access to land and other agricultural resources to guarantee adequate domestic production, whereas in urban areas, HFS is ensured by the availability of foods at accessible prices since there is dependency on purchasing foods rather than production (UNICEF 1998). The current continued increase in food prices, especially for staple foods have resulted in households employing food coping strategies to survive which often have a negative impact on nutritional status (Drimie, Faber, Vearey & Nunez 2013).

Access to quality, curative and preventive health services at affordable prices are key ingredients to good health. Despite the Bamako Initiative launched in Africa to address the health care crisis in the 1980s, many people still do not have access to affordable health care (UNICEF 1998). They sometimes defer timely and appropriate treatment due to high health care fees (UNICEF 1998). Although preventive health care and nutrition services are vital and cost effective they still have low demand in communities. A lack of ready access to a safe water supply, poor sanitation in and around the home and unhygienic handling of foods all have considerable implications on the spread of infectious diseases (UNICEF 1998).

The method by which a child is fed, nurtured, taught and guided has been recognised to also influence the growth of the child (UNICEF 1990). Caring tools such as adequate feeding, receiving essential health care at the appropriate time for both mother and child, emotional support and cognitive stimulation are essential for proper growth and development (UNICEF 1998).

2.2.3.3 Basic causes at societal level

The basic causes of malnutrition at the individual level are limited access to resources, environmental technology, and people (UNICEF 1998). Fulfilling all the requirements for proper nutrition is usually a challenge as a particular requirement is met at the expense of the other. For example, a woman spends excessive time producing food to achieve HFS and lacks time to care for her child. Non-discrimination against women in law and custom is likely to give them good access to resources, including credit, and the decision-making power that can enable them to make the best use of services for themselves and their children (UNICEF 1998). Political, cultural, religious, economic and social systems including the status of women limits the utilisation of potential resources (UNICEF 1998). For example in South Africa, many rural dwellers rely on social grants for survival (Smuts, Faber, Schoeman, Laubscher, Oelofse, Benade & Dhansay 2008) and these grants usually do not meet their needs.

2.2.4 Symptoms of malnutrition

The pathologic changes that occur in malnourished individuals include immunologic deficiency in the humoral and cellular subsystem as a result of protein deficiency and deficiency of immune mediators like tumour necrosis factor (Müller & Krawinkel 2005). Unstable metabolic activities resulting from carbohydrate insufficiency also play a role in

impaired intercellular degradation of fatty acids (Van Neste & Tobin 2004). Loss of subcutaneous fat and muscle due to endogenous mobilisation of energy and nutrients leads to primary and secondary amenorrhea, triangular face, extended abdomen and anal or rectal prolapse (Bhan, Bhandari & Bahl 2003). The body's ability to regulate temperature and store water is also lost (Alam, Hamadani, Dewan & Fuchs 2003; Van der Hoek, Feenstra & Konradsen 2002; Gracey 1999). Consequently, malnourished children become dehydrated, hypothermic and hypoglycemic faster and more severely than others do. Oedema, anaemia, hepatomegaly, lethargy, deficient immune system and loss of absorption and digestion capacity are other symptoms seen in severe cases of malnutrition (Müller & Krawinkel 2005; Alam *et al* 2003).

Although there is controversy regarding the most appropriate method to diagnose malnutrition, anthropometric measurements such as weight for height Z-score, MUAC and height for age Z-score are typical standard methods for assessing nutritional status in children (Manary & Sandige 2008; de Onis & Blössner 2003; WHO 1986). Weight for height Z-score compares a child's weight to that of a healthy reference population of children of the same height or length (Manary & Sandige 2008). It is expressed in units of standard deviation (SD) from the mean of the reference population and shows the extent of wasting in the examined individual (Prasad & Kochhar 2015). MUAC is also used in place of weight for height Z scores in the identification of malnutrition (WHO 1986). These standards were derived from international samples of healthy breastfed infants and young children raised in environments with no growth constraints (WHO 2006). Anthropometric measurements serve as indicators of malnutrition in children as children with weight for height measurements between -3SD and -2SD, MUAC values between 11.5 cm and 12.5 cm or height for age Z-score between -3SD and -2SD are regarded as having MAM. Those with weight for height measurements below -3SD, MUAC values less than 11.5 cm and 12.5 cm or height for age Z-score below -3SD are said to have SAM (Grover & Ee 2009; Manary & Sandige 2008) (Table 2.1).

Table 2.1 Classification of malnutrition using anthropometric measurements (Grover & Ee 2009; WHO & UNICEF 2009; Manary & Sandige 2008; WHO 2006)

Anthropometric index	Measurement (indicator)	Classification
Weight for height	Between -3SD and -2SD,	MAM
	Below -3SD	SAM
Middle Upper Arm Circumference (MUAC) (children age 6-60 months)	Between 11.5cm and 12.5cm	MAM
	Below 11.5cm	SAM
Height for age	Between -3SD and -2SD	MAM
	Below -3SD	SAM

Drowsiness, hypothermia, respiratory distress and severe dehydration are other characteristic symptoms of SAM (Grover & Ee 2009). However, Grover & Ee (2009) reported that MUAC measurements together with the presence of bilateral oedema are better parameters in measuring SAM than weight for height measurements. The short-term and long-term consequences of malnutrition are discussed next.

2.2.5 Short and long term effects of malnutrition

Growth is influenced by nutritional status and therefore important in reducing the incidence of morbidity and mortality in young children (de Onis & Blössner 2003). The adverse effects of nutrient deficiency in children could be either short or long-term. The short-term effects of malnutrition as classified by Swart, Sanders & McLachlan (2008, p130) include reduced/impaired brain development, poor growth and increased risk of disease (Figure 2.3).

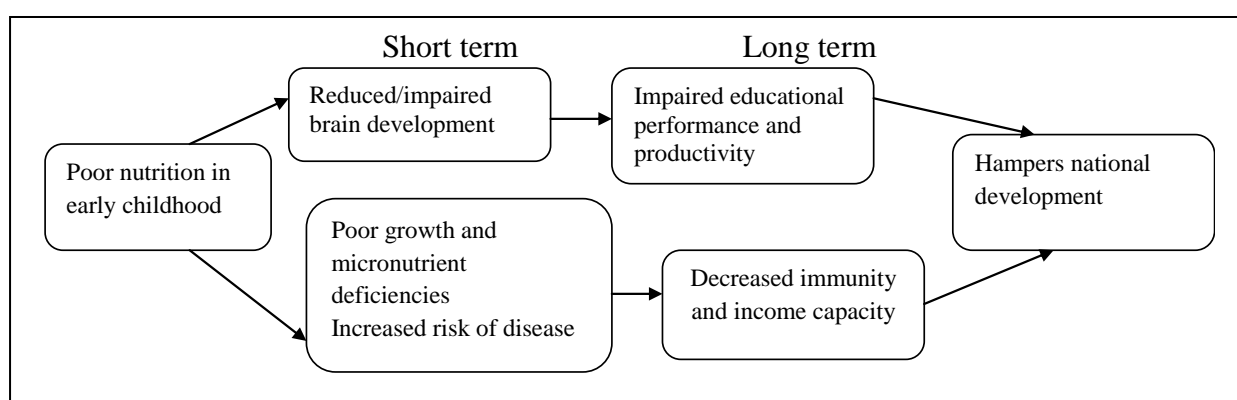


Figure 2.3 Short and long term effects of malnutrition (Swart *et al* 2008, p130; Victora, Adair, Fall, Hallal, Martorell, Richter & Sachdev)

Delayed cognitive development caused by direct structural damage to the brain results in impairment of infant motor development and exploratory behaviour (Murray, Veijola, Moilanen, Miettunen, Glahn, Cannon, Jones & Isohanni 2006). However, it is uncertain whether the cognitive effects continue into adolescence. Intrauterine growth retardation is also associated with impaired cognitive skills though this is dependent on certain environmental factors; for instance, studies from high-income countries showed that birth weight had little or no measurable effect on a child's cognitive performance (Hack 1998). However, an inverse relationship between height and head circumference at two years and the extent of educational achievement in adult women was reported in Guatemala (Li, Stein, Barnhart, Ramakrishnan & Martorell 2003). Persistence of early nutritional deficits and absence of catch-up growth in deprived environments can result in decreased immunity and income capacity, impaired educational performance and productivity and can also hamper national development (Bain *et al* 2013; Victora *et al* 2008).

2.2.6 Strategies for combating protein-energy malnutrition in South Africa

Various nutrition intervention programmes have been introduced to combat the prevailing prevalence of malnutrition worldwide. The South African Union government introduced a food support programme during World War II which provided foods such as milk and butter to the malnourished (Steyn & Temple 2008, p34). Food subsidisation, voluntary enrichment of maize meal and food aid were the nutrition interventions that were implemented before 1994 in South Africa (Iversen, Marais, du Plessis & Herselman 2012). However, these interventions were not available to black Africans (Steyn & Temple 2008, p33).

Furthermore, a PEM scheme was also implemented with the aim to combat malnutrition among children below 6 years in the early 1970s (Iversen *et al* 2012). The scheme provided skimmed milk powder, breast milk substitutes and energy-enriched instant maize meal, which were distributed through local clinics and health centers. However, the scheme was short-lived as a result of insufficient storage facilities, shortage of appropriate staff to implement, monitor and evaluate the scheme and use of foods that were not generally consumed by the targeted communities (Steyn & Temple 2008, p36).

In 1994, the South African Nutrition Committee recommended an Integrated Nutrition Strategy (INS) with three major components: health facility-based nutrition programmes; community-based nutrition programmes; nutrition, HIV and AIDS support programmes

(Iversen *et al* 2012; Swart *et al* 2008). The INS was saddled with the responsibility to resolve problems hindering previous nutrition and health approaches (Schönfeldt *et al* 2010). It served as a basis for the development of the Integrated Nutrition Programme (INP) (Iversen *et al* 2012). The INP employed a holistic approach in combating malnutrition based on the UNICEF Conceptual Framework (Steyn & Temple 2008, p40). Its target groups were malnourished children and their households; pregnant women and their families; families and households who were nutritionally at risk (Iversen *et al* 2012). The INP tackled the underlying causes of nutrition via direct and indirect nutrition interventions (DoH 2008). The direct intervention approach included; nutrition education and promotion, micronutrient supplementation, food fortification and disease-specific nutrition counselling and support. Provision of healthcare services, improved access to food, parasite control and provision of clean and safe water were the indirect nutrition interventions employed by the INP (DoH 2008). The main focus areas of the INP included; promotion, protection and support of breastfeeding, growth monitoring and promotion (GMP), micronutrient malnutrition control, disease-specific nutrition support, treatment and counselling, nutrition promotion, education and advocacy, contribution to household food security and food service management (Hendricks, Goeiman & Dhansay 2007). Figure 2.4 illustrates the constraints to the effectiveness of the INP.

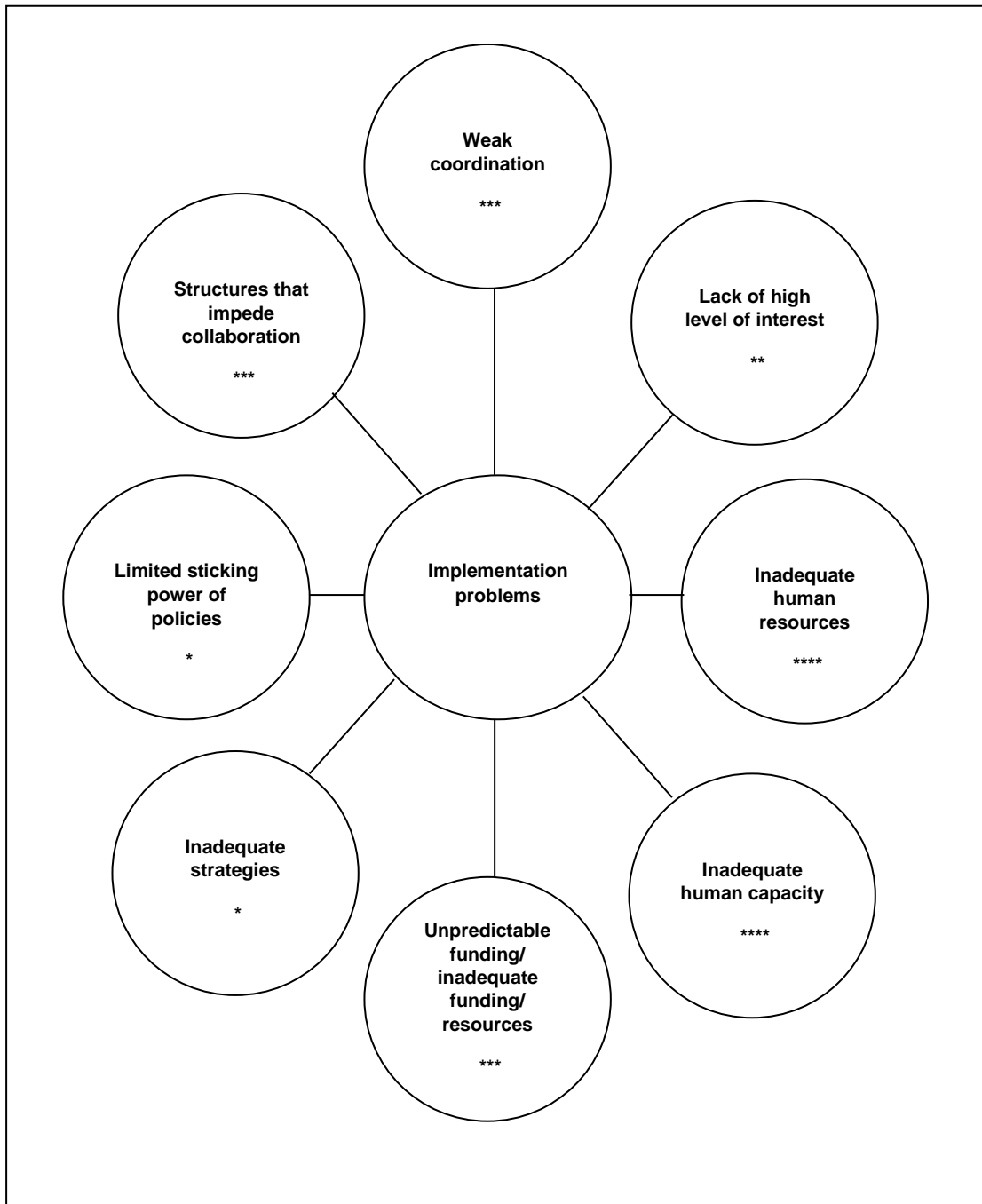


Figure 2.4 Core problems reducing the effectiveness of the INP (Adapted from Swart *et al* 2008).

**** significant contributor, *** moderate contributor, ** contributor, *possible contributor

The DoH also implemented various mandatory programmes such as food fortification, micronutrient supplementation, dietary diversification and other public health measures (Witten, Jooste, Sanders & Chopra 2004). The mandatory iodisation of salt and fortification

of maize meal and bread are examples of these food fortification programmes (Schönfeldt *et al* 2010).

Dietary patterns of children are key in determining their nutritional status. To ensure adequate nutritional intake especially in infants, their diet must include nutrient dense and easily digested foods. Complementary feeding patterns in South Africa are discussed next.

2.3 Complementary feeding

2.3.1 Global practices

Health, growth and development of infants and young children is solely dependent on optimum nutrition as good feeding practice is an antidote to malnutrition and early growth retardation (Michaelsen, Weaver, Branca & Robertson 2000). According to the WHO (2015), breast milk alone meets the complete nutritional requirements of infants for the first six months of life and exclusive breastfeeding is therefore recommended during this period. Exclusive breastfeeding means that the infant is fed with only breast milk and no other liquids or solids are given except for medication (WHO 2015). Although exclusive breastfeeding is recommended for the first 6 months of life, breastfeeding should continue for 2-3 years in addition to solids to achieve optimal growth, development and health (WHO 2002). Breast milk protects the infant against food allergies and prevents a variety of diseases and infections (Rolfes, Pinna & Whitney 2012, p454). Inappropriate breastfeeding in children, however, increases their susceptibility to recurring infections, growth retardation and early childhood mortality (WHO 2002).

Young infants do not have the ability to metabolise a wide variety of foods due to their digestive and excretory systems (Van der Merwe, Kluyts, Bowley & Marais 2007). As they grow, they gradually develop the ability to chew, swallow and digest a wide range of foods (Rolfes *et al* 2012, pp472-473). The ability to transfer solid foods from the front of the tongue to the pharynx is a milestone developed at about the age of 6 months (Pridham 1990). Breast milk also becomes insufficient in meeting the nutritional requirements after 6 months and hence the need for complementary foods (Allen 2012).

With complementary feeding, there is a gradual transition to solid foods. Infants are introduced to a variety of foods with varying texture, flavor, aroma and appearance (WHO 2011; Michaelsen *et al* 2000). Solids are needed to provide those nutrients which are no

longer supplied adequately by breast milk (Rolfes *et al* 2012, p473). Infants and children have small gastric capacity and hence should be fed with nutrient dense foods with proper consistency and sufficient frequency (Dewey & Brown 2003). Commercially processed complementary foods should meet applicable recommended standards by the Codex Alimentarius Commission and also the Codex Code of Hygienic Practice for Foods for Infants and Children (WHO & UNICEF 2003).

The age at which complementary foods are introduced to infants vary internationally despite recommendations by the WHO. Globally, less than 35% of infants are exclusively breastfed during their first four months of life (Allen 2012; WHO & UNICEF 2003). In developed countries such as the United States, infants between 4-5 months are fed with infant formula which constitute about 56% of their diet (Fox, Reidy, Novak & Ziegler 2006). Their major source of energy and nutrients is infant formula, breast milk and milk. Juices, fruit flavoured drinks and fortified foods supply many essential nutrients. Fortified grain-based foods and supplements make substantial contributions to their vitamin and mineral intakes (Fox *et al* 2006). In the United Kingdom, most infants are introduced to solid foods about 15 weeks after birth (Wright, Parkinson & Drewett 2004). Most mothers feel that breast milk alone is not sufficient for their babies and hence the need for additional foods.

As with most developed countries, exclusive breastfeeding in the first 6 months of life is sub optimally practiced in developing countries. For instance in Nepal and Tanzania, complementary foods are usually introduced to infants before 6 months of age (Allen 2012). These foods are porridges made from maize, rice, sorghum, millet or wheat (Figure 2.5) with the addition of large quantities of water due to their viscous nature.

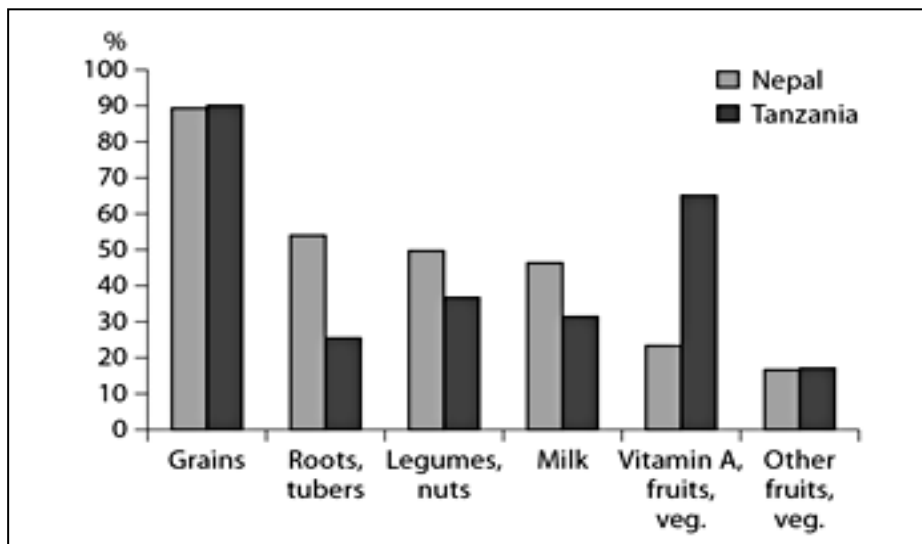


Figure 2.5 Foods consumed by Nepali and Tanzanian infants aged 9-11 months (Allen 2012)

However, the prepared foods have low nutrient density (Allen 2012). The inclusion of a suitable oil could provide essential fatty acids and increase the energy density but may also lower food intake and density of nutrients (Allen 2012). The absence of animal food sources in these complementary foods increases the risk of micronutrient deficiency. A study conducted in Lebanon by Batal, Boulghourjian, Abdallah & Afifi (2006) revealed that only about 23% of infants were exclusively breastfed for 6 months. Most of these infants were exclusively breastfed for only four months after which they were introduced to solid or semi-solid foods. Fruits were the first foods to be introduced followed by cereals, vegetables, dairy, dessert, meats, eggs and then legumes. Mothers were encouraged to feed their children starchy foods to boost the child's weight due to the cultural belief that fat babies are healthy (Churchill & Kanawati 1971, pp282-288). Bahraini housewives also introduced commercial baby foods to their infants at 3-6 months of age (Musaiger 1983).

The choice of foods and feeding practices is greatly influenced by traditional and cultural beliefs of mothers and caregivers (Nuss, Arscott, Bresnahan, Pixley, Rocheford, Hotz, Siamusantu, Chileshe & Tanumihardjo 2012; Sibeko, Dhansay, Charlton, Johns & Gray-Donald 2005). Many infants and children are mostly fed with plant-based staples, which usually have low nutrient density. Poor socio-economic status, lack of adequate knowledge and access to a variety of foods are some factors responsible for these feeding patterns (Lutter, Daelmans, Onis, Kothari, Ruel, Arimond, Deitchler, Dewey, Blössner & Borghi 2011). High mortality rates have been recorded in developing countries as a result of these

feeding practices (WHO 2000). Complementary feeding practices in South Africa are discussed in the next section.

2.3.2 South African practices

Various researchers have reported on the period during which complementary foods are introduced to South African infants. A study by Faber & Benadé (2007) reported that most South African infants were introduced to solid foods between the ages of 4-6 months. Exclusive breastfeeding for 6 months as recommended by the WHO was rarely practiced. Fifty-five percent of the 505 children in the cohort were first fed with soft porridge made with maize meal. Forty-one percent of the children received bottle milk feeds alone or alongside breast milk. Margarine, peanut butter, formula milk and fresh milk were some of the energy dense foods added to the porridge. Most of the mothers/caregivers in the study did not view commercially available infant cereals as an option due to high cost. Inappropriate infant feeding practices such as lack of exclusive breastfeeding, early introduction of complementary foods and incorrect preparation of formula feeds were commonly practiced among the subjects (Faber & Benadé 2007). A study conducted at Moretele district by Kruger & Gericke (2002) also showed that most of the mothers introduced solid foods to their children at 2-3 months of age and mixed family diets at 7-9 months. Exclusive breastfeeding was also very rare and children were fed mostly with maize-based cereal products. These foods were subjected to rigorous cooking conditions with excess water, which compromised the nutrient intake of the children. Formula powder and margarine were usually added to the cooked soft porridge (Kruger & Gericke 2002).

About 37 out of the 117 infants involved in a survey from a peri-urban community in South Africa were fed with breast milk substitutes and commercial infant cereal in their first month of life (Sibeko *et al* 2005). These mothers felt that they were not lactating enough to satisfy their infants. Further, infants in the central region of Limpopo Province also had a similar feeding pattern (Mamabolo, Alberts, Mbenyane, Steyn, Nthangeni, Delemarre-van De Waal & Levitt 2004). Most of the infants at 3 months of age were already taking infant formula and solid foods. Maize meal porridge and *mabella*, a sorghum porridge, were the most popular complementary foods. Fifty-two percent of the 219 infants received some other foods apart from breast milk by the end of the first month (Mamabolo *et al* 2004). About 65 infants of the subjects were stunted by the first month. The early introduction of complementary foods to

South African infants has been found to be a risk factor for malnutrition (Van der Merwe *et al* 2007).

In urban South African communities where commercial infant cereals were used, a high prevalence of micronutrient deficiencies were recorded (Faber & Wenhold 2007; Kruger & Gericke 2002). This is probably due to inadequate use of these infant cereals or failure to comply with the stated preparation procedures. Infant formulas were not used for long periods in rural areas because of the high costs (Benadé & Faber 2001). Cultural acceptance and affordability of fortified infant cereal by the lower socioeconomic sector may have an impact on the health, growth and development of infants and small children (Kerr, Dakishoni, Shumba, Msachi & Chirwa 2008).

2.3.3 Nutritional value of common South African complementary foods

Various commercially processed complementary foods are available on the shelf but there is low demand due to their relatively high cost (Faber & Benadé 2007). Cost effective alternatives are made from locally consumed cereals, legumes, fruits and vegetable. White maize grains seem to be the main ingredient in complementary foods for South African infants (Faber & Benadé 2007; Mamabolo *et al* 2004; Faber, Jogessar & Benadé 2001). It is usually processed into bulky foods with certain nutritional limitations. Unfortified white maize-based diets are rich in energy and fibre but deficient in essential amino acids such as lysine and tryptophan, vitamin A and minerals like calcium, iron and zinc (Duvenage & Schönfeldt 2007). White maize contains low amounts of phenylalanine, tyrosine, tryptophan, threonine, methionine, cysteine, leucine, valine, isoleucine and histidine and white maize-based diets may not meet the recommended dietary allowance (RDA) for these amino acids (Duvenage & Schönfeldt 2007). The phytate content of maize is also of major concern as it affects the bioavailability of minerals especially zinc, iron and calcium (Hotz & Gibson 2001). Although Kruger & Gericke (2002) found that margarine, infant formula, egg and peanut butter were added to maize meal porridge, it was diluted with water. Over dilution increases the risk of microbial contamination thus increasing the risk for a compromised health status in the child (Andresen, Rollins, Sturm, Conana & Greiner 2007).

The South African NFCS (1999) reported adequate protein intake in children 1-9 years old (Labadarios *et al* 2005). However, a large number of children had an inadequate intake of energy, vitamin A, vitamin C, thiamin, riboflavin, niacin, vitamin B6, vitamin B12, folic acid,

calcium, iron and zinc. Inadequate intake of these nutrients was worse in children from rural areas compared to children in urban areas. This was linked to food insecurity due to poor food accessibility or availability, leading to increased levels of stunting and underweight (Labadarios *et al* 2005). As mentioned earlier, there was a high dependence on maize as a food source especially for infants. The shortcomings of these feeding practices are discussed in the next section.

2.3.4 Shortcomings of South African complementary feeding practices

In South Africa, maize meal is an integral part of the infant diet. Most infants are usually weaned onto a soft maize meal porridge or *phutu*, a crumbly maize meal porridge (Mushaphi, Mbhenyane, Khoza & Amey 2008; Faber & Wenhold 2007; Benadé & Faber 2001). White maize based diets were reported to be the major complementary foods fed to infants (Mamabolo *et al* 2004; Kruger & Gericke 2002). Although yellow maize has a higher provitamin A carotenoid content it is perceived as being unfit for human consumption but suitable for animal feeds (Khumalo, Schönfeldt & Vermeulen 2011).

The prevalence of micronutrient deficiencies in South African children could be associated with the low nutrient density and bulkiness of maize meal-based complementary foods, which is a major part of the diet. The high phytate content of maize which is known to inhibit iron and zinc absorption is also a major concern (Davidsson 1998; Gibson & Ferguson 1998). In a study conducted by Faber & Benadé (2007) in rural KwaZulu-Natal, about 176 and 161 infants between the ages of 6-12 months were found to have iron deficiency anaemia and zinc deficiency respectively. The median iron and zinc intake was below the estimated average requirement (EAR). A total reliance on unfortified white maize could be a risk factor for vitamin A deficiency (VAD) in South African children (Labadarios *et al* 2005). Faber & Benadé (1998) reported that in rural KwaZulu-Natal, most infants were fed animal protein food sources only once a week. A lack of animal products in the diet could also result in iron, zinc and B vitamin deficiencies (Benadé & Faber 2001). An increase in the prevalence of childhood malnutrition was also reported by Smuts *et al* (2008) in a study conducted in the Eastern Cape and KwaZulu-Natal provinces. This may be attributed to inappropriate complementary feeding practices employed by caregivers (Smuts *et al* 2008). Animal proteins are generally more expensive than their plant protein counterparts. Although the use of animal proteins are considered better in terms of nutritional composition, plant proteins with comparable nutrient profile can be developed from underutilised crops such as bambara.

These underutilised species are cheap alternatives that could be used to make value added products at reduced cost, especially in the rural settings of South Africa. Considering the foregoing, there is need for a more affordable protein source, which may contribute to a reduction in the prevailing malnutrition epidemic. Bambara groundnut is a high quality protein food source, suitable for complementary feeding in resource-poor communities where animal protein sources are not readily available, leaving infants at increased risk of PEM.

2.4 Bambara groundnut as a food source

Bambara groundnut (*Vigna subterranea*) is a dicotyledonous legume indigenous to Africa and grown across the continent (Williams 1993, p14). It belongs to the family Fabaceae and sub family of Faboidea and is grown primarily for its edible seeds (Bamshaiye, Adegbola & Bamshaiye 2011). It is known as *Nyimo* beans in Zimbabwe, *Ntoyo ciBemba* in the Republic of Zambia, *Gurjiya*, *Okpa*, *Epa-Roro* or *Kwaruru* in Nigeria and *Izindlubu*, *Njugo* or *Jugo* beans in South Africa (Mabhaudhi, Modi & Beletse 2013; Bamshaiye *et al* 2011). In Africa, BGN is regarded as the third most important crop after groundnuts (*Arachis hypogea*) and cowpeas (*Vigna unguiculata*) [Department of Agriculture, Forestry And Fisheries (DAFF) 2011]. The plant has various agronomic benefits such as its high nutritional value, ability to produce in poor soils, high drought resistance and relative resistance to pests and diseases (Bamshaiye *et al* 2011; Nwanna, Enujiugha, Oseni & Nwanna 2005). However, it remains an underutilised crop in most parts of the world due to its hard to cook property and pronounced beany flavour (Fasoyiro, Widodo & Kehinde 2012).

2.4.1 Nutritional profile

Table 2.2 shows the chemical composition of some commonly consumed legumes, including bambara.

Table 2.2 Chemical composition of some commonly consumed legumes (Yusuf, Ayedun & Sanni 2008; Fasoyiro, Ajibade, Omole, Adeniyani & Farinde 2006; Amarteifio & Moholo 1998).

Legume	Carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)
African yam bean	50.02	24.19	5.04	3.31	7.20
Bambara groundnut	55.63	22.06	2.02	3.97	2.31
Benniseed	16.60	18.10	36.10	11.20	14.10
Cowpea	55.93	23.87	1.50	3.80	3.93
Groundnut	13.74	27.01	45.81	5.80	3.03
Lima bean	50.44	24.19	2.92	5.64	2.07
Marama bean	24.10	34.10	33.50	3.70	4.40
Mung bean	59.30	26.37	1.10	4.30	4.30
Pigeon pea	48.31	25.98	1.91	4.06	4.62
Soybean	25.19	37.27	17.79	4.86	5.05

Carbohydrates and proteins are the major nutrients in BGN (Table 2.2). It can be an inexpensive source of protein especially in communities where animal protein is not affordable (Bamshaiye *et al* 2011). The protein content of bambara is comparable to those reported for other pulses such as pigeon pea, lima bean, African yam bean and cowpea (Table 2.2). These values, however, may vary depending on the variety or cultivar. Up to 28% protein content has been reported for BGN grown in Southern Africa (Arise, Amonsou & Ijabadeniyi 2015), which appears higher when compared to values reported by Yusuf *et al* (2008) and Fasoyiro *et al* (2006) in Nigeria. This variation does not depend only on cultivar difference, but also on breeding efforts and growth environment. For instance a significant higher total dry matter content of bambara seeds cultivated under irrigated conditions compared to those subjected to drought conditions have been reported (Jørgensen, Ntundu, Ouédraogo, Christiansen & Liu 2011; Mwale, Azam-Ali & Massawe 2007). It is noteworthy that BGN has a higher methionine content than most legumes, including soybean (Bamshaiye *et al* 2011). Methionine, a sulfur-containing essential amino acid, is generally limiting in legumes (Bamshaiye *et al* 2011).

Minka & Bruneteau (2000) found bambara to be a rich source of polyunsaturated fatty acids (linoleic and linolenic acids) and saturated fatty acids (palmitic and stearic acids). Bambara compares well with soybean and groundnut in terms of mineral composition. Fasoyiro *et al*

(2012) and Fasoyiro *et al* (2006) reported similar sodium, calcium, potassium, phosphorus and iron values for soybean, groundnut and bambara. Despite its nutritional value, the nutrient limiting factors present in bambara are startling. Trypsin inhibitors, phytic acids and certain phenolic compounds such as tannins are some of these nutrient limiting factors (Brough, Azam-Ali & Taylor 1993). The tannins are located majorly in the seed coat and the seed colour correlates with the quantity of tannins present in the seed (Bamshaiye *et al* 2011). Tannins have the ability to precipitate protein by forming complexes with them and hence reduce protein digestibility (Hagerman, Riedl, Jones, Sovik, Ritchard, Hartzfeld & Riechel 1998). These antinutritional factors can be reduced and/or destroyed by various food processing methods (Bamshaiye *et al* 2011).

2.4.2 Physicochemical and functional properties

Bambara grains have been reported to vary in shapes, sizes and colours ranging from white, cream, yellow, red, purple brown and black (Williams 1993, p17). The coloration could be uniform, mottled, blotched or striped and they could be round or elliptical in shape with seed weight ranging between 280 and 320 g (Bamshaiye *et al* 2011; Williams 1993, p17). BGN is both nutritionally and economically important because it can serve as an inexpensive source of high quality protein for both humans and animals (Abiodun & Adepeju 2011). As a protein-rich legume, BGN flour possesses certain emulsification properties mainly due to its soluble and insoluble protein content (Eltayeb, Ali, Abou-Arab & Abu-Salem 2011). Electrostatic repulsions are formed on the surface of oil droplets due to the presence of proteins. These repulsions lead to a decrease in surface tension of the droplets and consequently the formation of stable emulsions (McClements 2004). Also, BGN flour and its protein isolate have been reported to have high water absorption capacity (Yusuf *et al* 2008). This is as a result of the presence of more polar hydrophilic parts than the hydrophobic parts. The reported water absorption capacity values were relatively higher than those reported for soybean, African yam bean and lima bean flours (Eltayeb *et al* 2011). Also, BGN flour has the ability to form stable foams at a pH of 6. These properties are important in the production of foods like porridges, purees and ice creams amongst others. Some of these foods could be used in complementary feeding. Bambara may therefore be a suitable ingredient in the production of complementary foods. Despite these attractive attributes, it is only cultivated by small-scale farmers, mostly women for the sustenance of their families (Mabhaudhi *et al* 2013).

2.4.3 Processing and utilisation of bambara groundnut

Both the mature and immature bambara seeds can be consumed (Bamshaiye *et al* 2011; Williams 1993, p14). The immature seeds, however, seem to be more palatable than the mature ones. Immature seeds can be eaten fresh or grilled and can also be boiled, shelled or unshelled (Williams 1993, p14) and also used in soups. Mature seeds are stored in its shell until required for use as shelled seeds are extremely susceptible to weevil infestation (DAFF 2011). Processing and utilisation of bambara differs amongst countries. In the western and northern parts of Côte d'Ivoire (Ivory Coast), BGN is an important snack and a food supplement (Hillocks, Bennett & Mponda 2012). In Botswana the immature grains are boiled in their pods with salt and consumed either singly or in combination with boiled maize grains (Bamshaiye *et al* 2011). The seeds are also made into a local pudding called *Moi Moi* or *Okpa* in some parts of Nigeria (Okpuzor, Ogbunugafor, Okafor & Sofidiya 2010). Mature seeds are pounded into flour and used to make cakes or mixed with cereals to prepare porridges. The pounded mature seeds are also made into balls which are fried and used to prepare stews. Furthermore, the mature seeds are sometimes soaked overnight and boiled until soft with or without capsicum pepper and salt. In Malawi, dry BGNs are consumed as decorticated, boiled and mashed nuts locally referred to as *Chipere* (Mwangwela, Makoka & Pungulani 2009).

Various studies have been carried out on the use of BGN in the formulation of complementary foods (Ijarotimi & Keshinro 2013; Ijarotimi, Oyewo & Oladeji 2009; Ijarotimi & Olopade 2009) and also in food fortification (Bankole, Tanimola, Odunukan & Samuel 2013; Nwosu 2013). However, studies on the use of BGN in complementary feeding in South Africa is scarce. In Nigeria, a complementary food was formulated using BGN and banana (Ijarotimi, Oyewo & Oladeji 2009; Ijarotimi & Olopade 2009). The product matched well in nutritional profile and functional characteristics with a well-known commercial weaning food brand. The authors pointed out that the consumption of the formulated puree was sufficient to meet the recommended dietary allowance (RDA) for energy, protein and minerals for infants and that the puree had a balanced essential amino acid profile. Bankole, Tanimola, Odunukan & Samuel (2013), similarly reported an improvement in the nutritional and rheological properties of *Garri*, a fermented cassava product fortified with BGN. An increase in the protein, ash and fat contents of the fortified *garri* was also observed. Composite flours have also been made with BGN flour and wheat flour in the production of biscuits (Nwosu 2013). This was done to reduce the total reliance on wheat flour for pastry.

Expectedly, the biscuit had a higher protein content than those made from pure wheat flour. Further, the possibility of producing a vegetable milk from bambara has also been investigated (Brough *et al* 1993). The milk had comparative sensory acceptability with milks from other vegetable sources like soybean, cowpea and pigeon pea. Nnam (2001), similarly observed an increase in the nutrient composition of a composite flour made from sprouted bambara, sprouted sorghum and fermented sweet potatoes. The protein, ash and mineral content of the composite flour increased with increasing BGN flour (Nnam 2001).

The hard shell of the mature dry seeds which reduces water permeability and antinutritional factors are some of the factors limiting the utilisation of this legume (Barimalaa & Anoghalu 1997). However, studies have shown that processes like soaking, germination, fermentation and roasting can help to overcome the hard to cook characteristics of bambara (Ijarotimi & Esho 2009). These processes can also destroy or reduce the antinutrients present in the grain. Afoakwa, Budu & Merson (2007), reported the effect of blanching, soaking and sodium hexametaphosphate salt on certain properties of canned BGN. Blanching and soaking of the seeds before canning reduced phytate and tannin content and the hardness of the grains. However, to maximize the nutritional potential of bambara, there has to be an increase in its utilisation. This is dependent on the awareness and acceptability of the grain by consumers. The acceptability of BGN in South Africa is discussed next.

2.4.4 Acceptability of bambara groundnut in South Africa

Bambara groundnut is an underutilised crop grown mainly for subsistence (Oyeyinka, Singh, Adebola, Gerrano & Amonsou 2015). It is grown by rural dwellers usually for home consumption in South Africa with Limpopo, Mpumalanga and KwaZulu-Natal provinces being the main producing areas (DAFF 2011). The underutilisation of many pulses including bambara may be associated with the limited research on the crop to unlock their potential (Oyeyinka *et al* 2015). In comparison with other well-known pulses such as dry bean consumed in South Africa, bambara remains a crop with less popularity and acceptability especially in urban settlements. This may be attributed to the limited research as previously indicated, hard to cook phenomenon and the strong beany flavor (Uvere, Uwaegbute & Adedeji 1999). To increase the utilisation and promote acceptability of BGN, the knowledge of its utilisation among South Africans needs to be assessed. Further, the potential utilisation of the grains as well as its associated health and nutritional benefits especially in

complementary foods should also be investigated. The next section reviews the desired attributes of complementary foods.

2.5 Desired attributes of complementary foods

2.5.1 Physical, nutritional and sensory properties

The organoleptic properties of a complementary food greatly influence its acceptability. Flavour and texture are key parameters in the initial acceptance of foods by infants as a result of their limited motor oral functioning (Nicklaus 2011; Carruth & Skinner 2002). The aroma of the formulated foods should compare with those of commercially available complementary foods in order for them to be accepted. Complementary foods with low water-holding capacity and hence low viscosity are desired. This enhances the consumption of larger food portion sizes, aids digestion and results in increased nutrient density (Nnam 2001). Efforts have been made to improve the amino acid profile of maize-based foods by complementing them with legumes. A significant increase in nutrient density was observed with the inclusion of soy, cowpea and groundbean tempe in a maize-based complementary food (Egounlety, Aworh, Akingbala, Houben & Nago 2002). Amino acids such as lysine and methionine deficient in cereals and legumes respectively, were found to be present in the cereal-legume food (Egounlety *et al* 2002).

2.5.2 Bambara groundnut as a complementary food

The use of BGN as a complementary food has the potential to improve nutrient intake in infants and young children. This seems plausible since previous studies on maize-based foods combined with BGN showed an improvement in the nutrient content, especially protein (Mbata, Ikenebomeh & Alaneme 2009). Various other studies on complementary foods reported an improvement in the nutrient profile of cereals and tubers fortified with legumes (Vilakati, MacIntyre, Oelofse & Taylor 2015; Sanoussi, Dansi, Bokossa-yaou, Dansi & Egounlety 2013; Tou, Mouquet-Rivier, Picq, Traoré, Trèche & Guyot 2007; Ijarotimi & Aroge 2005; Sanni, Onilude & Ibidapo 1999).

Bambara groundnut flour was found to influence the functional properties such as water binding and swelling capacity of composite flour blended from bambara, sorghum and sweet potato (Nnam 2001). In general, sensory properties of a food depend on the characteristics of the different components in the food (Ijarotimi & Aroge 2005). Therefore, the organoleptic properties, especially colour and aroma of the formulated food may also be influenced

depending on the variety of bambara used. Ijarotimi & Keshinro (2013) investigated the potential of fermented popcorn, African locust beans and bambara groundnut seed flour at different ratios in complementary foods. Blends with 70% fermented popcorn, 20% bambara groundnut and 10% African locust beans reportedly had superior nutrient profile compared to blends of either fermented popcorn and bambara groundnut or fermented popcorn and African locust beans. Hence, bambara groundnut is a promising component for formulating complementary foods.

2.6 Conclusion

PEM is detrimental at any point in life although its occurrence in early childhood has been shown to have lifelong effects. PEM is fairly common worldwide, but more prevalent in developing countries, including South Africa. Infections and diseases, ignorance and insufficient foods are some of the causes of PEM. Malnutrition is commonly observed in infants during the period of complementary feeding due to the use of weaning foods which lack energy and nutrients. Complementary feeding practices in South Africa largely involve the use of maize-based complementary foods, which do not completely meet the nutritional requirements of infants, especially protein requirements. BGN is usually referred to as a “complete food” due to its high nutrient density. Use of BGN in complementary feeding may reduce the prevalence of PEM, especially in poor rural communities. Presently, there are limited studies on the use of bambara in South African complementary foods. The potential to reduce the prevalence of PEM through the inclusion of BGN in complementary foods is also dependent on its acceptability to consumers. However, the acceptability of these complementary foods may be a challenge due to the unfamiliar sensory properties of the BGN to the majority of South Africans, including children and caregivers. Currently, it is not known whether or not and in what food form the caregivers in South Africa would be willing to use bambara in complementary feeding. Therefore, this study aimed to use BGN to formulate a complementary food with the potential to improve the nutritional status, especially protein and energy of populations at risk of malnutrition.

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CHAPTER 3

BACKGROUND TO STUDY DESIGN AND STUDY SITE

This chapter gives background information on the study design, study site and ethical approvals.

3.1 Study design

The study design is shown in Figure 3.1.

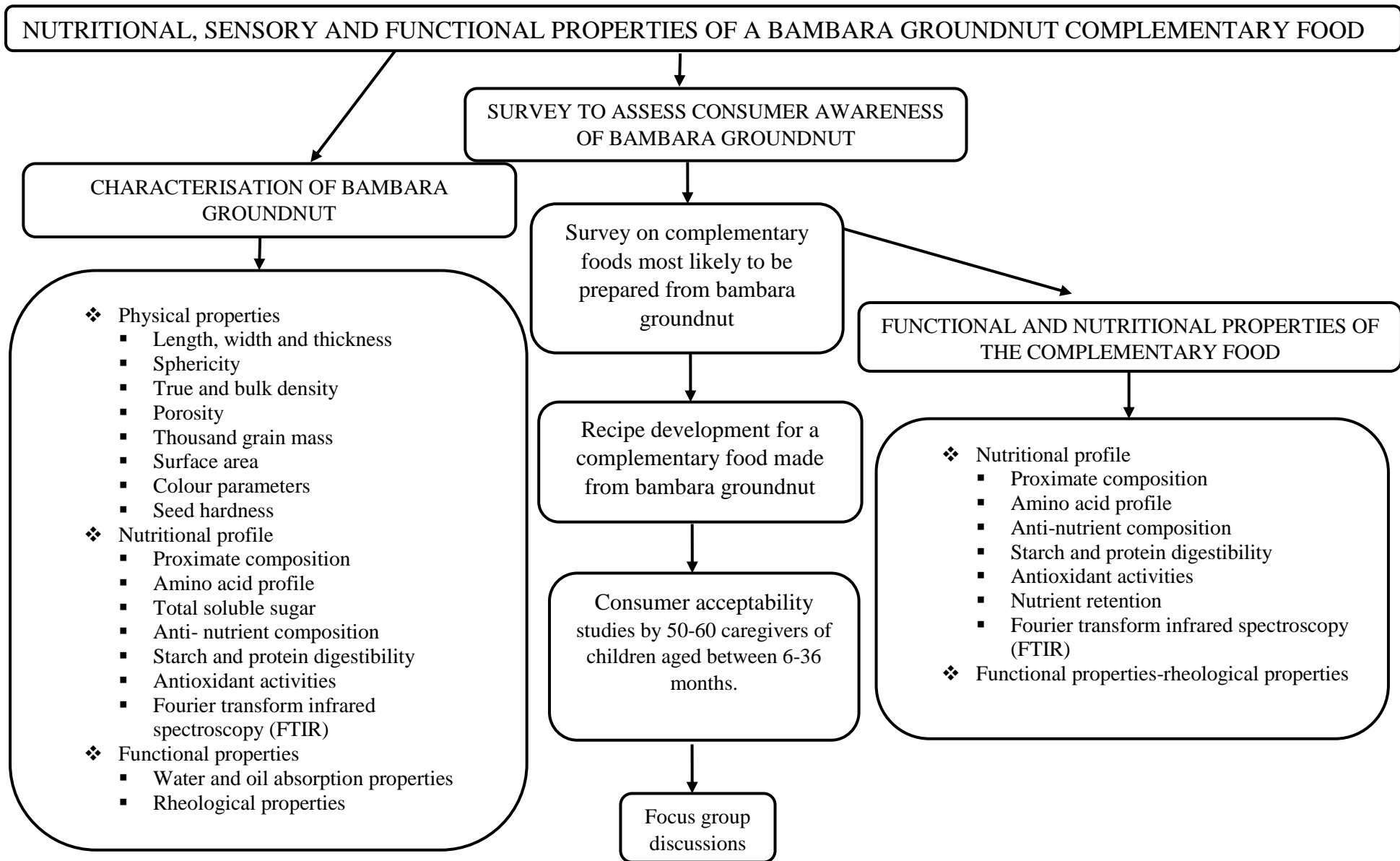


Figure 3.1 Study design

3.2 Background information on the study site

This study was conducted in KwaZulu-Natal (KZN), which is one of nine provinces in South Africa. KZN is situated in the southeast of the country, is 92 285 km² in area and has three geographic areas (Eeley, Lawes & Piper 1999). KZN has been described as one of the poorest provinces in South Africa with a high prevalence of malnutrition (Aliber 2003). The Health Systems Trust (HST) reported that 63 to 82% of households in KZN live on less than R800 per month (HST 2010). In 2008, about 2902 million children residing in KZN were reported be living in income poverty (DoH 2014). In addition about 28% of 6115 households were reported to be experiencing hunger in KZN in 2012 (Shisana *et al* 2013). According to Carter & May (1999), in South Africa, poverty is most prevalent among the black rural African population. Hence, a rural settlement in KZN with a large black African population was selected for this study.

The consumer awareness and acceptability study was conducted at uMshwathi Municipality, located on the northeast quadrant of the uMgungundlovu district in the KZN province (Figure 3.2). The Municipality covers an area of about 1 811 km² with a total population of about 106 374 and a gender ratio of 90 males per 100 females (uMshwathi Municipality 2010/2011). The site was selected because of its large black population (95.1%) compared to other races; Coloured (0.2%), Indian/Asian (1.7%), White (2.7%) and others (0.2%) as well as its classification as a rural settlement (uMshwathi Municipality 2010/2011).

The predominant language spoken by black Africans in uMshwathi local Municipality is isiZulu with agriculture, manufacturing and tourism being the dominant employment industries. Dependency ratio of the site is relatively high (61.2 per 100) with only about 32% of the working age (15-64 years) employed (uMshwathi Municipality 2010/2011).



Figure 3.2 Map of uMgungundlovu District Municipality showing the local municipalities

The caregivers used as subjects in this study were drawn from the paediatric outpatient clinic at the Gcumisa clinic, Swayimane (Figure 3.3). They were assessed on their awareness of BGN as a food source and also participated in the consumer acceptability study of the BGN complementary food product. The Gcumisa clinic is a primary health care facility that provides medical treatment to infants and children less than three years old (Info4africa 2014).



Figure 3.3 Site of consumer awareness and acceptability studies

3.3 Plant materials used in study

Two popularly cultivated BGN landraces (red and brown) were obtained from Buttercombe farm, Selous communal lands, Chegutu, Mashonaland West, Zimbabwe (Figure 3.4). The location received a mean annual precipitation of 650 mm and a mean temperature of 18.9°C during the 2014/2015 cropping season. A popular variety of dry bean (Figure 3.4) was used as a reference and was obtained from the Ukulinga research farm of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. The location received a mean annual precipitation of 790 mm and a mean temperature of 17.6°C during the 2013/2014 cropping season. The red and brown bambara landraces were selected because they are the most commonly consumed landraces in southern Africa.



Figure 3.4 A - red bambara, B - brown bambara, C - Ukulinga dry bean

3.4 Approvals to conduct study

Ethical approval was obtained from the University of KwaZulu-Natal, Humanities and Social Science Ethics Committee (HSS/0740/015D) (Appendix A). The Umgungundlovu health district issued a supporting letter for the research to be conducted (Appendix B). The Department of Health granted permission for the study to be conducted at Gcumisa clinic, Swayimane (HRKM95/12) (Appendix C).

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CHAPTER 4

¹PHYSICAL, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF GRAINS OF BAMBARA GROUNDNUT LANDRACES

Abstract

Bambara groundnut (*Vigna subterranea*) is an underutilised legume crop in SSA where undernutrition and poor health are prevalent. A few types (landraces) of BGNs are popular and are traditionally consumed in rural households. These households employ traditional methods to cultivate and process the bambara groundnuts into traditional food products, including weaning porridges and composites of whole or broken grains of bambara and maize. Knowledge of the physical, chemical and functional properties of the popular BGN landraces would enable their optimal processing and utilisation for human nutrition and health. In this study, the physical, nutritional and functional properties of grains of popular bambara landraces (varieties) were determined. The impact of different traditional processing methods on the nutritional and health-promoting potential (antioxidant properties) of bambara grains was also assessed. The physical, nutritional and antioxidant properties of the grains differed significantly ($p \leq 0.05$) with variety and processing methods. Red bambara had a significantly ($p \leq 0.05$) lower L^* colour value (26.64) compared to the brown variety (41.99) and the reference dry bean (45.69). Bambara grains had a relatively higher sphericity value (0.842 and 0.823 for brown and red variety, respectively) than the reference dry bean (0.585). The brown bambara grains displayed the highest resistance to cutting. Protein content of the bambara grains (23.25 g/100 g and 19.22 g/100 g for brown and red bambara respectively) were significantly ($p \leq 0.05$) lower than the reference dry bean (27.36 g/100 g). However, protein and carbohydrate content of all (dry bean and bambara) grains increased upon dehulling, whilst roasting slightly reduced the protein content of the bambara grains. All the processing methods employed significantly ($p \leq 0.05$) reduced anti-nutrient levels in the grains. These anti-nutritional factors likely had a negative effect on the bioavailability of BGN protein as *in vitro* protein digestibility of all the samples increased as their (anti-nutritional factors) levels decreased upon processing. Dehulling resulted in approximately 83% and 18% decrease in total phenolic content (TPC) and antioxidant activity

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of the grains, respectively. However, roasting increased the TPC of all the flours. Sifting further increased the TPC of the roasted reference bean but reduced those of the bambara samples. Bambara grains have the potential to make a significant contribution towards meeting the nutritional requirements of poor communities. Overall, the quality of the bambara grains was comparable to that of the reference dry bean. Bambara grain could therefore serve as a good source of both macro- and micro nutrients including antioxidants, especially in low socio-economic communities vulnerable to malnutrition.

4.1 Introduction

Bambara groundnut (*Vigna subterranea*) is a legume indigenous to Africa grown mainly for its edible seeds (Bamshaiye, Adegbola & Bamshaiye 2011). It has its origin in the Sahelian region of West Africa from the Bambara tribe where it got its name (Nwanna, Enujiugha, Oseni & Nwanna 2005). In Africa, BGN is regarded as the third most important crop after groundnuts (*Arachis hypogea*) and cowpeas (*Vigna unguiculata*) DAFF (2011).

The plant has various agronomic benefits such as its high nutritional value, ability to produce in poor soils, high drought resistance and relative resistance to pests and diseases (Bamshaiye *et al* 2011; Nwanna *et al* 2005). Its resistance to pests and diseases may be due to the presence of certain secondary metabolites (phenolic compounds) that act as biocides and influence yield stability (Hillocks, Bennett & Mponda 2012). Like most legumes, it can serve as an alternative and inexpensive source of high quality protein for both humans and animals. Bambara grains may show variable composition depending on origin, genetic, environmental and processing conditions. The protein (15-28%) and carbohydrate (42-67%) content of bambara grains (Arise, Amonsou & Ijabadeniyi 2015; Abiodun & Adepeju 2011; Yusuf, Ayedun & Sanni 2008) are similar to those of pigeon pea (Ghadge, Shewalkar & Wankhede 2010) and cowpea (Oyeyinka, Oyeyinka, Karim, Kayode, Balogun & Balogun 2013). Bambara has been reported to have comparable lysine content (0.419 g/gN) (Apata & Ologhobo 1994) with that of soybean (0.422 g/gN) (Petzke, Ezeagu, Proll, Akinsoyinu & Metges 1997) and superior methionine content (0.08 g/gN) (Apata & Ologhobo 1994) to soybean (0.069 g/gN) (Petzke *et al* 1997) and most legumes (Bamshaiye *et al* 2011).

Despite its potential, BGN remains a neglected and underutilised crop in SSA mainly cultivated for subsistence (Oyeyinka, Singh, Adebola, Gerrano & Amonsou 2015). In southern Africa, fresh bambara grains are consumed by boiling or eaten as a relish with maize meal porridge (Swanevelder 1998). Mature dried grains are milled into flours, which are cooked alone or included in a variety of dishes. According to Oyeyinka *et al* (2015) the underutilisation of many legume crops including bambara may be attributed to very limited information on their science and technology. The presence of beany flavours, processing challenges (particularly prolonged cooking time) and high antinutrient content have also been suggested to hinder the optimal utilisation of bambara as a food (Ijarotimi & Esho 2009). Furthermore, the bambara plant grows close to the ground and nuts are produced underground, hence making mechanised harvesting difficult (Hillocks *et al* 2012; Williams 1993, p19). The chemical properties, including nutritional and antioxidant properties, of legume grains are influenced by processing methods such as germination, fermentation, soaking, dehulling and roasting (Fasoyiro, Widodo & Kehinde 2012; Jain, Kumar & Panwar 2009). These processing methods have been found to have varying influence on nutrient and antioxidant properties of legumes (Xu & Chang 2008; Boateng, Verghese, Walker & Ogutu 2008; Jeong, Kim, Kim, Nam, Ahn & Lee 2004). For instance, some researchers reported that the tannin content of bambara grains increased after roasting (Ijarotimi & Esho 2009), while others reported a reduction in the tannin content (Barampama & Simard 1995). Besides changes in chemical properties, processing methods may also cause changes in functional properties such as water and oil absorption capacities (Adegunwa, Adebowale, Bakare & Kalejaiye 2014; Abiodun & Adepeju 2011; Yusuf *et al* 2008). Roasting bambara at 120°C for 20 min reportedly increased the water and oil absorption capacity of bambara flour from 174 to 210% and 150 to 170% respectively (Yusuf *et al* 2008).

The influence of processing methods on nutrient, functional and antioxidant properties may depend on legume type and processing methods. Boateng *et al* (2008), working with red kidney bean, black eyed pea, pinto bean and soybean observed varying effects of soaking and roasting on antioxidant properties of these legumes. Roasting was found to result in higher reduction (approx. 10%) in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity value of black eyed pea compared to soaking which caused approx. 2% reduction. However, roasting and soaking showed similar reduction (approx. 7%) in the DPPH value for kidney bean (Boateng *et*

al 2008). Other authors reported that the antioxidant activity of defatted sesame meal extract increased with increasing roasting temperature reaching a maximum activity when seeds were roasted at 200°C for 60 min (Jeong *et al* 2004). The physical properties of legume grains may be related to the chemical properties of the grains. In most reports, beans with darker seed coats, such as kidney beans and pinto beans have been found exhibiting significantly higher total phenolic content compared to those with lighter seed coats like soybeans (Boateng *et al* 2008; Barampama & Simard 1995). The physical properties of BGN and other grains are also important for the design of handling and processing equipment (Baryeh 2001; Olajide & Ade-Omowaye 1999). There are reports on the physical properties of bambara grains grown in different regions of sub-Saharan Africa (Mpotokwane, Gaditlhatlhelwe, Sebaka & Jideani 2008; Kaptso, Njintang, Komnek, Hounhouigan, Scher & Mbofung 2008; Baryeh 2001). A higher geometric mean diameter was reported for BGN grown in Cameroon (Kaptso *et al* 2008) compared to those grown in Botswana (Mpotokwane *et al* 2008). However, the geometric mean diameters for both bambara cultivars (Mpotokwane *et al* 2008; Kaptso *et al* 2008) were higher than the values reported for cowpea varieties (Kaptso *et al* 2008).

In southern Africa, there are a few types (landraces) of BGNs that are popular and traditional with rural households. These households use indigenous knowledge systems to cultivate and process the bambara grains into traditional food products, including weaning porridges and composites of whole or broken grains of bambara and maize. It appears that the popular bambara landraces have been selected on the basis of desirable properties, which are likely to include agronomic, processing, nutritional and sensory properties. Knowledge of the physical and chemical properties of the popular BGN landraces would enable their optimal processing and maximal utilisation for human nutrition and health. In this study, the physical, nutritional and functional properties of grains of popular bambara landraces were determined, and the effects of different processing methods on these properties assessed.

4.2 Materials and Methods

4.2.1 Bambara groundnut landraces

The bambara grains used in this study were the brown and red varieties (Figure 4.1). They were obtained from Selous communal lands, Mashonaland West, Zimbabwe. The location received a

mean annual precipitation of 650 mm and mean a temperature of 18.9°C during the 2014/2015 cropping season. A brown Ukulinga dry bean obtained from the Ukulinga research farm of the University of KwaZulu-Natal, Pietermaritzburg, South Africa was used as the control. The location received a mean annual precipitation of 790 mm and a mean temperature of 17.6°C during the 2013/2014 cropping season. These varieties were used to prepare complementary food products (bambara puree) (Chapter 5 and 6).

4.2.2 Physical properties of bambara grains

Grain of each of the BGN as well as the control Ukulinga dry bean were analysed for selected physical properties *viz*; sphericity, one thousand grain mass, bulk and true densities, porosity, surface area, colour and hardness.

4.2.2.1 Sphericity

Sphericity of the grains was determined as described by Olajide & Ade-Omowaye (1999). The length (L), width (W) and thickness (T) of one-hundred randomly selected seeds were measured with a micrometer reading to 0.001 mm. The geometric mean diameter D_p of the seed was calculated using the relationship: $D_p = [LWT]^{1/3}$ (Mohsenin 1970).

The degree of sphericity $\Phi = \frac{[LWT]^{1/3}}{L}$ (Mohsenin 1970).

4.2.2.2 True and bulk density

The bulk density (ρ_b) is defined as the ratio of the mass of a sample of grain to the solid volume occupied by the sample. The true density which is defined as the ratio between the mass of seed and true volume of seed was determined using the water displacement method as described by Mpotokwane *et al* (2008) The volume of water displaced was found by immersing a weighed quantity of bambara seed in the water.

4.2.2.3 Porosity

Porosity (ϵ) of the grain was determined as described by Baryeh (2001); given as $\epsilon = \frac{[(\rho_t - \rho_b)100]}{\rho_t}$

Where ρ_t is the true density and ρ_b is the bulk density.

4.2.2.4 Surface area

The surface area (S) of bambara grains was found by analogy with a sphere of the same geometric mean diameter (Olajide & Ade-Omowaye 1999):

$$S = \pi D_p^2$$

where D_p – geometric mean diameter

4.2.2.5 One thousand grain mass

One thousand grain mass of BGN was selected randomly from the sample lot and the weight measured (Baryeh 2001).

4.2.2.6 Colour

Colour measurement of the flour samples were determined using a colour flex on the basis of lightness (L^*), red-green (a^*) and yellow-blue (b^*) values. The instrument was calibrated against white and black coloured tiles before taking colour measurements.

4.2.2.7 Hardness

The hardness of the seeds was determined at approximately 15, 20 and 30% (db) moisture contents. The grains were brought to the desired moisture contents as described by Yalçın (2007) with some modifications. A calculated amount (Q) of distilled water was added to the grains and they were tightly sealed in polythene bags and kept at 4°C for 5 days.

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

Where W_i is the initial mass of grain (g), M_i is the initial moisture content of grains (% , dry basis) and M_f is the final moisture content (% , dry basis).

The hardness of the grains was measured using TA-XT2 Plus Texture Analyser (Stable Micro System, Goldalming, UK) as described by Afoakwa & Yenyi (2006) with slight modifications. Three seeds were placed longitudinally across the groove of the platform and the force required by the texture analyser probe, the Warner-Bratzler Blade to cut perpendicularly through the seeds was measured at a crosshead speed of 2.0 mm/s. The test was replicated five times and the average peak force recorded.

4.2.3 Nutritional composition of the bambara grains

The raw and processed (as described below) bambara grains were analysed for their nutritional composition using referenced methods. Analytical reagents were used and analysis of nutrients in samples was replicated thrice except otherwise stated.

4.2.3.1 Grain processing

The legume grains were divided into three equal portions. A portion of the grains were dry milled using a disc attrition mill (Retsch KG 5657, Haan, West Germany). Another portion of the grains were manually dehulled after soaking in water (1:4 w/v) for 12 h. The dehulled samples were oven dried (Prolab Air oven dryer) at 60°C for 24 h. The third part of the grains were roasted at 150°C for 35 min (Nti 2009) in a Juno air-o-steam oven. The processed flours were divided into two and a portion each of the flours were milled and sifted using a 355 µm sieve while the other was not. Both were packed and sealed in plastic bags and stored at 4°C for analysis.

4.2.3.2 Moisture

The moisture content of the samples was measured according to the AOAC Official Method 934.01 (2002). The samples were dried at 95°C for 72 hours in an air-circulated oven. The weight loss of the samples was used to calculate the moisture content.

4.2.3.3 Fat

The fat content of the samples was determined according to the Soxhlet procedure, using a Büchi 810 Soxhlet Fat extractor (Büchi, Flawil, Switzerland) according to the AOAC Official Method 920.39 (AOAC 2002). Petroleum ether was used for extraction.

4.2.3.4 Protein

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

4.2.3.5 Carbohydrate

Carbohydrate content was determined by difference (Yusuf *et al* 2008).

4.2.3.6 Ash

The total mineral content of the samples was determined by combusting the samples in a furnace set at 550 °C for 4 hours (AOAC 2002).

4.2.3.7 Minerals

Selected minerals were determined as described by Giron (1973) & AOAC (2002). Samples (1g) were dried for 2 hrs at 105 °C and ashed in a muffle furnace for a minimum of 4 h at 550 °C. The remaining ash was slowly hydrated with 2 ml deionized H₂O then 2 ml of concentrated HCl, diluted to 20 ml with deionized H₂O, then boiled to dryness in a water bath. The remaining ash was then hydrated a second time with 10 ml HCl (10 %), boiled vigorously and refluxed for about 5 min to ensure that minerals were brought into solution. After 24 h Ca, Mg, Cu, Fe, Zn and Mn were analyzed by atomic absorption spectrophotometry, K and Na by atomic emission spectrophotometry, and P by colorimetry.

4.2.4 Anti nutrient composition

4.2.4.1 Tannin content determination

Tannin content was determined using the modified vanillin-HCl method as described by Mazahib, Nuha, Salawa & Babiker (2013). Briefly, 0.2 g sample was extracted with 10 ml 1% (v/v) conc. HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to 1 ml extract and absorbance read after 20 min at 500 nm. A catechin standard curve was prepared expressing the results as catechin equivalents.

4.2.4.2 Phytic acid determination

Phytic acid content of the samples was determined as described by Aina, Binta, Amina, Hauwa Haruna, Hauwa, Akinboboye & Mohammed (2012). Briefly 2 g of sample was weighed into a 250 ml conical flask and soaked in 100 ml 2% conc. HCl for 3 h and then filtered with Whatman No. 1 filter paper. Ten milliliters of distilled water was added to 50 ml filtrate and 10 ml ammonium thiocyanate solution (0.3%) was added as indicator. The solution was titrated with

standard iron II chloride containing 0.00195 g iron/ml until the yellow colour persisted for 5 min.

The percentage phytic acid was expressed as:

$$\% \text{ Phytic acid} = y \times 1.19 \times 100$$

Where y = titre value \times 0.00195

4.2.5 Protein digestibility

Protein digestibility was assayed using a modified pH-drop procedure as described by Tinus, Damour, Van Riel & Sopade (2012). Ten milliliters of milli-Q water was added to flour samples containing equivalent of 62.5 mg protein. After 1 h at 37°C, the pH was adjusted to 8.0 with 0.1 mol/L NaOH and/or HCl. A multi-enzyme solution consisting 16 mg trypsin, 31 mg chymotrypsin and 13 mg protease was prepared with pH adjusted to about 8.0. One milliliter of the multi-enzyme solution was added to the 10 mL sample dispersion and pH of the digesta was recorded after 10 min using a pH meter. The change in pH at 10 min of digestion was used to calculate the percent in vitro protein digestibility (IVPD) using the following equation

$$\text{IVPD} = 65.66 + 18.10\Delta\text{pH}_{10\text{min}}$$

4.2.6 Antioxidant activity of bambara grains

4.2.6.1 Total phenolic content (TPC) determination

Phenols were determined as described by Tesfay, Bertling, Odindo, Workneh & Mathaba (2011) with slight modifications. Briefly, 0.2 g of BGN flour was mixed with 10 ml methanol [99.8% (v/v)] and vortexed for 30 s. The mixture was left overnight at room temperature and subsequently decanted to extract the free phenols. Membrane-bound phenols were released from the remaining flour residue by acid hydrolysis. A 10 ml portion of acidified (2 M hydrochloric acid) 60% (v/v) aqueous methanol was added to each sample, which was then incubated at 90°C for 90 min. Samples were allowed to cool before the supernatant was filtered and analysed for phenols. Free and membrane-bound phenols concentrations were determined by a spectrophotometer at 765 nm. Five milliliters of distilled water, 1 mL Folin Ciocalteu reagent, 10 mL 7 % sodium carbonate and 8 mL distilled water was added to 1 mL of the extract. The solution was incubated for 3 hours in a dark room and absorbance read. The result was expressed as catechin equivalents.

4.2.6.2 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay

Samples were extracted by adding 10 mL acidified methanol (1 % HCl in methanol) to 0.2 g of flour samples. The solution was allowed to stand for 1 h after which the extracts were decanted (Siwela, Taylor, de Milliano & Duodu 2007). ABTS radical scavenging assay was carried out as described by Adedapo, Jimoh, Koduru, Masika & Afolayan (2009). Stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulfate solution were prepared. Working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS^{•+} solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS^{•+} solution was prepared for each assay. Exactly 1 ml of extract was allowed to react with 1 ml of the ABTS^{•+} solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer (Shimadzu UV 1800). The percentage inhibition was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = [(\text{Ab}_{\text{Scontrol}} - \text{Ab}_{\text{Ssample}})/(\text{Ab}_{\text{Scontrol}})] \times 100$$

where; $\text{Ab}_{\text{Scontrol}}$ is the absorbance of ABTS radical + methanol; $\text{Ab}_{\text{Ssample}}$ is the absorbance of ABTS radical + sample extract/standard.

4.2.6.3 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging assay was done as described by Moodley, Amonsou & Kumar (2015) with slight modification. Briefly, a solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of the extracted sample. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically using a Shimadzu UV 1800 at 517 nm. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{Radical scavenging activity (\%)} = [(\text{Ab}_{\text{Scontrol}} - \text{Ab}_{\text{Ssample}})/(\text{Ab}_{\text{Scontrol}})] \times 100$$

Where $\text{Ab}_{\text{Scontrol}}$ is the absorbance of DPPH radical+methanol; $\text{Ab}_{\text{Ssample}}$ is the absorbance of DPPH radical+sample extract/standard.

4.2.7 Functional properties of flours

4.2.7.1 Water and oil absorption capacities

The water absorption capacity (WAC) and oil absorption capacity (OAC) of the flour samples was determined using methods described by Elkhailifa, Schiffler & Bernhardt (2005). Two grammes each of the BGN flour samples was weighed into a pre-weighed centrifuge tube and 20 ml of distilled water was added. For oil absorption, 20 ml sunflower oil was added. Samples were mixed and allowed to stand for 30 min at $25 \pm 2^\circ\text{C}$ before being centrifuged at $4000\times g$ for 25 min. Excess water or oil was decanted by inverting the tubes over absorbent paper and samples were allowed to drain. The weights of water and oil bound in the samples were determined by difference.

4.2.7.2 Dynamic rheology

This was measured using the modified method of Oyeyinka *et al* (2015). The samples (10% w/v) were gelatinised at 95°C for 30 min in centrifuge tubes and rapidly transferred into the Rheolab cup. Samples were allowed to equilibrate at 60°C for 10 min and measurement was taken at shear rates ranging between 10 and 1000 s^{-1} . The data was fitted into a Power-Law Model (Barnes, Hulton & Walters 1989, pp11-35) as follows:

$$\tau = k\gamma^n$$

Where τ is the shear stress (Pa), k is the consistency coefficient, $(\text{Pa}\cdot\text{s})^n$, γ is the shear rate (s^{-1}) and n is the flow behaviour index.

4.2.8 Statistical analyses

All experiments were conducted in triplicate except otherwise indicated. Data were analysed using analysis of variance (ANOVA) and means were compared using the Fischer's Least Significant Difference Test ($p \leq 0.05$).

4.3 Results and Discussion

4.3.1 Physical properties of bambara grains

Bambara groundnuts and Ukulinga bean showed significant differences ($p < 0.05$) in their physical properties (Table 4.1).

Table 4.1 Physical properties of bambara grains and Ukulinga dry bean

Parameter	Ukulinga Dry bean	Brown bambara	Red bambara
Length (mm)	16.054 ^a ± 1.09	12.112 ^c ± 1.57	13.524 ^b ± 1.57
Width (mm)	6.120 ^c ± 0.56	9.601 ^b ± 1.00	9.974 ^a ± 1.78
Thickness (mm)	8.411 ^c ± 0.59	9.005 ^b ± 0.93	10.171 ^a ± 0.99
Mean diameter (mm)	9.373 ^c ± 0.57	10.136 ^b ± 0.97	11.098 ^a ± 1.11
Sphericity	0.585 ^c ± 0.02	0.842 ^a ± 0.06	0.823 ^b ± 0.04
Bulk density (g/cm ³)	0.716 ^a ± 0.04	0.706 ^a ± 0.12	0.676 ^b ± 0.03
True density (g/cm ³)	1.110 ^b ± 0.000	1.227 ^a ± 0.04	1.213 ^a ± 0.04
Porosity (%)	35.47 ^b ± 0.42	42.42 ^a ± 1.79	44.28 ^a ± 1.78
1000 grain mass (g)	639.30 ^b ± 0.027	579.10 ^c ± 0.010	758.67 ^a ± 0.002
Surface area (mm ²)	277.04 ^c ± 3.30	325.77 ^b ± 6.21	390.81 ^a ± 7.46
L*	45.69 ^a ± 0.01	41.99 ^b ± 0.49	26.64 ^c ± 0.02
a*	12.20 ^c ± 0.01	17.72 ^b ± 0.04	19.92 ^a ± 0.03
b*	17.04 ^b ± 0.01	28.09 ^a ± 0.68	13.17 ^b ± 0.01

Mean ± SD. Means with different superscripts in a row are significantly different ($p \leq 0.05$); L*-lightness, a*-red-green and b*-yellow-blue (HunterLab values)
SD: Standard deviation

The reference Ukulinga bean was longer (average length: 16.054 mm) than the brown (average length: 12.112 mm) and red bambara (average length: 13.524 mm) grains. The slight variation in the size (length and thickness) of bambara grains may be attributed to differences in the genetic factors. Previous studies similarly reported higher length for red bambara compared to other landraces (Mpotokwane *et al* 2008; Nape 1995).

Both red and brown bambara showed significantly ($p \leq 0.05$) higher widths, thickness, mean diameter surface area and sphericity than the Ukulinga bean. According to Eke, Asoegwu & Nwandikom (2007), grains with sphericity value greater than 0.7 are close to the shape of a sphere. The high sphericity values (approx. 0.833) of the bambara grains compared to that of Ukulinga bean (0.585) suggest that bambara grains are closer to a sphere than the reference bean. Thus, bambara grains will roll well rather than slide during conveyance in hoppers or other transport medium. Grain sphericity is important in the design of hoppers chutes and other storage facilities (Mpotokwane *et al* 2008).

The thousand grain mass of the legume grains were higher in the order red bambara > Ukulinga bean > brown bambara, while bulk density was higher in the order Ukulinga bean > brown bambara > red bambara. The red bambara had the highest porosity among the legume samples.

The physical properties including length, width, thickness, sphericity and a thousand grain mass of bambara grains in this study agrees with previous reports on bambara (Mpotokwane *et al* 2008; Baryeh 2001). Mpotokwane *et al* (2008) reported an average length of 0.012 m, width of 0.0096 m and thickness of 0.0092 m for five BGN landraces grown in Botswana. The lightness (Hunter L value) of brown bambara grain was similar to that of dry bean, but almost double that of the red variety. The relatively lower Hunter L value of red bambara grain, which showed correspondingly higher redness as indicated by its Hunter “a” value (19.92), could possibly be attributed to its higher phenolic content (Table 4.3). Phenolic substances, especially tannins, have been suggested to influence grain colour since they are concentrated in the seed coat (Bamshaiye *et al* 2011).

The hardness of the legumes decreased in the order brown bambara > red bambara > Ukulinga dry bean (Figure 4.2).

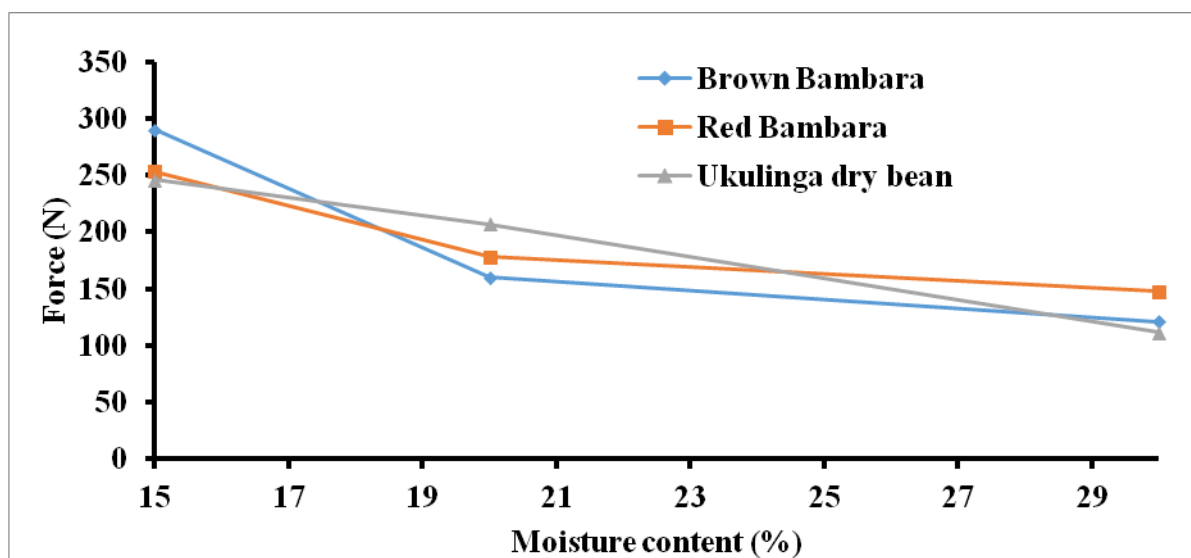


Figure 4.1 Effect of moisture content on the hardness of legume seeds

The force required to cut the grains reported in this study are similar to those reported by Ajayi & Lale (2000) for different bambara varieties. As expected, the hardness of all the legumes reduced with increasing moisture. An increase in moisture content from 15 to 20% decreased the hardness of the seeds by approximately 45, 29 and 15% for the brown bambara, red bambara and, Ukulinga bean respectively. Other studies have reported a reduction in sorghum and

sunflower grain hardness with increase in moisture content from 15 to 30% (Jafari, Khazaei, Arabhosseini, Massah & Khoshtaghaza 2011; Mwithiga & Sifuna 2006). Conditioning of legume grains may be essential in facilitating dehulling and milling processes.

4.3.2 Nutritional composition of the bambara grains

Data on proximate composition of bambara and the Ukulinga bean showed that protein and carbohydrate are the major components of the grains for both raw and dehulled grains (Table 4.2a).

Table 4. 2a Nutrient composition of bambara grains and Ukulinga dry bean (g/100 g)

Sample	Processing	Moisture	Protein	Fat	Ash	Carbohydrate
Brown bambara	Raw	8.71 ^c ± 0.19	23.25 ^e ± 0.01	7.09 ^d ± 0.52	4.52 ^b ± 0.09	56.42 ^c ± 0.74
Brown bambara	Dehulled	4.50 ^f ± 0.30	25.48 ^d ± 0.16	8.14 ^b ± 0.01	4.57 ^b ± 0.01	57.33 ^b ± 0.46
Brown bambara	Roasted	5.15 ^{ef} ± 0.21	22.81 ^f ± 0.31	8.53 ^{ab} ± 0.11	4.79 ^a ± 0.02	58.72 ^b ± 0.14
Red bambara	Raw	10.90 ^b ± 0.15	19.22 ^h ± 0.18	7.58 ^c ± 0.05	4.18 ^d ± 0.03	58.13 ^b ± 0.11
Red bambara	Dehulled	5.80 ^e ± 1.67	20.64 ^g ± 0.43	8.29 ^b ± 0.22	4.06 ^b ± 0.11	61.21 ^a ± 2.39
Red bambara	Roasted	5.94 ^e ± 0.08	18.88 ^h ± 0.25	8.86 ^a ± 0.24	4.14 ^d ± 0.05	62.18 ^a ± 0.31
Ukulinga dry bean	Raw	14.79 ^a ± 0.27	27.36 ^c ± 0.24	1.53 ^f ± 0.10	4.70 ^a ± 0.13	51.61 ^d ± 0.26
Ukulinga dry bean	Dehulled	3.20 ^g ± 0.32	28.34 ^b ± 0.10	1.58 ^f ± 0.11	4.34 ^c ± 0.11	62.54 ^a ± 0.46
Ukulinga dry bean	Roasted	7.43 ^d ± 0.15	29.45 ^a ± 0.28	2.62 ^e ± 0.38	4.06 ^d ± 0.50	56.44 ^c ± 0.74

Mean ± SD

Means with different superscripts in a column are significantly different ($p \leq 0.05$)

SD: Standard deviation

The protein content of raw Ukulinga bean (27.36 g/100 g) was significantly ($p \leq 0.05$) higher than that of red (19.22 g/100 g) and brown (23.25 g/100 g) bambara grains. After dehulling, both the reference bean and the bambara grains had higher protein content than the raw samples. The inner layers of the legume grain are largely comprised of cotyledon tissues, which contain a large proportion of the grain protein. Therefore, the observed increase in protein concentration upon dehulling may be due to an increase in the components of the grain inner layers (Abiodun & Adepeju 2011; Nti 2009). Some authors, working with bambara and other legumes, similarly reported higher protein content for dehulled grains compared to the undeulled samples (Abiodun & Adepeju 2011; Ghavidel & Prakash 2007). Although the dehulled grains showed

higher protein concentration as indicated above, dehulled Ukulinga bean had relatively higher (approx. 1.3 times) protein content than the dehulled bambara grains. Roasting on the other hand reduced the content of the bambara grains but increased that of the reference dry bean. A reduction in protein content after roasting bambara grains has also been reported in literature (Yusuf *et al* 2008; Yagoub & Abdalla 2007). The fat and ash contents of bambara and Ukulinga bean were generally low. While the ash contents (approx. 4.44 g/100 g) were not very different among the bambara and Ukulinga bean, for both raw and dehulled, the fat contents showed some variations. Both raw and processed bambara grains had higher fat content (raw; approx. 7.34 g/100 g, dehulled approx. 8.22 g/100 g, roasted approx. 8.70 g/100 g) than the Ukulinga bean (approx. 1.91 g/100 g). The proximate composition of the bambara grains in this study is similar to previous reports for bambara and other legumes like pigeon pea, African yam bean and lima bean (Yusuf *et al* 2008; Omoikhoje, Bamgbose & Aruna 2006; Fasoyiro, Ajibade, Omole, Adeniyani & Farinde 2006; Barampama & Simard 1993).

The mineral composition of bambara and Ukulinga bean samples are presented in Table 4.2b.

Table 4.2b Nutrient composition of bambara grains and Ukulinga dry bean

Sample	Processing	ADF*	NDF*	Ca*	Mg*	K*	Na*	P*	Fe**	Mn**	Cu**	Zn**
Brown bambara	Raw	11.90 ^{cd} ±	32.71 ^b ±	0.053 ^b	0.171 ^{bc} ±	1.669 ^a ±	0.008 ^{bc}	0.456 ^c	47.46 ^c ±	15.33 ^b ±	8.33 ^c ±	27.75 ^b
		0.33	0.46	± 0.001	0.000	0.010	± 0.001	± 0.002	0.17	0.03	0.58	^c ±
Brown bambara	Dehulled	9.31 ^{de} ±	32.06 ^b ±	0.038 ^d	0.175 ^b ±	1.662 ^a ±	0.009 ^{bc}	0.501 ^b	25.13 ^d ±	12.57 ^{bc} ±	9.00 ^c ±	28.33 ^b
		0.59	0.18	± 0.000	0.002	0.017	± 0.001	± 0.003	0.08	0.04	0.00	^c ±
Brown bambara	Roasted	19.23 ^a ±	57.54 ^a ±	0.051 ^b	0.171 ^{bc} ±	1.622 ^a ±	0.003 ^c	0.442 ^d	18.27 ^e ±	12.65 ^{bc} ±	8.01 ^{cd} ±	29.52 ^b
		5.39	9.10	± 0.002	0.002	0.019	± 0.001	± 0.001	1.18	0.28	0.20	± 0.06
Red bambara	Raw	10.63 ^{c-e}	21.44 ^{cd} ±	0.048 ^b	0.166 ^c ±	1.467 ^c ±	0.025 ^{ab}	0.287 ^f	25.44 ^d ±	11.00 ^c ±	6.00 ^{ef} ±	23.94 ^b
		± 0.32	0.91	± 0.001	0.004	0.064	± 0.002	± 0.005	0.65	0.00	0.73	± 0.27
Red bambara	Dehulled	7.13 ^e ±	28.72 ^{bc} ±	0.027 ^e	0.157 ^d ±	1.476 ^c ±	0.030 ^a	0.290 ^f	10.62 ^f ±	8.33 ^d ±	7.00 ^{de} ±	25.48 ^b
		0.55	0.78	± 0.001	0.003	0.017	± 0.004	± 0.007	0.09	0.58	0.00	^c ±
Red bambara	Roasted	17.03 ^{ab} ±	53.95 ^a ±	0.045 ^c	0.169 ^{bc} ±	1.484 ^c ±	0.002 ^c	0.274 ^g	24.81 ^d ±	12.05 ^c ±	5.74 ^f ±	24.10 ^c
		1.61	9.48	± 0.001	0.001	0.029	± 0.000	± 0.003	1.24	1.23	0.21	± 1.25
Ukulinga dry bean	Raw	8.04 ^c ±	18.34 ^d ±	0.147 ^a	0.179 ^a ±	1.570 ^b ±	0.021 ^{ab}	0.548 ^a	82.92 ^a ±	25.82 ^a ±	13.00 ^b ±	43.00 ^a
		0.46	0.84	± 0.005	0.001	0.008	± 0.002	± 0.009	0.80	0.08	0.00	± 1.73
Ukulinga dry bean	Dehulled	7.62 ^e ±	61.62 ^a ±	0.048 ^b	0.160 ^d ±	1.463 ^c ±	0.027 ^{ab}	0.550 ^a	59.92 ^b ±	26.33 ^a ±	14.33 ^a ±	41.00 ^a
		0.47	0.16	± 0.000	0.002	0.012	± 0.008	± 0.005	0.20	0.00	0.49	± 0.00
Ukulinga dry bean	Roasted	13.94 ^{bc}	56.32 ^a ±	0.144 ^a	0.158 ^d ±	1.250 ^d ±	0.014 ^{a-c}	0.389 ^e	58.33 ^b ±	12.96 ^{bc} ±	12.67 ^b ±	30.24 ^b
		± 1.35	8.33	± 0.001	0.000	0.019	± 0.004	± 0.002	2.24	3.73	0.24	± 7.46

Mean ± SD

Means with different superscripts in a column are significantly different (p ≤ 0.05)

ADF- Acid Detergent Fibre, NDF- Neutral Detergent Fibre, *g/100 g, **mg/kg

SD: Standard deviation

Generally, the individual mineral content of all the samples differed significantly ($p \leq 0.05$). The values reported in this study were higher than those of previous studies (Ijarotimi & Esho 2009; Amarteifio & Moholo 1998). This variation may be attributed to the different landraces used and planting conditions. Bambara landraces had comparable individual mineral content with the reference dry bean except for iron which was significantly ($p \leq 0.05$) lower in the bambara landraces. Processing had generally decreased the mineral content levels of all the samples. Abiodun & Adepeju (2011) also reported a decrease in mineral content of dehulled bambara samples. Studies have reported that adequate intake of essential minerals could promote health, growth and cognitive development in children and adults (Ivanovic, Leiva, Pérez, Almagia, Toro, Urrutia, Inzunza & Bosch 2002; Bhan, Sommerfelt & Strand 2001). Bambara groundnut could therefore be a good source of minerals for both adults and children.

4.3.3 Protein digestibility

The effect of processing on the in-vitro protein digestibility is reported in Table 4.3.

Table 4.3 *In-vitro* protein digestibility

Samples	Treatments	IVPD g/100 g	% Increase
Brown bambara	-	71.18 ^c ± 5.76	-
Brown bambara	Sifted	77.52 ^{c-e} ± 0.64	8.91
Brown bambara	Dehulled	82.95 ^{b-e} ± 0.13	16.54
Brown bambara	Dehulled sifted	92.90 ^{ab} ± 13.95	30.51
Brown bambara	Roasted	83.76 ^{b-e} ± 4.60	17.67
Brown bambara	Roasted, sifted	84.03 ^{b-c} ± 0.38	18.05
Red bambara	-	81.41 ^{b-e} ± 2.04	-
Red bambara	Sifted	88.83 ^{a-c} ± 2.56	9.11
Red bambara	Dehulled	90.46 ^{ab} ± 13.57	11.12
Red bambara	Dehulled, sifted	99.78 ^a ± 5.25	22.56
Red bambara	Roasted	81.14 ^{b-e} ± 0.90	0.11
Red bambara	Roasted, sifted	83.12 ^{b-e} ± 0.64	2.10
Ukulinga dry bean	-	74.17 ^{de} ± 3.32	-
Ukulinga dry bean	Sifted	81.41 ^{c-f} ± 1.27	9.76
Ukulinga dry bean	Dehulled	84.12 ^{b-f} ± 0.26	13.42
Ukulinga dry bean	Dehulled, sifted	98.24 ^a ± 3.07	32.45
Ukulinga dry bean	Roasted	89.19 ^{a-c} ± 2.56	20.25
Ukulinga dry bean	Roasted, sifted	88.46 ^{a-c} ± 1.28	19.27

Mean ± SD

Means with different superscripts in a column are significantly different ($p \leq 0.05$)

SD: Standard deviation

Low digestibility of seed protein is one of the main drawbacks limiting the nutritional quality of food legumes. The *in-vitro* protein digestibility (IVPD) observed in this study is similar to those reported for other legumes like kidney beans, chick peas and faba bean (El-Hady & Habiba 2003). All the processing methods employed in this study significantly ($p \leq 0.05$) increased the digestibility of the grains. However, a maximum increase in protein digestibility was observed in dehulled, sifted samples. This may be linked to the lower levels of anti-nutrients in the dehulled-sifted samples (Table 4.4). Tannins and phytic acid have been said to be negatively correlated with protein digestibility (Ghavidel & Prakash 2007). Loss of these anti-nutrients could probably have created a large space within the food matrix to enhance enzymatic activity and consequently increase IVPD (Rehman & Shah 2005). Ghavidel & Prakash (2007) also reported an increased IVPD after dehulling. Heat treatments have been shown to be effective in destroying protease inhibiting activity and lectin (haemagglutinating) activity (Alonso, Aguirre & Marzo 2000). This may explain the increase in IVPD of the roasted samples.

4.3.4 Effect of processing on the antinutrient and antioxidant content and antioxidant activities
Tannins and phytic acids are some of the predominant antinutrients in legumes. The tannin and phytic acid content of the studied legumes are presented in Table 4.4.

Table 4.4 Effect of processing on antioxidant content and activities

Grain type	Treatment	Sieving	Phytic acid*	Tannin*	Membrane bound phenol**	Free Phenol**	Total phenol**
Brown bambara	Raw	-	781.23 ± 0.45 ^a	5.17 ± 0.36 ^a	0.14 ± 0.03 ^{bc}	0.05 ± 0.01 ^{ab}	0.19 ± 0.02 ^{bc}
Brown bambara	Dehulled	-	525.98 ± 0.98 ^{cde}	3.34 ± 0.50 ^{bc}	0.02 ± 0.00 ^e	0.01 ± 0.00 ^{fg}	0.03 ± 0.00 ^e
Brown bambara	Roasted	-	549.19 ± 0.59 ^{bcd}	3.80 ± 0.62 ^b	0.23 ± 0.01 ^a	0.03 ± 0.02 ^{b^cdef}	0.21 ± 0.01 ^{ab}
Brown bambara	Raw	Sifted	556.92 ± 0.15 ^{bcd}	4.96 ± 0.12 ^a	0.16 ± 0.02 ^{bc}	0.04 ± 0.03 ^{abc}	0.20 ± 0.03 ^b
Brown bambara	Dehulled	Sifted	518.24 ± 0.45 ^{cde}	2.79 ± 0.78 ^{cd}	0.04 ± 0.00 ^e	0.02 ± 0.00 ^{cdefg}	0.06 ± 0.00 ^e
Brown bambara	Roasted	Sifted	518.25 ± 0.66 ^{cde}	3.19 ± 0.32 ^{bc}	0.15 ± 0.01 ^{bc}	0.04 ± 0.01 ^{abcde}	0.19 ± 0.02 ^{bc}
Red bambara	Raw	-	587.86 ± 0.47 ^{bc}	5.26 ± 0.44 ^a	0.18 ± 0.08 ^b	0.04 ± 0.01 ^{abcd}	0.22 ± 0.08 ^{ab}
Red bambara	Dehulled	-	394.49 ± 0.19 ^{ef}	3.47 ± 0.31 ^{bc}	0.03 ± 0.00 ^e	0.01 ± 0.00 ^{efg}	0.04 ± 0.00 ^e
Red bambara	Roasted	-	433.16 ± 0.89 ^{dde}	3.64 ± 0.49 ^b	0.17 ± 0.01 ^{bc}	0.06 ± 0.02 ^a	0.23 ± 0.03 ^{ab}
Red bambara	Raw	Sifted	440.90 ± 0.19 ^{de}	5.09 ± 0.37 ^a	0.13 ± 0.04 ^c	0.02 ± 0.01 ^{defg}	0.15 ± 0.05 ^{cd}
Red bambara	Dehulled	Sifted	278.46 ± 0.39 ^f	3.15 ± 0.68 ^{bc}	0.03 ± 0.00 ^e	0.03 ± 0.01 ^{bcdefg}	0.05 ± 0.01 ^e
Red bambara	Roasted	Sifted	278.46 ± 0.39 ^f	3.24 ± 0.28 ^{bc}	0.15 ± 0.01 ^{bc}	0.04 ± 0.01 ^{abcd}	0.19 ± 0.01 ^{bc}
Ukulinga dry bean	Raw	-	672.95 ± 0.03 ^{ab}	3.04 ± 0.12 ^{bcd}	0.09 ± 0.01 ^d	0.05 ± 0.00 ^{ab}	0.13 ± 0.01 ^d
Ukulinga dry bean	Dehulled	-	541.45 ± 0.61 ^{bcd}	1.84 ± 0.04 ^e	0.02 ± 0.00 ^e	ND	0.02 ± 0.00 ^e
Ukulinga dry bean	Roasted	-	541.45 ± 0.45 ^{bcd}	2.11 ± 0.08 ^{de}	0.17 ± 0.01 ^{bc}	0.04 ± 0.00 ^{abcdef}	0.19 ± 0.01 ^{bc}
Ukulinga dry bean	Raw	Sifted	611.07 ± 0.59 ^{bc}	2.33 ± 0.16 ^{de}	0.14 ± 0.01 ^{bc}	0.04 ± 0.03 ^{b^cdef}	0.18 ± 0.02 ^{b^cd}
Ukulinga dry bean	Dehulled	Sifted	417.69 ± 0.39 ^{de}	1.84 ± 0.04 ^e	0.03 ± 0.01 ^e	ND	0.03 ± 0.02 ^e
Ukulinga dry bean	Roasted	Sifted	433.16 ± 0.31 ^{de}	1.88 ± 0.08 ^e	0.18 ± 0.04 ^{bc}	0.04 ± 0.01 ^{abc}	0.22 ± 0.04 ^{ab}

Mean ± SD

Means with different superscript in a column are significantly different ($p \leq 0.05$); *mg/100 g; **mg/g of catechin equivalent, db

ND-not detected

SD: Standard deviation

Red bambara varieties had the highest tannin content followed by the brown bambara samples. This may be linked to its lighter colour (26.64) as tannins have been found to influence the colour of grains (Siwela *et al* 2007). Dark coloured legumes have been reported to contain more tannins than light coloured ones (Bamshaiye *et al* 2011). The tannin content of the raw grains varied from 3.04-5.26 g/100 g. The tannin content of the three grain legumes were within the range (1.10-8.99 g/100 g) as reported in previous studies on legumes (Fasoyiro *et al* 2006; Amarteifio & Moichubedi 1997). The higher tannin content in the red bambara grain compared to the brown bambara grain and the reference bean corresponded with its higher total phenolic content (0.22 mg/g) (Table 4.4). Tannins are polyphenols which are associated with the seed coat colour (Bamshaiye *et al* 2011). The variations in the tannin content between the bambara landraces could be due to genetic factors since they were grown in the same location under the same environmental conditions. Fasoyiro *et al* (2006) also reported variations in tannin content for different varieties of African yam bean, lima bean and pigeon pea.

Generally, dehulling or roasting alone or in combination with sifting decreased the tannin content of the studied grains. A reduction in tannin content has been reported for roasted Canadian and Egyptian cowpea and kidney bean samples (Khattab & Arntfield 2009). However, in the current study, dehulling in combination with sifting resulted in a higher reduction of the tannin content of the grains when compared with roasting alone or roasting in combination with sifting. A similar trend was observed for BGN in a previous study (Adegunwa *et al* 2014).

The phytic acid content of the studied grains was highest for raw brown bambara (781.23 mg/100 g) and lowest for raw red bambara (587.86 mg/100 g). The phytic acid contents of the bambara grains are similar to that reported by Yagoub & Abdalla (2007) for bambara grown in Sudan. Dehulling or roasting alone or in combination with sifting significantly ($p \leq 0.05$) reduced the phytic acid, tannin, and total phenolic content (TPC) of the legume grains (Table 4.4). Dehulling and roasting led to a 32% reduction in phytic acid content of the bambara grains. However, a lower reduction (approx. 19%) in phytic acid content was recorded for roasted and dehulled Ukulinga bean. A possible explanation for the higher reduction in phytic acid content of the bambara grains compared to the reference bean is that phytic acid is concentrated in the hull of the bambara grains, while it is concentrated in the cotyledon of the Ukulinga bean. This may

explain the higher phytic acid content of the dehulled reference bean (541.45 mg/100 g) compared to the dehulled brown (525.98 mg/100 g) and red (394.49 mg/100 g) bambara (Table 4.4). Some authors similarly reported a reduction in phytic acid contents of bambara after dehulling (Abiodun & Adepeju 2011; Omoikhoje *et al* 2006). Higher reductions in phytic acid contents were observed when the dehulled and roasted grains were further sifted.

Unlike roasting, which had varied effects on TPC of the studied grains, dehulling reduced the TPC of all the studied grains by approximately 83% (Table 4.4). Other studies on legumes similarly reported a reduction in TPC after dehulling (Han & Baik 2008; Ghavidel & Prakash 2007). A substantial amount of phenolic compounds are reported to be associated with the hull (Xu & Chang 2008). Therefore, the reduction in TPC upon dehulling indicates that most of the phenolic substances in the grains were lost due to the removal of the hulls. Sifting also had varied effects on the TPC of all the samples. With the exception of red bambara, sifting slightly increased the TPC of the other samples. Roasting increased the TPC of the reference bean and bambara grains, however, the TPC of the roasted bambara grains decreased significantly ($p \leq 0.05$) after sifting, whilst that of the dry bean increased. Phenolics have been reported to be located in different parts of a grain depending on the variety (Xu & Chang 2008), but they have often been found concentrated in the hull (Jain *et al* 2009; Han & Baik 2008). During sifting, flour particles are separated based on their particle sizes. The hulls of the legume grains are likely to have a larger particle size and are therefore retained in the sieve. This may account for the loss of phenolic substances in the sifted flours. Differences in the effect of sifting on the flours may be due to the different distribution of phenolic compounds in the samples. The increased TPC of the roasted legume flours in this study is similar to those reported for roasted defatted sesame seed (Jeong *et al* 2004). According to these authors, roasting may have cleaved and liberated phenolic compounds thus increasing the TPC of the samples.

The free radical scavenging activities (FRSA) of the raw and processed grains are shown in Figures 4.2 and 4.3.

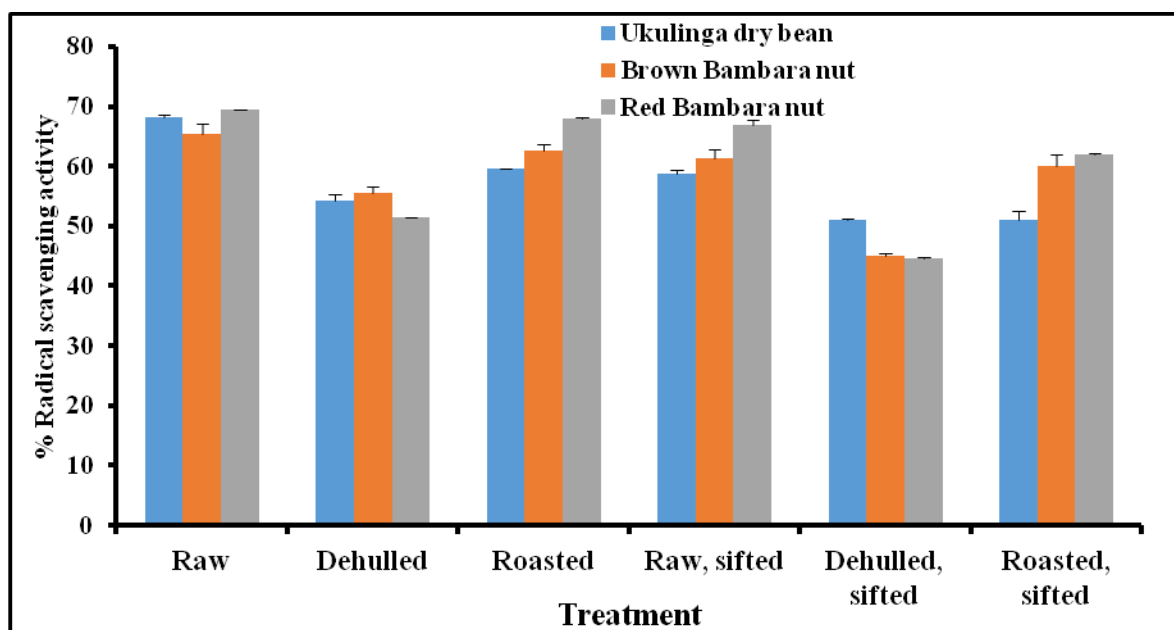


Figure 4.2 Effect of processing on % DPPH radical scavenging activity of the legumes. Error bars indicate standard deviation (n=3)

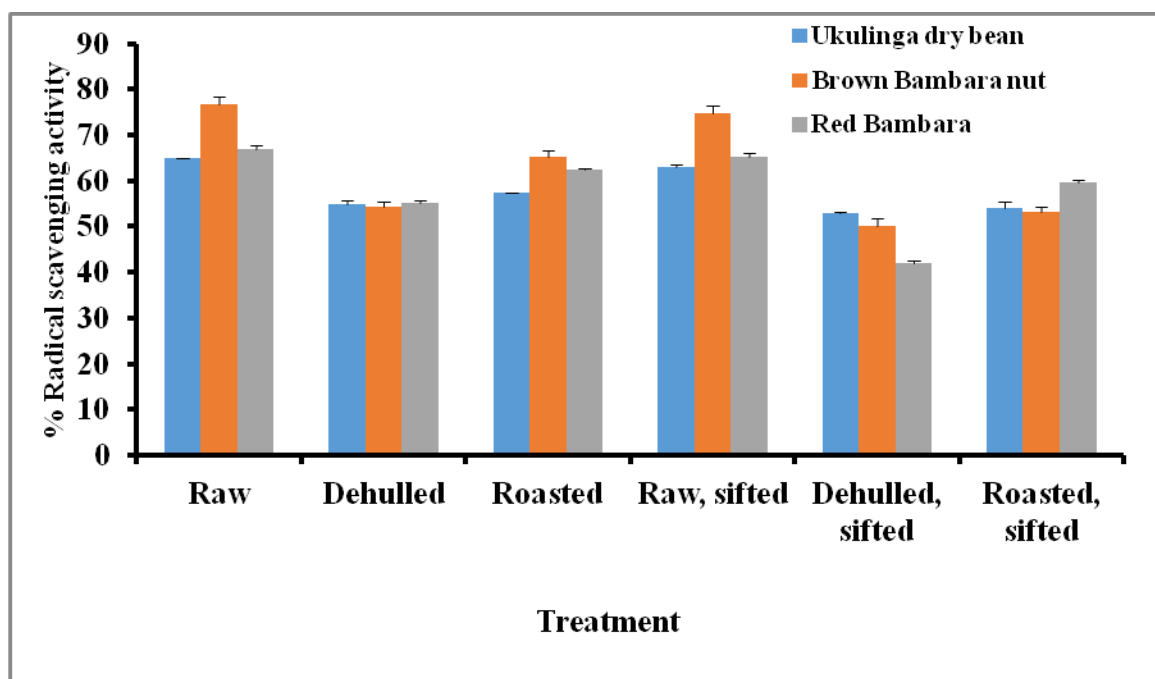


Figure 4.3 Effect of processing on ABTS radical scavenging activity of legume samples. Error bars indicate standard deviation (n=3)

The raw legume grain had DPPH FRSA ranging between 65 and 68% and ABTS FRSA ranged between 64 and 76%. This is in agreement with values reported by Ademiluyi & Oboh (2011)

for BGN. Dehulling and roasting decreased the % FRSA (DPPH and ABTS) of all the samples. Sifting further decreased the antioxidant activity of all the samples. Dehulling followed by sifting led to a greater decrease in the %FRSA of all the samples than roasting. Siddhuraju & Becker (2007) also reported a decrease in %FRSA of dry heated cowpea samples. The antioxidant activities of these samples may be linked to their TPC. For instance, the raw red bambara grain which had the highest TPC (Table 4.4) had a relatively higher DPPH FRSA than the other two samples. Reduction in the antioxidant activities of the samples after dehulling and sifting may be as a result of the loss of phenolic substances during processing, whilst the reduction in antioxidant activity during roasting could have been a result of decomposition of larger molecular weight phenolic compounds, particularly tannins. High-tannin grains have been reported to exhibit higher antioxidant activity than low-tannin grains (Siwela *et al* 2007).

4.3.5 Functional properties of flours

4.3.5.1 Water and oil absorption properties

Water absorption capacity of flours influence functional and sensory properties of food substances (Sreerama, Sashikala, Pratape & Singh 2012). Water absorption capacity of the raw and processed samples is presented in Figure 4.4.

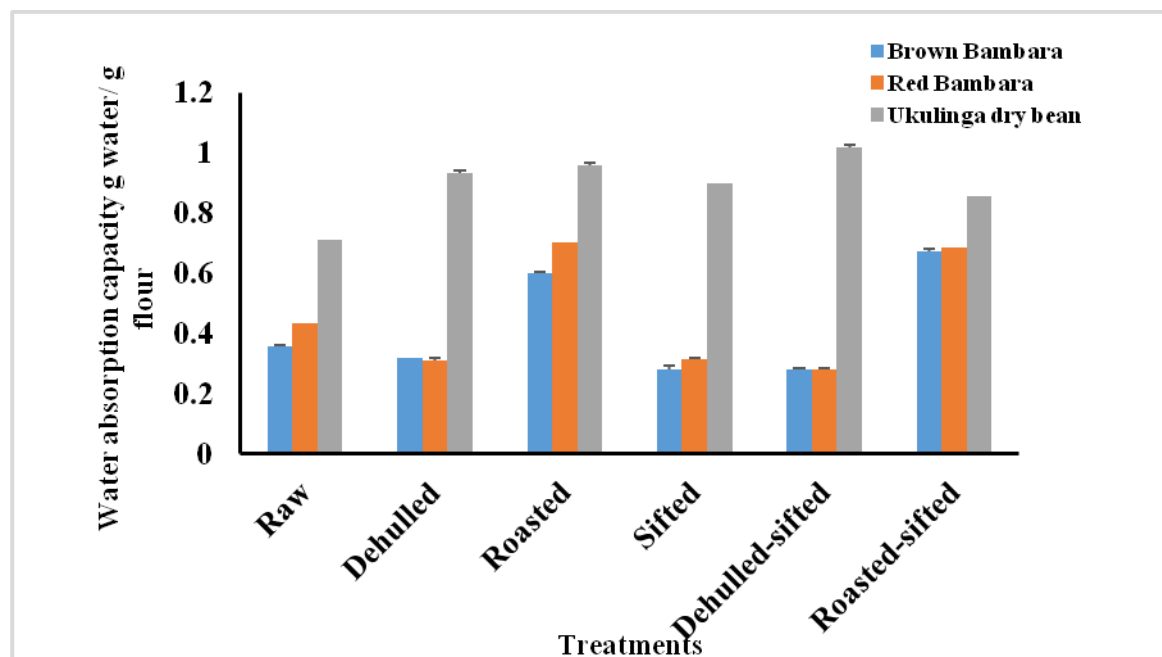


Figure 4.4 Water absorption capacity of raw and processed samples

The reference dry bean showed the highest ability to absorb water compared to both raw and processed bambara, which could be due to its higher protein content. Furthermore, the protein in the dry bean may have more polar amino acid residues, which could also have influenced its ability to absorb water (Yusuf *et al* 2008). The WAC values obtained in this study were slightly lower than those reported for dehulled cowpea samples (Abu, Muller, Duodu & Minnaar 2005).

Dehulling reduced the WAC of the bambara flours which may be attributed to leaching of water binding sites during soaking and subsequent dehulling of the samples. However, WAC of the reference bean increased upon dehulling. Roasting caused a significant increase (89%, 58% and 20% respectively for brown bambara, red bambara and the reference dry bean) in WAC. A similar trend was reported for roasted BGN flours by Onwuka & Abasiokong (2006). Adegunwa *et al* (2014) also reported an increased WAC for roasted BGN. The increased WAC could be as a result of protein depolymerisation. Proteins depolymerise into sub units with more water binding sites during high temperature treatments (Abu, Duodu & Minnaar 2006; Akubor, Isiolokwu, Ugbane & Onimawo 2000). This may result in a corresponding increase in the WAC of roasted samples. Degradation of starch to simpler molecules such as dextrans and sugars with higher affinity for water during high temperature treatment may also be responsible for the increased

WAC. Moisture loss during roasting may be responsible for the higher WAC of roasted bambara. An approximate 24% and 28% reduction in WAC of raw and dehulled bambara samples respectively was observed after sifting. Sifting is a mechanical separation technique which separates components based on particle sizes. Water absorption capacity is a critical property of proteins in viscous foods (Sreerama *et al* 2012). The lower WAC of bambara samples compared to the reference bean may be advantageous as it may result in food products with lower viscosity. Complementary foods with low viscosity are desirable as they may be easily ingested by children (WHO 2003). Hence, bambara may be a suitable ingredient in complementary food formulations.

The ability of flours to absorb and retain oil may enhance flavour retention and improve mouthfeel (Kaur, Kaushal & Sandhu 2013). Oil absorption capacity of the raw and processed sample is presented in Figure 4.5.

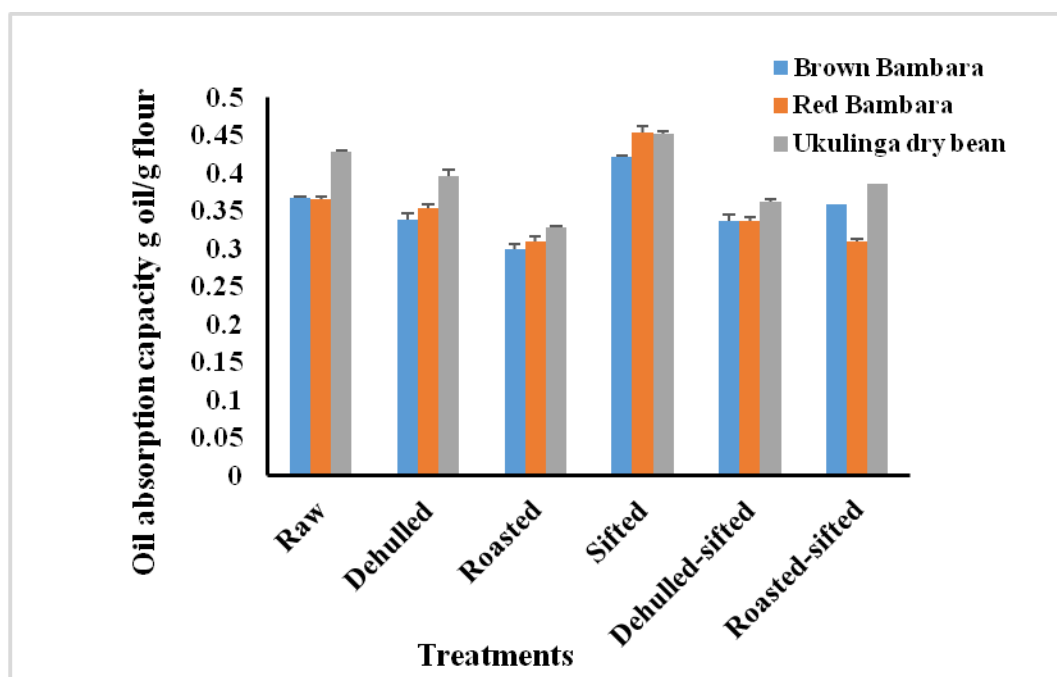


Figure 4.5 Oil absorption capacity of raw and processed samples

Oil absorption capacity of all the samples were generally lower than their corresponding WAC. A similar trend was observed for taro and soybean (Kaur *et al* 2013). Oil absorption capacity of

all raw and roasted-sifted samples increased after sifting while a decrease was observed in the dehulled-sifted samples. Sifting is a mechanical separation technique which separates components based on their particle sizes. The decrease in the OAC after sifting of dehulled samples may be due to removal of oil binding sites during the mechanical separation together with the hull that had been previously removed.

4.3.5.2 Rheological properties

The flow properties of flour samples were measured at different shear rates using a rheometer. Power law was applied to describe the flow properties of the flours. The flow properties followed the Power-Law model with a fit of $r^2 \geq 0.7$ for bambara samples and $r^2 \geq 0.3$ (Table 4.5) for the reference dry bean. The effect of dehulling and sifting on the viscosity of bambara flour are indicated by Power law coefficients.

Table 4.5 Effect of different processing methods on the viscosity of bambara flour and reference bean as indicated by Power law coefficients

Sample	Treatments	n	k	r ²
Brown bambara	-	0.021 ^{c-e} ± 0.001	26.71 ^{a-e} ± 0.31	0.79 ^{ab} ± 0.01
Brown bambara	Sifted	0.023 ^{c-e} ± 0.001	22.70 ^{a-e} ± 0.14	0.77 ^{ab} ± 0.04
Brown bambara	Dehulled	0.024 ^{cd} ± 0.005	37.56 ^{ab} ± 0.78	0.75 ^{ab} ± 0.05
Brown bambara	Dehulled, sifted	0.024 ^{cd} ± 0.002	29.90 ^{a-e} ± 0.07	0.74 ^{ab} ± 0.04
Brown bambara	Roasted	0.056 ^a ± 0.000	1.58 ^e ± 0.45	0.87 ^{ab} ± 0.05
Brown bambara	Roasted, sifted	0.029 ^{cd} ± 0.003	11.23 ^{b-e} ± 4.60	0.78 ^{ab} ± 0.02
Red bambara	-	0.014 ^{de} ± 0.009	44.95 ^a ± 0.81	0.72 ^{ab} ± 0.06
Red bambara	Sifted	0.024 ^{cd} ± 0.001	34.53 ^{a-c} ± 0.35	0.76 ^{ab} ± 0.04
Red bambara	Dehulled	0.021 ^{c-e} ± 0.005	44.08 ^a ± 0.03	0.71 ^{ab} ± 0.00
Red bambara	Dehulled, sifted	0.021 ^{c-e} ± 0.001	40.46 ^{ab} ± 0.98	0.71 ^{ab} ± 0.05
Red bambara	Roasted	0.030 ^{cd} ± 0.015	3.90 ^{de} ± 2.74	0.84 ^{ab} ± 0.15
Red bambara	Roasted, sifted	0.029 ^{cd} ± 0.001	7.35 ^{c-e} ± 1.46	0.77 ^{ab} ± 0.02
Ukulinga dry bean	-	0.006 ^e ± 0.000	33.32 ^{a-d} ± 0.12	0.51 ^{bc} ± 0.01
Ukulinga dry bean	Sifted	0.026 ^{cd} ± 0.003	25.81 ^{a-e} ± 0.40	0.72 ^{ab} ± 0.04
Ukulinga dry bean	Dehulled	0.017 ^{de} ± 0.003	47.08 ^a ± 0.60	0.39 ^c ± 0.05
Ukulinga dry bean	Dehulled, sifted	0.037 ^{bc} ± 0.004	23.14 ^{a-e} ± 0.80	0.75 ^{ab} ± 0.02
Ukulinga dry bean	Roasted	0.052 ^{ab} ± 0.000	1.87 ^e ± 0.01	0.78 ^{ab} ± 0.00
Ukulinga dry bean	Roasted, sifted	0.047 ^{ab} ± 0.004	1.61 ^e ± 0.56	0.77 ^{ab} ± 0.04

All samples displayed a shear thinning behavior as “n” was less than 1 indicating that the flour samples have pseudo-plastic behaviour. Dehulling, roasting and sifting of raw flour samples increased “n” for the bambara landraces. However, while sifting of dehulled samples did not

significantly ($p \leq 0.05$) affect these values, a decrease in “n” was observed after sifting roasted samples. Processing except dehulling reduced the Power-Law consistency index “k” of all the samples. “k” is a measure of the viscous properties of samples as a function of shear rates (D'Silva *et al* 2011). This implies that the processed samples were more shear thinning than the raw samples (D'Silva *et al* 2011).

From Figures 4.6 and 4.7, the viscosities of all the samples reduced with increasing shear rate, which also confirms the shear thinning behavior of all the samples.

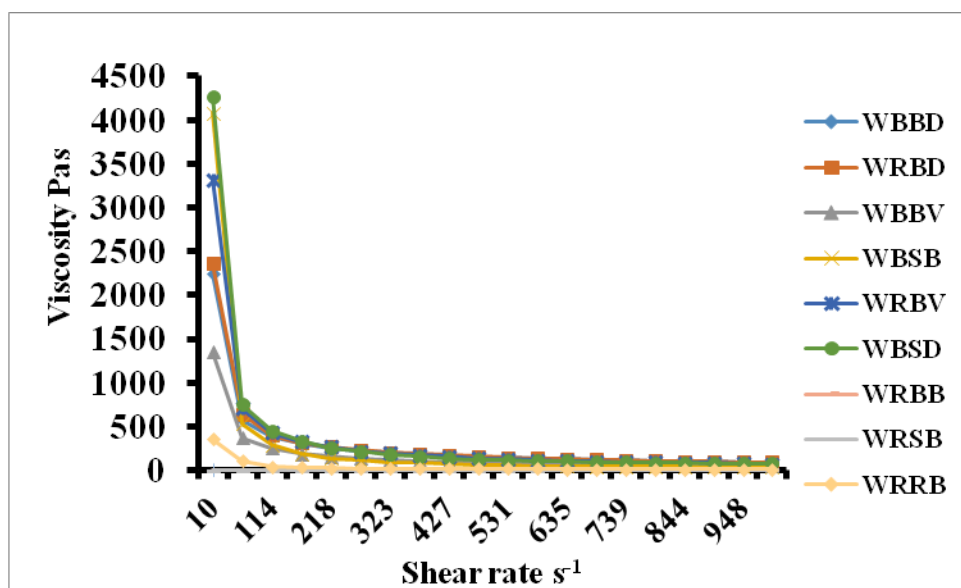


Figure 4.6 Effect of different processing methods on the viscosity of bambara flour

WBBD - Dehulled brown bambara flour; WRBD - Dehulled red bambara flour; WBBV - Raw bambara flour; WBSB - Raw dry bean flour; WRBV - Raw red bambara flour; WBSD - Dehulled dry bean flour; WRBB - Roasted brown bambara flour; WRSB - Roasted dry bean flour; WRRB - Roasted red bambara flour

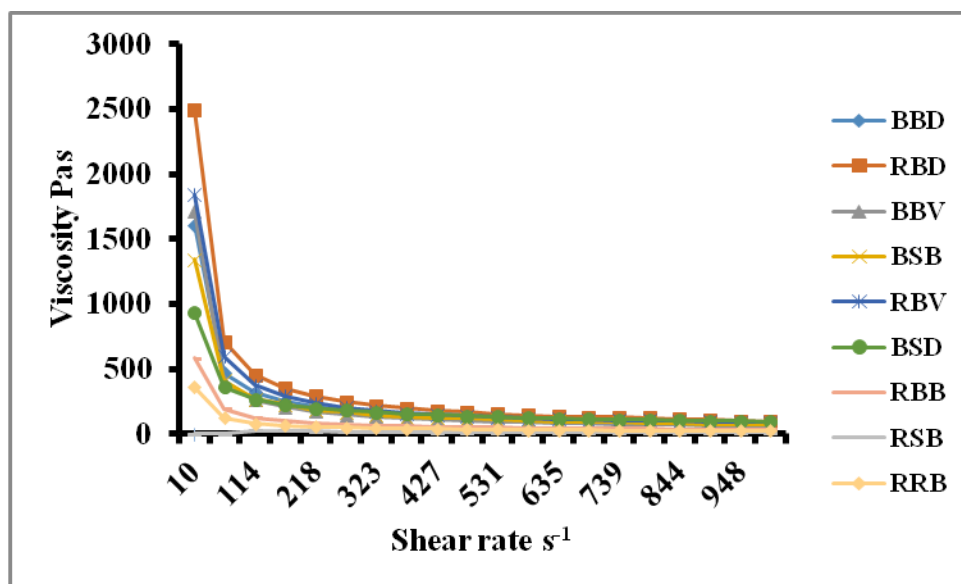


Figure 4.7 Effect of different processing methods combined with sifting on the viscosity of bambara flour

BBD - Dehulled, sifted brown bambara flour; RBD - Dehulled, sifted red bambara flour; BBV- Raw, sifted bambara flour; BSB- Raw, sifted dry bean flour; RBV- Raw, sifted red bambara flour; BSD - Dehulled, sifted dry bean flour; RBB - Roasted, sifted brown bambara flour; RSB - Roasted, sifted dry bean flour; RRB - Roasted, sifted red bambara flour

Lower viscosity of the roasted samples could be as a result of polysaccharide degradation during roasting. Monosaccharide constituents may have been chemically modified by participating in heat enhanced reactions like the Maillard reaction and caramelisation (Sharma, Gujral & Rosell 2011)

4.4 Conclusions

Grains of BGN landraces popular in Southern Africa possess different physical and chemical (including nutritional and antioxidant) properties. Generally, these grains are good sources of antioxidants (largely phenolic compounds) and nutrients, including protein. Thus, they could be suitable for inclusion in the diets of population groups that are vulnerable to undernutrition and poor health. Different processing methods have varying impact on the nutritional and antioxidant properties of the grain types. Dehulling significantly ($p \leq 0.05$) increased the protein and carbohydrate content of the samples, but caused a decrease in the tannin and total phenolic

content and antioxidant activity of the grains. Dehulling also led to a greater decrease in antioxidant activity relative to roasting. The increase in protein and carbohydrate content observed in the dehulled samples was more in the brown bambara landrace than the red one. Also, percentage increase in protein digestibility was more in the brown bambara landrace (approx. 17%) than the red one (approx. 11%). Roasting in combination with sifting also caused a higher reduction in phenolic content and antioxidant activity of bambara grains than roasting alone. Therefore, it is recommended that appropriate methods of processing be established and used on specific popular grain types for optimal nutritional and health benefits.

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CHAPTER 5

²CONSUMER AWARENESS AND ACCEPTABILITY OF A BAMBARA GROUNDNUT COMPLEMENTARY FOOD IN RURAL KWAZULU-NATAL, SOUTH AFRICA

Abstract

Protein energy malnutrition (PEM) in children is a major public health concern in most developing countries. PEM results from an inadequate diet, specifically a lack of protein and energy. In developing countries animal protein sources are poorly consumed due to high cost while plant sources of protein are consumed far more widely. Inclusion of affordable plant proteins, especially legumes in the diets of vulnerable groups may reduce PEM prevalence. BGN, an underutilised legume indigenous to Africa is a rich source of protein and carbohydrate. In this study, the consumer awareness and acceptability of BGN as a protein source was determined. Its potential for use in complementary feeding was also assessed. Black African female caregivers participated in the consumer awareness survey (n=70), sensory evaluation (n=64) and focus group discussion (n=16). The participants were drawn from a rural community in KwaZulu-Natal province, South Africa. Consumer awareness of BGN and acceptability of pureed samples made from BGN and common dry bean (reference) were assessed using a questionnaire and a sensory evaluation respectively. A focus group discussion was held with some of the participants to further determine their perception of the BGN puree. The survey participants were not familiar with BGN and its preparation methods. The sensory attributes, including overall acceptability, of the BGN puree compared well with that of the reference. Grain colour significantly ($p \leq 0.05$) influenced overall acceptability of the puree ($p \leq 0.05$). The overall acceptability of the bambara puree increased with the age of the caregivers. The caregivers expressed willingness to use the BGN in complementary feeding if it was accessible, affordable, and beneficial to health. BGN is not a familiar legume in KwaZulu-Natal and utilisation is seemingly limited due to poor market availability and knowledge on cooking methods. However, there is potential for its use as a protein source in complementary foods. An

² Publication based on this research chapter:

Oyeyinka AT, Pillay K & Siwela M. Consumer awareness and acceptability of bambara groundnut as a protein source for use in complementary foods in rural KwaZulu-Natal. South African Journal of Clinical Nutrition (accepted for publication).

improvement in the market availability and nutrition programmes to highlight the nutritional benefits of bambara are required to improve its utilisation.

5.1 Introduction

Protein energy malnutrition (PEM) has been reported to be a major cause of deaths in children globally (Grover & Looi 2009) and is a concern in most developing countries (Müller & Krawinkel 2005; de Onis, Monteiro, Akré & Clugston 1993). PEM results from an inadequate intake of protein and energy and could manifest as either moderate acute malnutrition (MAM) or severe acute malnutrition (SAM). It is rife among children due to their high energy and protein requirements relative to their body weight (Kulkarni & Metgud 2014). PEM appears more frequently during the weaning period, which is usually between the age of 4-6 months and leads to growth retardation and increased susceptibility to infectious diseases (Prasad & Kochhar 2015).

Good feeding practices can prevent malnutrition and early growth retardation (Michaelson, Weaver, Branca & Robertson 2000). Breast milk alone is sufficient to meet the nutritional requirements of infants for the first six months of life (WHO 2015). Beyond six months, infants gradually develop the ability to chew, swallow and digest a wide variety of foods (Rolfes, Pinna & Whitney 2012, pp472-473). Breast milk also becomes nutritionally insufficient at this age, hence the need for complementary foods. Complementary foods should be nutrient dense as infants have a small gastric capacity (Dewey & Brown 2003). However, in most developing countries, these foods are of low nutritional quality and consist mainly of cereal-based porridges (Allen 2012). For instance, Allen (2012) reported a high dependence on maize, rice, sorghum and millet for preparing complementary foods in developing countries. In South Africa, soft porridge made from maize meal seems to be the most common complementary food especially among the rural populations (Faber & Benadé 2007). The choice of complementary foods is subject to cultural acceptance and affordability, which consequently influences the nutritional status of infants and children (Kerr, Dakishoni, Shumba, Msachi & Chirwa 2008). Animal food sources which are expensive are usually excluded especially in low socio-economic regions (Lutter, Daelmans, Onis, Kothari, Ruel, Arimond, Deitchler, Dewey, Blössner & Borghi 2011). From the aforementioned, it is imperative to consider alternative protein food sources to address

PEM, especially in developing countries. The inclusion of affordable plant proteins, especially legumes in the diets of vulnerable groups may be one way to achieve this.

Bambara groundnut (*Vigna subterranea*) is a legume indigenous to Africa and grown mainly for its edible grains (Bamshaiye, Adegbola & Bamshaiye 2011). It is a rich source of protein (15-28%) and carbohydrates (57-67%), comparable to those reported for other legumes like cowpea (19.61%) and pea (23.5%) (Oyeyinka, Singh, Adebola, Gerrano & Amonsou 2015). It has a better methionine profile than most legumes, including soybean, and can serve as an affordable source of protein for population groups vulnerable to PEM, particularly the poor rural populations in countries of SSA, including South Africa (Bamshaiye *et al* 2011). However, BGN is an underutilised crop grown mainly for subsistence. Its underutilisation can be attributed to its hard-to-cook defect, its strong beany flavour as well as gas forming properties (Hillocks, Bennett & Mponda 2012; Bamshaiye *et al* 2011). It is well-known that the acceptance of a crop as a food source by consumers is dependent on their knowledge of the crop, its processing technologies, nutritional benefits, as well as availability and cultural acceptability (Feldmann & Hamm 2015). According to Feldmann & Hamm (2015) consumer knowledge of local food influences attitudes and translates into purchase behaviour. Hence, there is a need to investigate the consumer awareness of bambara grain as a potential protein source. Although BGN is grown in some areas of South Africa, especially rural areas (Department of Agriculture, Forestry and Fisheries (DAFF) 2011), it appears that, as in other sub-Saharan African countries, it is grossly underutilised as a food. There is a lack of information on the consumer awareness and acceptance of BGN as a protein source in rural KwaZulu-Natal. It is not known whether BGN would be acceptable for use as a complementary food by caregivers of children vulnerable to PEM. In this study, the consumer awareness of BGN as a protein source and acceptability of pureed samples made from BGN and common dry bean (*Phaseolus vulgaris*) were assessed.

5.2 Materials and Methods

This was a cross-sectional study which employed a mixed method approach to determine the consumer awareness and acceptability of BGN as a protein source and to assess its potential for use in complementary feeding.

5.2.1 Plant materials

Two BGN landraces (red and brown) were used in this study. The landraces were selected because they are among the most commonly consumed landraces in Southern Africa. A dry bean (*Phaseolus vulgaris*), (Ukulinga dry bean variety) commonly consumed in South Africa was used as a reference.

5.2.2 Consumer awareness of bambara groundnut as a food source

5.2.2.1 Pilot study

A pilot study was conducted prior to the main consumer awareness study. This was done to detect and correct any methodological errors associated with the preparation of the survey questionnaires. Ten black African female caregivers were recruited by research assistants to participate in the pilot study. The caregivers were recruited from the paediatric unit of Gcumisa clinic at Swayimane, which was the site for the study. Participants from the pilot study were not allowed to participate in the main study. To ensure that subjects from the pilot study did not participate in the main study, the main study was conducted in a different week to the pilot study (most patients have monthly follow-up visits close to the same date each month). The pilot study detected typographical errors in the isiZulu version of the survey questionnaire which were subsequently corrected. Questionnaires were initially printed in English (Appendix D) and isiZulu (Appendix E), however, from the pilot study, it was observed that participants preferred the questionnaires in isiZulu, hence, subsequent questionnaires were printed in isiZulu only.

5.2.2.2 Consumer awareness of bambara groundnut

A total of 70 black African female caregivers who were outpatients of the paediatric unit of the Gcumisa clinic at Swayimane, uMgungundlovu district of uMshwathi municipality participated in the consumer awareness survey. Third year students from the Department of Dietetics and Human Nutrition, University of KwaZulu-Natal were recruited as research assistants and trained. Research assistants showed the outpatients the grains and explained the study to them after which volunteers were recruited. Participants were requested to sign a consent form prepared in English (Appendix F) and translated into isiZulu (Appendix G) before the questionnaires were administered. The volunteers were given the survey questionnaires and were requested to fill it in to assess their awareness of BGN as a protein source and also to identify the complementary

foods that they would likely prepare using BGN. The recipe used to prepare the identified complementary foods was obtained from the participants during the survey. The survey results indicated that if participants were to use bambara to make a complementary food, it would be in the form of a puree. A bambara puree was therefore evaluated for its acceptability for use as a complementary food.

5.2.3 Optimisation of bambara groundnut cooking conditions

The bambara grains were subjected to following cooking conditions;

- Whole grains were boiled without prior treatment until soft
- Whole grains were soaked in hot water (100°C) or tap water (19°C) for 6 hours
- Whole grains were soaked in hot water (100°C) or tap water (19°C) for 12 hours
- Whole grains were soaked in hot water (100°C) or tap water (19°C) for 18 hours
- Whole grains were soaked in hot water (100°C) or tap water (19°C) for 24 hours.

The soaked grains were drained and then boiled in fresh water, until soft.

5.2.4 Sensory evaluation

5.2.4.1 Pilot study

A pilot study of the sensory evaluation and focus group discussions was conducted before the main study. The time required for the samples to be warmed in the microwave oven in order to achieve the ideal serving temperature for sensory evaluation was decided during the pilot study. Ten black African female caregivers participated in the pilot study. To ensure that participants from the pilot study did not participate in the main study, the pilot study was conducted in a different week to the main study as previously explained. Focus group discussions were conducted after the sensory evaluation, using some of the sensory evaluation panelists. The outcomes of the pilot study were as follows: (i) questionnaires were modified and it was also observed that participants preferred questionnaires written in isiZulu (Appendix H) rather than in English (Appendix I); (ii) a standardised recipe was developed (Appendix J); (iii) the procedure to be followed during the focus group discussions was confirmed; (iv) it was decided that the main study would be conducted at an earlier time than the pilot study, so that sufficient participants could be recruited to participate in the study; (v) the time required for warming the samples to reach the ideal temperature for the sensory evaluation was determined as 15 s.

5.2.4.2 Preparation of complementary food (puree)

Preliminary trials in the laboratory showed that soaking the grains in hot water for 18 h at room temperature reduced cooking time by approximately 53%. Hence, this was used in the puree preparation. The two BGN varieties and the reference dry bean were all prepared using a standard recipe as described by the survey participants (Appendix J). The purees were prepared by a black African woman living in a rural area in the Umgungundlovu District with experience in cooking dry bean puree (Figure 5.1).



Figure 5.1 Preparation of bambara groundnut puree in the Food Processing Laboratory

This ensured that the foods were culturally acceptable to the participants. The foods were prepared in the Food Science Laboratory, University of KwaZulu-Natal, Pietermaritzburg. The puree was prepared by soaking 250 g of grains in 750 mL water for 18 h. Thereafter, the soaked grains were drained and boiled in 2.5 L of tap water for about 150 mins and 1 mL of salt was added. It was then cooked on low heat for about 15 min and mashed into a puree using a wooden spoon. The samples were then transported to the research site in air-tight containers. No seasoning was added to the bambara puree complementary food during its preparation. This was

in line with the recipes received for the bambara puree from the participants during the survey and complementary feeding guidelines.

5.2.4.3 Sample coding, serving order and sensory evaluation set-up

The consumer acceptability study was conducted using 64 black African female caregivers from the paediatric unit of Gcumisa clinic at Swayimane, uMgungundlovu district of uMshwathi municipality. The panelists were seated some distance from each other in the evaluation room at ambient temperature and were asked not to communicate during the sensory evaluation session. This was done to prevent the panelists from influencing each other's responses. The three pureed samples were assigned a unique three-digit code obtained from a Table of Random Numbers (Heymann 1995). The three digit codes were known to the researcher, but not to the panelist and research assistants, to prevent bias. The serving order of the puree samples was determined by a Table of Random Permutations of Nine (Heymann 1995). Each participant was served 35 ± 5 ml of each sample in a polystyrene cup. Each sample was warmed for 15 s before being served to the participant. Each panelist was provided with a pencil, a cup of water to rinse the palate between samples, a consent form (Appendix K & L) and the sensory evaluation questionnaire in isiZulu (Appendix I), the local language in KwaZulu-Natal. The English version of the consent form (Appendix K) and sensory evaluation questionnaire (Appendix H) were translated into isiZulu by a translator proficient in both languages. The questionnaire made use of a five-point facial hedonic scale (1=very bad; 5=very good), and assessed the following attributes: taste, texture, aroma, colour and overall acceptability. The hedonic scale is a popular sensory evaluation tool that indicates the degree of likeness (Bergara-Almeida, Aparecida & Da Silva 2002).

The participants were required to fill in a consent form before starting the evaluation. A research assistant explained the consent form in detail in isiZulu to all the participants. The participants were asked if they understood everything that was on the consent form before they signed the forms. If anything was unclear it was explained before the participant signed. After the consent forms were signed, the research assistant explained the questionnaire in isiZulu to the participants. All the sensory attributes were explained to the participants. If the explanation for a sensory attribute was unclear the research assistant repeated the explanation for that sensory

attribute. Illiterate participants were helped by research assistants if they required further assistance to complete the questionnaire. Figure 5.2 shows participants completing the questionnaire with the help of research assistants.



Figure 5.2 Subjects completing the five-point facial hedonic questionnaire

5.2.5 Focus group discussions

Focus group discussions were conducted in a designated room within the clinic premises to further determine the perceptions of caregivers about the use of BGN puree as a complementary food. Sixteen participants were randomly selected from the caregivers who had volunteered to participate in the sensory evaluation (Figure 5.3). The participants were divided into two groups of eight. The ideal size for a focus group discussion is between 7-12 individuals (Bogart, Tickle-Degnen & Joffe 2012). Focus group discussion questions were generated prior to the study in English (Appendix M) and translated into isiZulu (Appendix N). Participants gave consent to participate in the discussion and for the discussion to be recorded by signing consent forms before the commencement of the discussions (see Appendix O & Q for the consent documents in English and Appendix P & R for the consent document in isiZulu). The discussions were facilitated by a research assistant who was fluent in isiZulu. The discussions were recorded using

a digital voice recorder. The recordings were later translated into English verbatim by the focus group discussion facilitator.



Figure 5.3 Some participants at the focus group discussion

5.2.6 Reduction of bias and data quality control

Various steps were taken to reduce bias during the preparation of the purees. These included calibrating the balance that was used to weigh the dry ingredients, using the same measuring cup and spoons to measure the ingredients. Pots of the same type and size were used in food preparation. The pureed food was transported in air-tight containers to the study site. The same amount of sample (15 ml) was served to all the participants. All the samples were heated for the same duration just before evaluation. Sample labeling and serving order were randomised and panelists were not allowed to communicate during the sensory evaluation session. Sensory evaluation data was captured on a spread-sheet and cross-checked to ensure that all the data was entered correctly. The focus group discussions that were recorded using a digital voice recorder were translated and cross-checked by an isiZulu-speaking person for accuracy.

5.2.7 Ethics approval

Ethical approval was obtained from the University of KwaZulu-Natal, Humanities and Social Science Ethics Committee (HSS/0740/015D) (Appendix A). The Umgungundlovu Health District issued a supporting letter for the research to be conducted (Appendix B). The Department of Health granted permission for the study to be conducted at Gcumisa clinic, Swayimane (HRKM95/12) (Appendix C).

5.2.8 Statistical analysis

Data was analysed using Statistical Package for Social Sciences (SPSS) version 19.0. One-way analysis of variance (ANOVA), Fischer's Least Significant Difference (LSD) and linear regression were used to analyse the data. A p-value of ≤ 0.05 was considered significant.

5.3 Results and Discussion

5.3.1 Consumer awareness of bambara groundnut as a protein source

The number and age of the caregivers are presented in Table 5.1. Most of the caregivers were below 36 years old.

Table 5.1 Number and age of caregivers who participated in the survey and sensory evaluation

Age group (years)	n (%)*	n (%)**
16-25	14 (20.0)	19 (29.7)
26-35	33 (47.1)	20 (31.3)
36-45	7 (10.0)	7 (10.9)
46-55	8 (11.4)	9 (14.1)
56-65	7 (10.0)	8 (12.5)
66-75	1 (1.4)	1 (1.6)

* Survey participants n=70; **Sensory participants n=64

The results of the survey on the consumer awareness of BGN as a food source are presented in Table 5.2.

Table 5.2 Consumer awareness of bambara groundnut as a food source (n=70)

Survey questions	n	%
Have you seen it before?		
Yes	45	64.3
No	25	35.7
Have you eaten it before?		
Yes	43	61.4
No	27	38.6
Reasons for not eating it;		
The grains are not available	8	11.4
I do not know the plant	10	14.3
Don't know how to use it	8	11.4
Other: It causes acne	1	1.4
Willingness to purchase if it is more nutritious than common dry bean		
Yes	64	91.4
No	6	8.6
Willingness to purchase if it was cheaper than common dry bean		
Yes	65	92.9
No	5	7.1
Willingness to pay more for bambara than common dry bean		
Yes	43	61.4
No	27	38.6
Willingness to include bambara in family cooking		
Yes	64	91.4
No	6	8.6
Willingness to include bambara in infants' meal		
Yes	50	71.4
No	20	28.6

This study revealed that BGN is a less popular legume compared to the dry bean, which is in agreement with previous research (Bamshaiye *et al* 2011). Survey participants who had not previously seen and used bambara grains were fewer (37%) than those who were familiar (64%) and had consumed it (61%) (Table 5.2). Although most of the survey participants were familiar with bambara, some of them had never cooked it. Chi-square analysis showed that there was significant differences ($p \leq 0.05$) in participants' responses to using bambara groundnut. Unavailability of BGN and lack of knowledge on preparation methods and lack of knowledge of its nutritional value were some of the reasons for not using it. Some participants did not consume it due to superstitious beliefs that it would cause skin reactions like acne. The majority of the

participants (91%) were willing to purchase the grain for its nutritional benefits and 92.9% of the participants would buy bambara if it was cheaper than the common dry bean. Neumark-Sztainer, Story, Perry & Casey (1999) also reported that cost, cultural beliefs and health benefits are some of the factors that influence food choices. Seventy-one percent of the participants showed willingness to include the bambara in infant complementary foods if it were readily available at grocery stores. Therefore, BGN could serve as a source of affordable food protein especially in low socio-economic regions.

5.3.2 Optimisation of bambara groundnut cooking conditions

Cooking time is an important quality characteristic usually used to evaluate the cooking quality of dry legumes (Xu & Chang 2008). Table 5.3 shows the result of the optimisation of BGN cooking conditions.

Table 5.3 Optimisation of bambara groundnut cooking conditions

Brown bambara groundnut									
Soaking time (hour)	0	6	6	12	12	18	18	24	24
Soaking water type*	None	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
Cooking time (min)	260	208	226	161	170	120	135	117	132
Red bambara groundnut									
Soaking time (hr)	0	6	6	12	12	18	18	24	24
Soaking water type*	None	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold
Cooking time (min)	240	190	210	130	150	115	131	110	127

* Hot = 100°C; Cold = 17°C

Generally, the red bambara landrace had a shorter cooking time compared to the brown landrace. Heat and mass transfer in substances have been reported to be dependent on surface area (Mubaiwa, Fogliano, Chidewe & Linnemann 2016). The red bambara landrace used in this study had a significantly ($p \leq 0.05$) higher surface area (390.81 mm^2) than the brown landrace (325.77 mm^2) (Table 4.1). Further, a larger force was required to cut through the grains of the brown bambara landrace (Figure 4.2). This may be responsible for the shorter cooking time observed in the red landrace. Reyes-Moreno, Paredes-López & Gonzalez (1993) also indicated that uptake of water in legume grains during cooking is greatly influenced by the seed variety, size and density. The appreciable amount of pectic substances in the cotyledons of legumes provides principal

adhesion which holds cells together (Reyes-Moreno *et al* 1993). They influence the permeability of water into the grains and consequently affect cooking properties (Reyes-Moreno *et al* 1993).

Soaking of the grains before cooking reduced cooking time for all the samples. The temperature of the soaking water had a significant effect on the cooking time of all the samples. Grains soaked in hot water had shorter cooking times than those soaked in tap water. The hot water may have influenced the rate of water absorption in all the samples. Cooking improves heat transfer properties as well as water absorption and water holding capacity of the grains. This in turn promotes the movement of water molecules into the cell wall causing dissolution of cell wall components and softening of tissues (Mubaiwa *et al* 2016). However, the nature of the polymers and their linkages dictate the wall polysaccharide solubility and texture hardening (Pirhayati, Soltanizadeh & Kadivar 2011). These factors increase the time required for water absorption during soaking and cooking resulting in a longer cooking time. Although there is an increased awareness of the need for consumption of healthy foods, demand for convenient and time-saving food products is also on the increase (Osman, Osman, Mokhtar, Setapa, Shukor & Temyati 2014). It seems plausible to say that the characteristic long cooking time and amount of energy that is required for cooking BGN is one reason for its underutilisation. This was also evident in the consumer acceptability study reported in Chapter 5. It appears that the optimal soaking condition is to use hot water for 18 hours. This is because beyond a soaking time of 18 hours, the cooking time was not very different. This condition was therefore used in the preparation of the puree for this study.

5.3.3 Sensory acceptability of bambara groundnut puree

Sensory attributes play a major role in the acceptance of new food products (Nti 2009). The mean sensory acceptability scores recorded for the puree made with BGN were comparable to that of the reference dry bean (Table 5.4).

Table 5.4 Sensory acceptability of bambara groundnut and the reference dry bean puree

Puree	Sensory attribute				
	Taste	Texture	Aroma	Colour	Overall acceptability
Brown bambara	3.80 ^a ± 0.84	3.66 ^a ± 1.02	3.80 ^a ± 0.78	3.73 ^a ± 0.82	3.92 ^a ± 0.80
Red bambara	3.48 ^a ± 1.02	3.33 ^a ± 1.04	3.39 ^b ± 0.79	3.41 ^b ± 0.83	3.54 ^b ± 1.01
Ukulinga dry bean	3.58 ^a ± 0.85	3.55 ^a ± 0.91	3.59 ^{ab} ± 0.79	3.67 ^{ab} ± 0.67	3.68 ^{ab} ± 0.83

Mean ± SD

Mean with different superscripts along a column are not significantly different ($p \leq 0.05$)

SD: Standard deviation

However, significant differences were observed between the bambara samples in terms of aroma, colour and overall acceptability. Puree made from red bambara landrace had lower sensory ratings than that of the brown landrace and the reference bean. This is in agreement with a study on complementary foods made from different bambara landraces (Nti 2009) The authors also observed a lower rating for complementary foods prepared from highly pigmented bambara landraces. The reference dry bean was popular among participants and had a colour similar to that of the brown bambara. The similarity in the colour of the grains may have influenced the preference for brown bambara over the red bambara landrace. Generally, the overall acceptability of the samples increased with the age of the participants (Figure 5.4). This could be because the older participants were more familiar with bambara than the younger participants were.

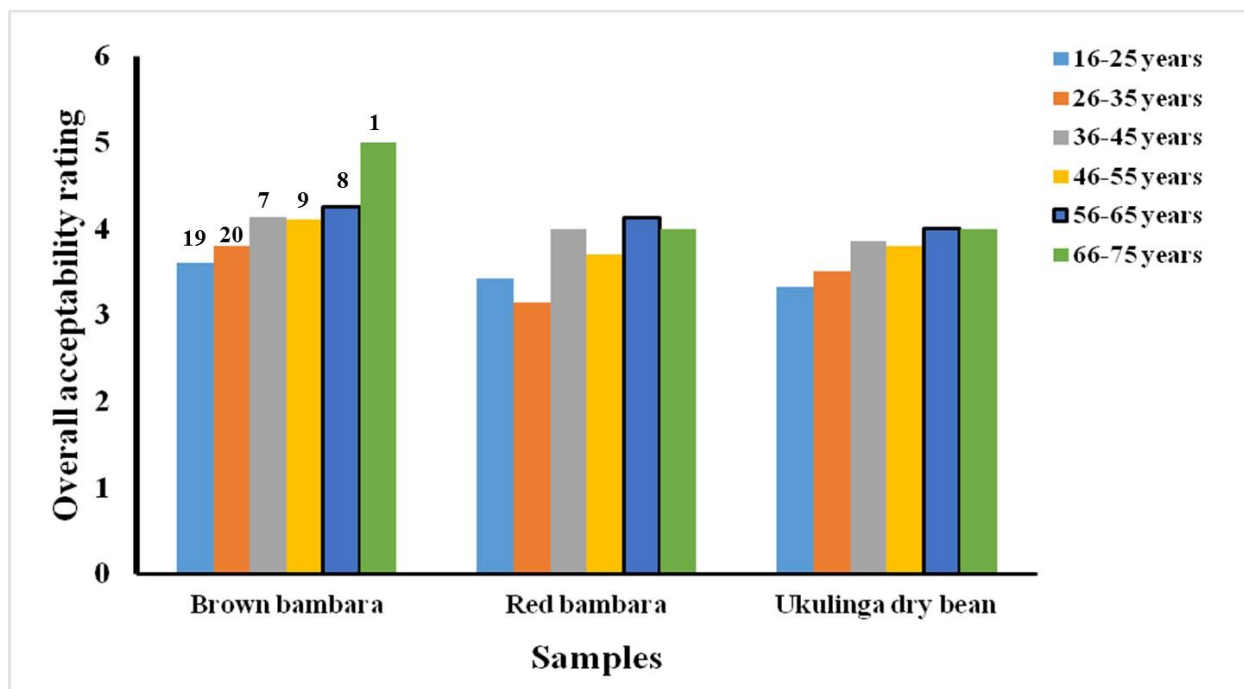


Figure 5.4 Relationship between age group and overall acceptability of bambara and dry bean purees

*Values on bar chart represents number of participants in each age group

Consequently, chances of caregivers including bambara in children's diets may be high. It has been reported previously that older caregivers are key determinants of early childhood care patterns especially in Africa (Kerr *et al* 2008). Table 5.5 shows the relationship between sensory attributes and the overall acceptability of the puree.

Table 5.5 Linear regression coefficients showing the relationship between other sensory attributes and the overall acceptability of the puree

Sensory attribute	Standard error	t-stat	p-value*
Brown bambara puree			
Taste	0.980	6.594	0.000
Texture	0.073	1.268	0.210
Aroma	0.095	2.075	0.042
Colour	0.080	0.168	0.868
Ukulinga dry bean puree			
Taste	0.122	2.600	0.012
Texture	0.122	2.278	0.026
Aroma	0.121	1.963	0.054
Colour	0.121	0.533	0.596
Red bambara puree			
Taste	0.135	4.201	0.000
Texture	0.134	1.429	0.158
Aroma	0.112	-0.567	0.573
Colour	0.125	0.935	0.353

*Level of significance at $p \leq 0.05$

Linear regression analysis showed that there was a linear relationship between the colour, acceptability and overall acceptability of the brown bambara and the reference dry bean, while aroma acceptability was linearly related to the overall acceptability of the red bambara (Table 5.4). Hence, sensory properties of bambara landraces should be determined and landraces with more acceptable sensory properties be selected for making bambara puree.

5.3.4 Focus group discussion

Caregivers are important determinants of a child's feeding pattern and may influence the health status of a child (Kerr *et al* 2008). Unavailability of BGN is a major factor affecting its utilisation. Participants identified changes in soil and climate conditions and lack of proper knowledge on cultivation techniques as some of the factors responsible for its unavailability. Overall, the caregivers had positive perceptions about the sensory characteristics of the bambara puree (Table 5.6) which is in agreement with previous findings (Hillocks *et al* 2012; Bamshaiye *et al* 2011).

Table 5.6 Caregivers' perceptions of bambara groundnut as a food source

Discussion topics	Themes	Concepts	Responses	Quotes
Familiarity of consumers with bambara groundnut	Consumer perception about bambara groundnut as a food source	Reasons for its poor utilisation	Market availability of bambara groundnut is an important factor affecting its popularity and utilisation. Older participants were able to easily identify the grain and describe its utilisation. Participants associated the unavailability of bambara groundnut with changes in the soil conditions, which does not favour the cultivation of the plant. Other factors like climate conditions, time of planting and seed viability also influence plant growth. Extension programmes to improve the cultivation and sensitisation on accrued benefits of bambara may be necessary. This would enlighten farmers on the appropriate planting conditions for bambara groundnut to maximise yield.	<p><i>"Local name Izindlubu."</i></p> <p><i>"Soil does change as back in the days it was grown here but now it is not."</i></p> <p><i>"If we get hold of the seeds we would most definitely plant them."</i> <i>"They also fertilize the soil, or so it is said so we would grow them."</i></p>
Willingness to use bambara as a complementary food	Willingness	<ul style="list-style-type: none"> • Sensory properties • Health implications 	Participants likened the sensory characteristics of bambara groundnut to that of the common dry bean. They were willing to include bambara in both family and children's meals. However, they had certain concerns about bambara groundnut causing gastrointestinal side effects in children who consumed it.	<p><i>"Bambara groundnut seeds were very delicious."</i></p> <p><i>"Old people can accept the bambara but children might have problem."</i></p>
	Preparation methods	<p>Could be boiled and eaten as snack or made into curry or porridge.</p> <p>Could also be included in other foods like <i>uphuthu</i>, <i>izinkobe</i> and <i>samp</i></p>		<p>Bambara could be used to make <i>izinkobe</i> (Bambara groundnut and dried mealies)</p>
Likelihood of purchase	Willingness to buy bambara if it was cheaper and for its health benefits	Availability at grocery stores and affordability	Participants were willing to buy bambara to prepare children's meals. However, this is subject to its availability at grocery stores and also affordability. The participants mentioned that bambara is not commonly found at grocery stores. They were also willing to buy this grain because of its nutritional benefits since highly nutritious foods are required for proper growth and development.	<i>"Foods are needed, especially those with high nutrient content."</i>

The caregivers identified the unavailability of Bambara groundnut as the key factor limiting its utilisation. They stated that they could eat BGN alongside other foods like *uphuthu*, *izinkobe* and *samp*. Bambara was used by this community at one stage and had reduced over time due to lack of planting seeds and inadequate knowledge on cultivation practices. Although some of the caregivers had concerns about the likelihood of infants experiencing gastrointestinal problems if they ate the Bambara puree, most of them were willing to feed their children the Bambara puree for its nutritional benefits. They were also willing to include BGN in their farming system if they had access to viable planting seeds. Market availability of Bambara therefore needs to be improved.

5.4 Conclusion

BGN is not a popular grain at Swayimane, KwaZulu-Natal province and its utilisation seems to be limited due to poor market availability and knowledge on cooking methods. However, Bambara has the potential to be used as a protein source in infant complementary feeding due to its sensory attributes being comparable to that of the common dry bean. Nutrition education on the nutritional benefits of Bambara and improved market availability may help to improve its overall utilisation.

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CHAPTER 6

FUNCTIONAL AND NUTRITIONAL PROPERTIES OF A BAMBARA GROUNDNUT COMPLEMENTARY FOOD (PUREE)

Abstract

Complementary foods are required to meet the nutritional requirements of infants after their first six months of life. In most developing regions, they are usually made from local staples such as cereals and legumes. Nutritional and functional properties of complementary foods are key quality attributes. The aim of this study was to assess the nutritional and functional properties a BGN complementary food (puree). Red bambara landrace had a shorter cooking time compared to the brown landrace. Soaking of the grains in water prior to cooking significantly ($p \leq 0.05$) decreased the cooking time. Brown bambara puree had a protein content (23.36 g/100 g) comparable to that of the reference dry bean puree (23.23 g/100 g) whilst, the red bambara landrace puree had a significantly ($p \leq 0.05$) lower protein content (19.48 g/100 g). The brown bambara variety had higher concentrations of amino acids than the red landrace. The glycemic carbohydrate and fibre contents of all the samples increased with cooking, whilst a decrease was observed in the mineral content. Cooking caused an increase in the amino acid concentration of the bambara landraces for most of the amino acids tested, but the methionine contents of the two bambara landraces and the reference bean decreased by approx. 90 and 20%, respectively. An approximate 14% increase in fat content was observed in the bambara landraces after cooking while that of the reference bean decreased by 16%. Cooking caused a significant increase in the total phenolic content (TPC) of the grains and a decrease in phytic acid and tannin contents, which seems to have resulted in an increase in the *in vitro* starch and protein digestibility, respectively, of all the samples. The apparent viscosities of paste of cooked grains were 5 times lower than that of their raw grains. Thermal processing had a varying effect on the conformation of proteins as observed in the FTIR spectra of all samples. With the exception of minerals, nutrients are retained when BGN grain is soaked and then cooked into a puree, whilst antinutritional factors are reduced. BGN puree is a nutritious complementary food that has the potential for use in place of animal protein, especially in developing regions where animal food sources are generally less accessible than in developed regions.

6.1 Introduction

Complementary foods are introduced to infants when breast milk is no longer enough to meet their nutritional needs. Beyond six months of age, infants require complementary foods

with enough energy and nutrients in addition to breast milk to meet their physiological requirements (Islam, Ahmed, Peerson, Mollah, Khatun, Dewey & Brown 2016). In most of the developing regions, complementary foods are formulated using local staples such as cereals (Ikujenlola 2008; Ikujenlola & Fashakin 2005). In some instances, these cereals are used either alone or as composites with legumes such as soybean (Ijarotimi & Famuewa 2006). The major reason for combining these basic food groups i.e. cereals and legumes is to complement each other in terms of their limiting amino acids. Cereals are deficient in lysine, while legumes are deficient in the sulfur-containing amino acid methionine. The adoption of a complementary food in specific regions of the world may vary with socio-cultural practices, the knowledge of the nutrient composition of the complementary food as well as the availability of the raw materials used in the formulation. Furthermore, the acceptability of complementary foods is reported to be influenced by the functional properties of the ingredients used in their formulation (Usman, Bolade & James 2016). Functional properties such as water absorption and viscosity are important in determining acceptability and utilisation. They are also very important for ensuring that the complementary foods are appropriate for the growing child (Usman *et al* 2016).

Okoye & Ojobor (2016) suggest that the ideal ingredients for the formulation of low cost complementary foods must be obtained from dietary staples that are readily available and affordable in the region of interest. In SSA, some underutilised legumes, such as BGN, seem more attractive for use in complementary foods relative to the traditionally used legumes such as the common dry bean. Bambara groundnut (*Vigna subterranea*) is indigenous to southern Africa and is traditionally grown by farmers for subsistence. It is a more affordable source of protein (17-24.6%) and carbohydrate (56.5-59.5%) compared to cowpea and is highly drought tolerant (Hillocks, Bennett & Mponda 2012). The use of bambara in combination with cereals in the formulation of complementary food has been reported previously (Oluwatofunmi, Ishola & Bamidele 2015; Ijarotimi & Keshinro 2012). A survey conducted in rural KwaZulu-Natal province of South Africa revealed that caregivers (n=70) were willing to use BGN complementary food for their infants. The majority of the surveyed caregivers indicated that they would process the BGN alone into a complementary food and would not combine it with a cereal. On the other hand, no published reports could be found on the use of BGN alone in preparing complementary foods.

A recent study by Islam *et al* (2016) found that infants consumed fewer meals per day and at the same time greater amounts of complementary foods per meal when they received diets with lower energy density. Unlike the conventional complementary foods made from cereals which are high in energy and lack protein, BGN may be an ideal ingredient for the preparation of complementary food with a good balance of protein and energy. The objective of this study was to investigate the functional and nutritional properties of a complementary food formulated using two BGN landraces.

6.2 Materials and Methods

6.2.1 Materials

Two BGN landraces (red and brown) were used in this study. The landraces were selected because they are among the most commonly consumed landraces in southern Africa. A dry bean (*Phaseolus vulgaris*), (Ukulinga dry bean variety) commonly consumed in South Africa was used as a reference.

6.2.2 Preparation of the complementary food (puree)

The two BGN landraces and the reference dry bean puree were all prepared using a standard recipe as described by the survey participants (Appendix J). The purees (Figure 6.1) were prepared by a black African woman living in a rural area in the Umgungundlovu District who had experience of cooking dry bean puree. Details of the method of preparation of the puree have been described in Chapter 5, section 5.2.3.3.

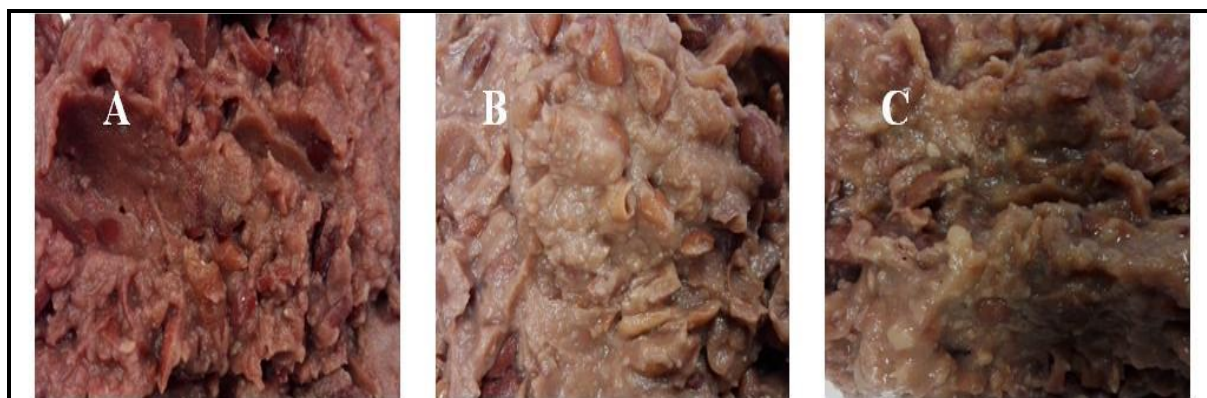


Figure 6.1 A - red bambara puree, B - brown bambara puree, C - Ukulinga dry bean puree

6.2.3 Nutritional composition of cooked bambara groundnut

The nutritional composition of the bambara puree and the reference dry bean were determined using standard methods as previously described (Chapter 4, section 4.2.3).

6.2.3.1 Amino acid determination

The amino acid profile of the samples was analysed by the Pico-Tag method (Millipore Corporation 1986, 1987) using a Waters Breeze High-Performance Liquid Chromatography (HPLC) with Empower software (Waters, Millipore Corp., Milford, MA). Samples (400 mg) were hydrolysed with 6 N HCl for 24 hours and then derivatized with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl (PTC) amino acids, which were analysed by reverse phase HPLC. The derivatized samples were separated on cationic column (4.6x150 mm) using a gradient of sodium citrate buffers (pH 3.45 and pH 10.85) at a flow rate of 0.45 mL/minute.

6.2.3.2 *In vitro* starch digestibility

In vitro starch digestibility was determined according to Oyeyinka, Singh, Venter & Amonsou (2016). Briefly, porcine pancreatic α -amylase (3.89 g) was dispersed in deionised water (25.7 ml), centrifuged for 10 min at 2500 x g, and 18.7 ml of supernatant collected. Amyloglucosidase (1 ml) diluted in deionised water (2 ml) was added to the supernatant. The enzyme solution was freshly prepared for the digestion analysis. Aliquots of guar gum (10 ml, 5 g/l) and sodium acetate buffer (5 ml, 0.5 M) were added to the starch samples (0.5 g, dry basis) in plastic centrifuge tubes. Seven glass balls (10 mm diameter) and 5 ml of enzyme solution were then added to each tube, following the incubation in a water bath (37°C) with agitation (170 rpm). Aliquots (0.5 ml) were taken at intervals and mixed with 4 ml of 80% ethanol, and the glucose contents in the mixture were measured using glucose oxidase and peroxidase assay kits. Nutritional starch fractions based on digestibility were: rapidly digestible starch (RDS), which represents the portion of starch that was hydrolysed within 20 min of incubation; slowly digestible starch (SDS), the starch hydrolysed between 20 and 120 min; and resistant starch (RS), was estimated as the starch not digested after 120 min of incubation.

$$\text{RDS} = \text{Glucose (20 min)} \times 0.9$$

$$\text{SDS} = \text{Glucose (120-20 min)} \times 0.9$$

$$\text{RS} = \text{TS} - (\text{RDS} + \text{SDS}) \times 0.9$$

*TS - total starch

6.2.3.3 Protein digestibility

Protein digestibility studies was carried out as described by Tinus, Damour, Van Riel & Sopade (2012). Details of this method are described in Chapter 4, section 4.2.5.

6.2.3.4 Anti-nutritional factors

Tannin content was determined using the modified vanillin-HCl method as described by Mazahib, Nuha, Salawa & Babiker (2013) and phytic acid content was according to Aina, Binta, Amina, Hauwa Haruna, Hauwa, Akinboboye & Mohammed (2012). Details of the methods are described in Chapter 4, section 4.2.4.

6.2.4 Total phenolic content (TPC) and antioxidant activities of bambara groundnut

Total phenolic content (TPC) was determined as described by Tesfay, Bertling, Odindo, Workneh & Mathaba (2011) with slight modifications. Antioxidant activity was measured by the ABTS radical scavenging assay and DPPH radical scavenging assay as described previously (Chapter 4, section 4.2.6).

6.2.5 Functional properties of flour

6.2.5.1 Water and oil absorption capacities

The water absorption capacity (WAC) and oil absorption capacity (OAC) of the puree flour samples was determined using methods described by Elkhalfifa, Schiffler & Bernhardt (2005). Details of the methods are described in Chapter 4, section 4.2.7.1.

6.2.5.2 Dynamic rheology

The rheological properties of the cooked samples were measured according to Oyeyinka *et al* (2015) as described previously (Chapter 4, section 4.2.7.1).

6.2.5.3 Fourier Transform Infrared Spectroscopy (FTIR)

Protein extraction

Protein concentrates from the raw and cooked samples were extracted using a method described by (Arise, Amonsou & Ijabadeniyi 2015). Raw and cooked bambara groundnuts and reference bean flours were defatted with n-hexane in the ratio 1:5 (flour: solvent) on a magnetic stirrer (3 h) at speed of 198 g. The defatted flours were allowed to dry in a fume hood overnight to remove the residual hexane. They were then suspended in water at 1:10 (flour to water ratio) and pH was adjusted to pH 8.0 with 1 M NaOH to facilitate protein

solubilisation. The suspension was stirred for 4 h at 32°C in a shaking water bath (Scientific, 132A, Pretoria, South Africa) and centrifuged at 4000 g for 30 min (temperature: 4°C). After centrifugation and recovery of the supernatant, the precipitate was re-suspended in half the volume of initial water and the extraction process repeated. The supernatants were pooled together and their pH adjusted to pH 4.0 with 0.5 M HCL to precipitate the protein, which was recovered by centrifugation at 5000 g for 30 min at 4°C. The protein concentrate was freeze dried and kept at 4°C until required.

Fourier Transform Infrared spectroscopy (FTIR)

An attempt was made to analyse the changes in protein molecule conformation and interactions between protein and anti-nutritional factors during the processing of the bambara complementary food (puree). It was thought that the changes would be indicative of changes in protein digestibility. The analysis was carried using the modified method of Falade & Oyeyinka (2014). Accurately, 3 mg of the flour was mixed with 150 mg of fourier transform infrared-grade potassium bromide (KBR) and pressed using a manual press for 20 min to obtain a transparent pellet. The pellet formed was then transferred into the FTIR system to obtain the spectra.

6.2.6 Statistical analysis

All experiments were repeated three times. Data were analysed using analysis of variance (ANOVA) and means were compared using Fischer's Least Significant Difference (LSD) test. A p-value of ≤ 0.05 was considered significant.

6.3 Results and discussion

6.3.1 Nutritional composition of bambara puree

The nutritional composition of the bambara puree is reported in Table 6.1a.

Table 6. 1a Nutritional composition of bambara and Ukulinga dry bean puree compared to the raw grains (g/100 g)

Sample	Processing	Moisture	Protein	Fat	Ash	Carbohydrate
Brown bambara	Raw	8.71 ^c ± 0.19	23.25 ^b ± 0.01	7.09 ^d ± 0.52	4.52 ^b ± 0.09	56.42 ^e ± 0.74
Brown bambara	Cooked	3.96 ^e ± 0.05	23.36 ^b ± 0.06	8.11 ^b ± 0.06	4.47 ^b ± 0.05	60.10 ^c ± 0.41
Red bambara	Raw	10.90 ^b ± 0.15	19.22 ^d ± 0.18	7.58 ^c ± 0.05	4.18 ^c ± 0.03	58.13 ^d ± 0.11
Red bambara	Cooked	3.18 ^f ± 0.06	19.48 ^c ± 0.06	8.71 ^a ± 0.05	3.90 ^d ± 0.03	64.74 ^b ± 0.78
Ukulinga dry bean	Raw	14.79 ^a ± 0.27	27.36 ^a ± 0.24	1.53 ^e ± 0.10	4.70 ^a ± 0.13	51.61 ^f ± 0.26
Ukulinga dry bean	Cooked	6.63 ^d ± 0.07	23.23 ^b ± 0.12	1.28 ^e ± 0.12	2.85 ^e ± 0.07	66.01 ^a ± 0.22

Mean ± SD

Means with different superscripts in a column are significantly different ($p \leq 0.05$)

SD: Standard deviation

Protein (19.22-27.36 g/100 g) and carbohydrate (51.61-66.01g/100 g) are the major component of raw and cooked bambara grains as well as the reference bean (Table 6.1a). Fat (1.28-8.71 g/100 g) and ash (2.85-4.70 g/100 g) contents were generally low. Cooking did not significantly ($p \leq 0.05$) change the protein content of brown bambara, but changed that of red bambara slightly. However, the protein content of the control bean reduced significantly ($p \leq 0.05$) after cooking by approximately 15% (Table 6.1a). Ekpenyong & Borchers (1980) found that the protein content of winged beans (*Psophocarpus tetragonolobus*) increased by 14% after cooking. Other authors found minimal losses (0.86-2.63%) in protein concentration of different legumes after cooking (Rehman & Shah 2005). Differences may be observed in the protein content of cooked grains depending on the grain type, the extent to which soluble solids are lost during soaking and cooking (Ekpenyong & Borchers 1980), partial removal of certain amino acids and other nitrogenous compounds via leaching during soaking (Rehman & Shah 2005; Clawson & Taylor 1993; Lyimo, Mugula & Elias 1992).

The fibre content of raw and cooked bambara, measured as acid detergent fibre (ADF) or neutral detergent fibre (NDF) and individual mineral elements are presented in Table 6.1b.

Table 6. 1b Nutrient composition of bambara and Ukulinga dry bean puree compared to the raw grains (g/100 g)

Sample	Processing	ADF*	NDF*	Ca*	Mg*	K*	Na*	P*	Fe**	Mn**	Cu**	Zn**
Brown bambara	Raw	11.90 ^b ±	32.71 ^c ±	0.053 ^c ±	0.171 ^b ±	1.669 ^a ±	0.008 ^a ±	0.456 ^b ±	47.46 ^c ±	15.33 ^b ±	8.33 ^c ±	27.75 ^{bc}
		0.33	0.46	0.001	0.000	0.010	0.001	0.002	0.17	0.03	0.58	± 0.21
Brown bambara	Cooked	20.77 ^a ±	40.98 ^b ±	0.052 ^c ±	0.163 ^c ±	1.516 ^c ±	0.011 ^a ±	0.426 ^c ±	13.88 ^c ±	12.49 ^c ±	10.41 ^b ±	27.77 ^{bc}
		4.20	0.11	0.000	0.001	0.015	0.003	0.003	0.21	0.00	0.21	± 1.21
Red bambara	Raw	10.63 ^{bc} ±	21.44 ^d ±	0.048 ^c ±	0.166 ^c ±	1.467 ^c ±	0.025 ^a ±	0.287 ^e ±	25.44 ^d ±	11.00 ^c ±	6.00 ^d ±	23.94 ^c ±
		0.32	0.91	0.001	0.004	0.064	0.002	0.005	0.65	0.00	0.73	0.27
Red bambara	Cooked	19.61 ^a ±	63.24 ^a ±	0.048 ^c ±	0.155 ^d ±	1.339 ^d ±	0.014 ^a ±	0.272 ^f ±	27.54 ^d ±	10.33 ^c ±	9.09 ^c ±	24.10 ^{cc}
		2.05	0.28	0.001	0.002	0.011	0.003	0.001	0.21	0.00	0.21	± 1.19
Ukulinga dry bean	Raw	8.04 ^c ±	18.34 ^d ±	0.147 ^a ±	0.179 ^a ±	1.570 ^b ±	0.021 ^a ±	0.548 ^a ±	82.92 ^a ±	25.82 ^a ±	13.00 ^a ±	43.00 ^a ±
		0.46	0.84	0.005	0.001	0.008	0.002	0.009	0.80	0.08	0.00	1.73
Ukulinga dry bean	Cooked	21.31 ^a ±	42.98 ^b ±	0.134 ^b ±	0.122 ^e ±	0.805 ^e ±	0.011 ^a ±	0.376 ^d ±	65.69 ^b ±	16.42 ^b ±	6.57 ^d ±	30.71 ^b
		1.05	2.49	0.007	0.000	0.017	0.002	0.003	0.08	0.00	0.58	± 0.91

ADF- Acid Detergent Fibre, NDF- Neutral Detergent Fibre, *g/100 g, **mg/kg

Mean ± SD

Means with different superscripts in a column are significantly different (p ≤ 0.05)

SD: Standard deviation

The NDF values of bambara and the control bean were generally higher than ADF for both raw and cooked grains. This is because NDF measures most of the structural components in plant cells such as lignin, hemicellulose and cellulose, but not pectin (Lattimer & Haub 2010). ADF on the other hand measures highly indigestible parts of forage excluding hemicelluloses (Lattimer & Haub 2010). The higher NDF values of the grains suggest that bambara as well as the control bean are good sources of fibre especially hemicellulose. The levels of ADF and NDF were substantially higher in the cooked bambara grains compared to their raw counterparts, suggesting that cooking may have facilitated the release of the fibre components, thereby facilitating their analysis. Alternatively, this could be an apparent increase in fibre which is due to compensation for the decrease in the concentration of other components, especially protein.

Potassium (K), phosphorus (P) and calcium (Ca) were the major minerals detected in raw and cooked bambara grains (Table 6.1b). Previous studies similarly found Ca, K and P as major minerals in legumes, including BGN (Fasoyiro, Ajibade, Omole, Adeniyani & Farinde 2006). Among these minerals, K was the most predominant in the raw and cooked grains. Other minerals were present in relatively smaller quantities. The Ca, P and Magnesium (Mg) contents of raw bambara grains in the current study are similar to values reported for nine varieties of BGN grown in Botswana, Namibia and Swaziland (Amarteifio, Tibe & Njogu 2006). However, the K contents of bambara grains in the current study were about 1.2 to 4 times higher than values previously reported for bambara (Fasoyiro *et al* 2006), marama bean, mung bean and tepary bean (Amarteifio & Moholo 1998). Differences in mineral composition may be attributed to varietal differences and possibly growing conditions. The raw control dry bean showed significantly ($p \leq 0.05$) higher Ca, Mg, P, Iron (Fe), Manganese (Mn), Copper (Cu) and Zinc (Zn) contents than the bambara grains ($p \leq 0.05$).

Cooking generally reduced the mineral content of the grains. However, cooking had no significant effect on the Ca, Na and Zn contents of brown and red bambara grains as well as the Fe content of red bambara. Ekpenyong & Borchers (1980) similarly found that cooking had no effect on the Fe content of winged bean seeds. Raw (82.92 mg/kg) and cooked (65.69 mg/kg) control bean had substantially higher Fe contents than those of brown bambara (raw: 47.46 mg/kg, cooked: 13.88 mg/kg) and red bambara (raw: 27.54 mg/kg, cooked: 25.44 mg/kg). Several researchers reported the leaching of minerals during soaking or cooking of leguminous grains at different rates (Alajaji & El-Adawy 2006; El-Adawy 2002). Variations

observed in the leaching rate of minerals during cooking may depend on the method of cooking. According to El-Adawy (2002), microwave cooking resulted in the greatest retention of all minerals in legumes followed by autoclaving and then boiling. In the current study, the cooking losses of Mg (approx. 6%) and K (approx. 9%) in brown and red bambara were much lower compared to the losses in the control bean (Mg: 31%, K: 48%). BGN could therefore serve as a good source of both macro and micro nutrients especially among vulnerable population groups such as infants and pregnant women from poor, developing countries. The differences in retention rates of the minerals could also have been due to differences in their interaction with other components of the food matrix; the mineral elements that are more tightly bound to other components would have higher retention rates.

6.3.2 Amino acids composition of the raw and cooked grains

The amino acid content of raw and cooked BGN and the reference bean is presented in Table 6.2. A typical chromatogram showing integrated amino acids peaks in BGN is presented in Figure 6.2.

Table 6.2 Amino acid composition of raw and cooked bambara samples g/100 g

Sample	Processing	Essential amino acids								Non-essential amino acids								
		His	Thr	Val	Ile	Leu	Phe	Lys	Met	Ser	Arg	Gly	Asp	Glu	Ala	Pro	Cys	Tyr
Brown bambara	Raw	0.53 ^{a-}	0.57 ^{bc}	0.80 ^{cd}	0.63 ^c	1.34 ^{a-}	1.02 ^a	1.21 ^{ab}	0.10 ^a	0.87 ^{bc}	1.19 ^{ab}	0.73 ^{ab}	2.13 ^{ab}	2.94 ^a	0.70 ^{ab}	0.83 ^{ab}	0.01 ^a	0.28 ^a
		^c ±	±	±	±	^c ±	±0.1	±	±	±	±	±	±	±	±	±	±	±
Brown bambara	Cooked	0.02	0.14	0.05	0.07	0.12		0.24	0.01	0.17	0.21	0.03	0.51	0.71	0.10	0.05	0.01	0.05
		0.64 ^a	0.55 ^c	0.89 ^{bc}	0.69 ^{bc}	1.43 ^{ab}	1.27 ^a	0.91 ^{ab}	0.00 ^b	0.90 ^{a-}	1.30 ^a	1.00 ^a	2.00 ^{a-}	2.83 ^{ab}	0.74 ^{ab}	1.12 ^a	0.01 ^a	0.23 ^{ab}
Red bambara	Raw	0.42 ^c	0.38 ^c	0.65 ^d	0.42 ^d	1.04 ^c	0.90 ^a	0.68 ^b	0.10 ^a	0.61 ^d	0.80 ^c	0.65 ^b	1.33 ^c	1.93 ^b	0.50 ^c	0.71 ^b	0.03 ^a	0.21 ^{a-}
		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	^c ±
Red bambara	Cooked	0.01	0.04	0.04	0.11	0.08	0.07	0.33	0.04	0.02	0.02	0.04	0.15	0.11	0.05	0.01	0.04	0.00
		0.50 ^{bc}	0.44 ^c	0.66 ^d	0.53 ^{cd}	1.12 ^{bc}	0.98 ^a	0.90 ^{ab}	0.02 ^b	0.72 ^{cd}	0.95 ^{bc}	0.79 ^{ab}	1.68 ^{bc}	2.34 ^{ab}	0.61 ^{bc}	0.92 ^{ab}	0.02 ^a	0.13 ^c
Ukulinga dry bean	Raw	0.07	0.01	0.06	0.09	0.23	0.29	0.04	0.02	0.01	0.13	0.17	0.02	0.07	0.03	0.19	0.02	0.04
		0.59 ^{ab}	0.83 ^a	1.05 ^a	0.89 ^a	1.66 ^a	1.19 ^a	1.54 ^a	0.12 ^a	1.15 ^a	1.21 ^{ab}	0.86 ^{ab}	2.53 ^a	3.08 ^a	0.83 ^a	0.88 ^{ab}	0.02 ^a	0.29 ^a
Ukulinga dry bean	Cooked	0.07	0.09	0.13	0.06	0.18	0.16	0.30	0.01	0.15	0.16	0.10	0.33	0.40	0.11	0.13	0.01	0.01
		0.55 ^{ab}	0.76 ^{ab}	1.00 ^{ab}	0.82 ^{ab}	1.59 ^a	1.15 ^a	1.52 ^a	0.10 ^a	1.08 ^{ab}	1.08 ^{a-}	0.85 ^{ab}	2.32 ^{ab}	2.66 ^{ab}	0.78 ^{ab}	0.85 ^{ab}	0.01 ^a	0.18 ^{bc}
		±	±	±	±	±	±	±	±	^c ±	±	±	±	±	±	±	±	±
		0.02	0.06	0.03	0.03	0.04	0.11	0.33	0.00	0.05	0.06	0.13	0.08	0.13	0.03	0.15	0.01	0.03

Mean ± SD

Means with different superscripts in a column are significantly different (p ≤ 0.05)

SD: Standard deviation

His- histidine; Thr- threonine; Val- valine; Ile- isoleucine; Leu- leucine; Phe- Phenylalanine; Lys- lysine; Met- methionine; Ser- serine; Agr- arginine; Gly- glycine; Asp- asparagine; Glu- glutamic acid; Ala- alanine, Pro- proline; Cys- cysteine; Tyr- tyrosine

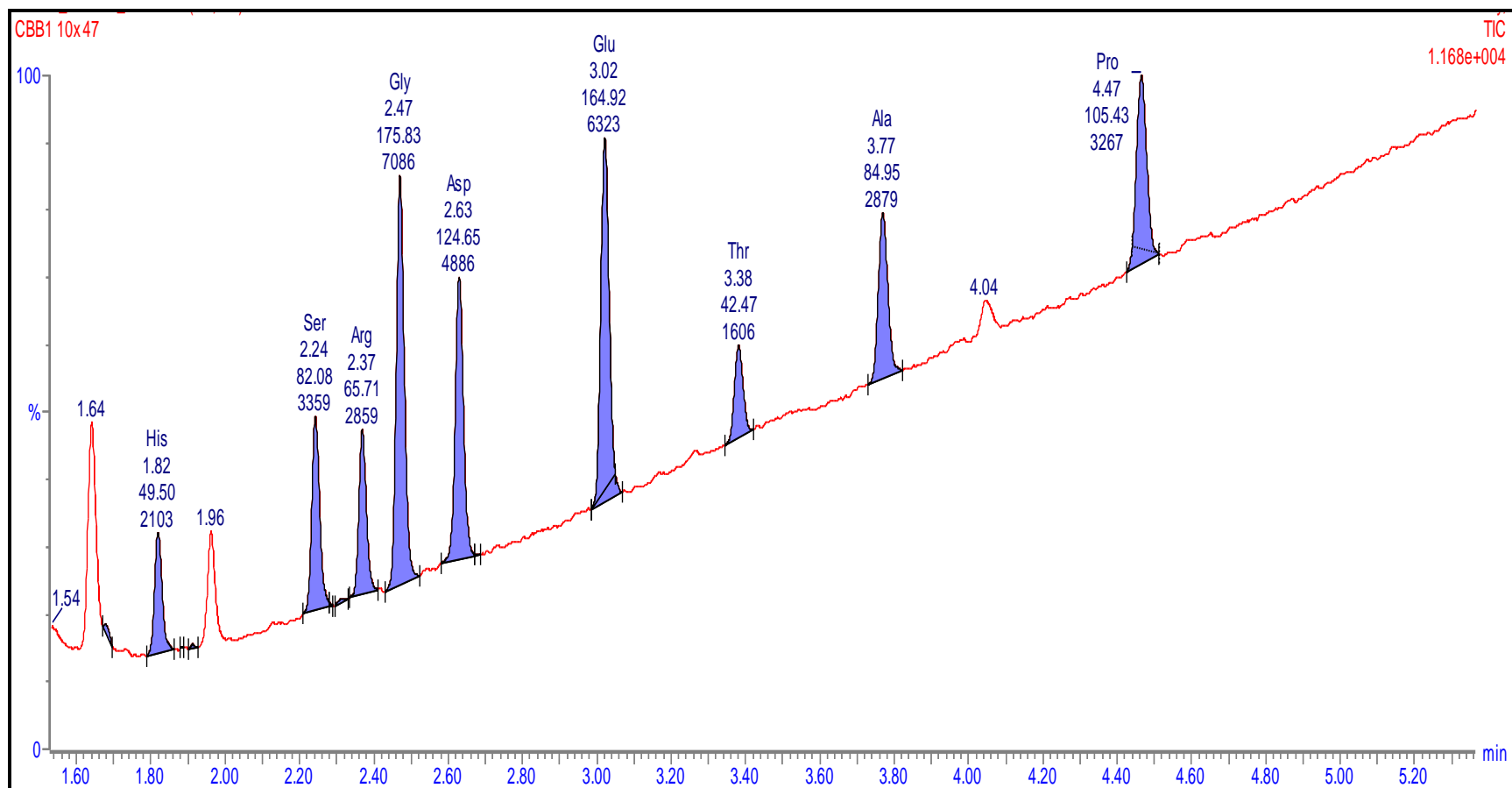


Figure 6.2 Typical processed chromatogram showing some of the integrated amino acid peaks

Generally, the raw reference bean had higher concentrations of essential and non-essential amino acids than the raw bambara landraces. Brown BGN had higher concentrations of amino acids than the red landrace. Among the essential amino acids, histidine, isoleucine and leucine were predominant, followed by valine, phenylalanine, lysine, isoleucine and threonine. The protein quality of the raw bambara grains and reference bean and their corresponding purees was evaluated in terms of essential amino acid scoring pattern using WHO/FAO/UNU standards (WHO 2002) (Table 6.3).

Table 6.3 Comparison of essential amino acid composition of bambara groundnut and reference dry bean with amino acid scoring pattern

Sample	Protein	Essential amino acids						
		His	Thr	Val	Ile	Leu	Phe	Lys
Raw brown bambara	23.25 ^a	0.53 ^b 23 ^c	0.57 25	0.80 34	0.63 27	1.34 58	1.02 44	1.21 52
Cooked brown bambara	23.36	0.64 27	0.55 24	0.89 38	0.69 30	1.43 61	1.27 54	0.91 39
Raw red bambara	19.22	0.42 22	0.38 20	0.65 34	0.42 22	1.04 54	0.90 47	0.68 35
Cooked red bambara	19.48	0.50 26	0.44 23	0.66 34	0.53 27	1.12 57	0.98 50	0.90 46
Raw Ukulinga dry bean	27.36	0.59 22	0.83 30	1.05 38	0.89 33	1.66 61	1.19 43	1.54 56
Cooked Ukulinga dry bean	23.23	0.55 24	0.76 33	1.00 43	0.82 35	1.59 68	1.15 50	1.52 65
Amino acid scoring pattern (mg/g protein requirement)^d								
0.5 years		20	31	43	32	66	N/A	57
1-2 years		18	27	42	31	63	N/A	52
3-10 years		16	25	40	31	61	N/A	48

a- g/100 g, dry weight; b- Amino acid content (g/100 g, db); c -Amino acid concentration in mg/g protein; rounded off to a whole number; d- WHO (2002); N/A =Not available; His- histidine; Thr- threonine; Val- valine; Ile- isoleucine; Leu- leucine; Phe- phenylalanine; Lys- lysine

The sulfur-containing amino acid methionine was found to be the most limiting in the studied samples and this confirms previous reports on legumes being poor sources of methionine (Iqbal, Khalil, Ateeq & Sayyar Khan 2006). However, it was previously reported that BGN had a superior methionine content compared to soybean (Apata & Ologhobo 1994). BGN

could be used to supplement other grains such as cereals to obtain a nutritional balance of amino acids. Cooking caused an increase in the amino acid concentration in the bambara landraces for most of the amino acids tested. This result agrees with those reported by Khattab, Arntfield & Nyachoti (2009) for Canadian and Egyptian cowpea, kidney bean and pea. The reason for these slight increases after cooking could be degradation of some other amino acids. However, a decrease was observed in the concentration of methionine after cooking. Alajaji & El-Adawy (2006); Khalil & Mansour (1995) also reported a decrease in the sulfur-containing amino acids after cooking of chickpea and faba bean respectively. On the other hand, the concentration of all amino acids tested reduced in the reference dry bean. Breeding of high protein varieties which special attention to the limiting amino acids could be another means of overcoming the nutritional deficiencies of BGN and most legumes.

6.3.3 *In vitro* starch digestibility

As stated earlier, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) depending on the rate and extent of *in vitro* digestion. RDS and SDS fractions are digested in the small intestine at different rates while the RS fraction is not enzymatically digestible and therefore fermented in the large intestine. *In vitro* starch digestibility assays allows for the determination of nutritionally important starch fractions. Table 6.4 shows the effect of cooking on the *in vitro* starch digestibility of BGN and the reference dry bean.

Table 6.4 Effect of cooking on *in vitro* starch digestibility

Sample	RDS	SDS	RS
Raw brown bambara	23.68 ^d ± 0.02	47.65 ^a ± 0.92	28.67 ^c ± 0.94
Cooked brown bambara	70.42 ^b ± 0.05	10.70 ^c ± 0.10	18.88 ^e ± 0.15
Raw red bambara	21.72 ^e ± 0.04	47.96 ^a ± 0.04	30.31 ^b ± 0.08
Cooked red bambara	72.85 ^{a±} 0.58	9.72 ^c ± 0.45	17.43 ^f ± 0.13
Raw Ukulinga dry bean	17.18 ^f ± 0.08	46.21 ^b ± 0.06	36.61 ^a ± 0.03
Cooked Ukulinga dry bean	69.54 ^c ± 0.06	10.29 ^c ± 0.19	20.09 ^d ± 0.06

RDS- rapidly digestible starch, SDS- slowly digestible starch, RS- resistant starch

Mean ± SD

Means with different superscripts in a column are significantly different ($p \leq 0.05$)

SD: Standard deviation

RDS and SDS fractions of the samples significantly ($p \leq 0.05$) increased after cooking indicating an increase in starch digestibility. A similar trend was observed in cooked black gram, chickpea and mung bean (Kaur, Sandhu, Ahlawat & Sharma 2015; Zia-ur-Rehman 2007). Cooking has been suggested to increase the susceptibility of legume starch to pancreatic amylase activity (Zia-ur-Rehman 2007). This was suggested to be due to gelatinisation of starch during cooking and reduction of antinutritional factors (Table 6.6) (Giuberti, Gallo, Cerioli, Fortunati & Masoero 2015). The rupture of starch granules during cooking facilitates amylolysis and hence decreases the levels of resistant starch fractions (Yagoub & Abdalla 2007). A decrease in the RS fractions could increase the proportion of starch that the body is able to utilise.

6.3.4 *In vitro* protein digestibility (IVPD)

Low digestibility of the protein found in legumes is one of the main shortcomings in their nutritional quality. Table 6.5 shows the effect of cooking on the protein digestibility of BGN and the reference dry bean.

Table 6.5 *In vitro* protein digestibility

Samples	IVPD g/100 g	% Increase
Raw brown bambara	71.18 ^b ± 5.76	-
Cooked brown bambara	77.97 ^a ± 6.14	9.54
Raw red bambara	81.41 ^a ± 2.04	-
Cooked red bambara	82.33 ^a ± 4.86	1.13
Raw Ukulinga dry bean	74.17 ^b ± 3.32	-
Cooked Ukulinga dry bean	75.25 ^a ± 10.75	1.46

Mean ± SD

Means with different superscripts in a column are significantly different ($p \leq 0.05$)

SD: Standard deviation

Cooking increased the *in vitro* protein digestibility (IVPD) of all the samples. However, a higher increase was observed in the cooked brown bambara (9.54%) than the red bambara and the reference dry bean (1.13% and 1.46%, respectively). This increase in IVPD for all the samples may be linked to the decrease in the antinutrient content (Table 6.7) and also structural fragmentation of long polymer chains of native proteins, including antinutritional factors such as trypsin inhibitors and lectins during thermal processing (Patil, Brennan,

Mason & Brennan 2016; Kalpanadevi & Mohan 2013). Heat treatment is said to be capable of destroying heat labile protease inhibitors and also denature proteins, thus opening the structure (especially globulins) and thereby making them less resistant to proteases (Khattab *et al* 2009). A similar trend was reported for soaked cooked *Vigna unguiculata* subsp. *Unguiculata* (Kalpanadevi & Mohan 2013). On the other hand, a decrease in IVPD after cooking of lentil cultivars was reported by Sulieman, Hassan, Osman, El Tyeb, El Khalil, El Tinay & Babiker (2008). This decrease was due to protein aggregation during thermal treatment.

6.3.5 Effect of cooking on the antinutrient and antioxidant content and antioxidant activity

bambara grains had higher phytic acid, tannin, membrane bound phenol and TPC than the control bean after cooking (Table 6.6). Cooking generally reduced the phytic acid and tannin contents of the grains, but increased the membrane bound phenol and total phenolic contents (Table 6.6). Free phenolic compounds were not detected in the cooked grains. Phenolic compounds in legumes may exist in bound forms as conjugates with protein, sugars or fatty acids. These conjugates may dissociate after cooking leading to an increase in the total fraction of phenolics (Gujral, Sharma, Gupta & Wani 2013; Randhir, Kwon & Shetty 2008). Hence, the increase in the TPC of the grains after cooking could be due to dissociation of conjugates formed due to the leaching of non-phenolic soluble components such as mono-, di- and oligosaccharides, soluble fibres and proteins in the grains. Furthermore, Dewanto, Wu, Adom & Liu (2002) also suggested that phenolic compounds which accumulate in vacuoles are released during thermal processing due to the breakdown of cellular constituents and membranes. Although there are reports where the TPC reduced after pressure cooking of common beans (Rocha-Guzmán, González-Laredo, Ibarra-Pérez, Nava-Berumen & Gallegos-Infante 2007) and boiling of chickpea (Xu & Chang 2008), some authors also found an increase in TPC of yellow pea, green pea (Han & Baik 2008), soybean (Gujral *et al* 2013; Han & Baik 2008) and common bean (Fernandez, Elias, Braham & Bressani 1982), after cooking. Differences in the result recorded in the literature including that of the present study could be attributed to varying degrees of heat treatment used, possible formation of insoluble complexes between phenolic compounds and protein or polysaccharides as well as variation in the degree of leaching of non-phenolic soluble components as previously noted.

Table 6. 6 Effect of cooking on ant nutrient and antioxidant content

Grain type	Phytic acid*		Tannin*		Membrane bound phenol**		Free Phenol**		Total phenol**	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Brown bambara	781.23 ^a ±0.45	92.82 ^a ± 0.00	5.17 ± 0.36 ^a	0.31 ^a ± 0.01	0.14 ± 0.03 ^{bc}	0.44 ^a ± 0.01	0.05 ± 0.01 ^{ab}	ND	0.19 ± 0.02 ^{bc}	0.44 ^a ± 0.01
Red bambara	587.86 ± 0.47 ^{bc}	69.62 ^b ± 0.74	5.26 ± 0.44 ^a	0.29 ^a ± 0.02	0.18 ± 0.08 ^b	0.41 ^a ± 0.02	0.04 ± 0.01 ^{abcd}	ND	0.22 ± 0.08 ^{ab}	0.41 ^a ± 0.02
Ukulinga dry bean	672.95 ± 0.03 ^{ab}	69.62 ^b ± 0.04	3.04 ±0.12 ^{bcd}	0.20 ^a ± 0.17	0.09 ± 0.01 ^d	0.36 ^b ± 0.02	0.05 ± 0.00 ^{ab}	ND	0.13 ± 0.01 ^d	0.36 ^b ± 0.02

Mean ± SD. Means with different superscript in a column are significantly different ($p \leq 0.05$); *mg/100 g; **mg/g of catechin equivalent, db.

ND-not detected

SD: Standard deviation

Cooking resulted in a substantial reduction (approx. 94%) of the tannin content of all the grains, whilst phytic acid decreased by approximately 89% after cooking (Table 6.6). Tannins and phytic acid are sensitive to heat and may be lost to varying degrees during soaking. Bambara grains and the control bean were soaked prior to cooking. Losses in phytic acid and tannins may have occurred due to leaching during soaking and extensively due to the heat applied during cooking. Siddhuraju & Becker (2001) reported between 4-8% reductions in phytic acid content due to leaching in two varieties of mucuna beans (*Mucuna pruriens*). According to these authors, phytic acid with low molecular weight and ionic in nature are more susceptible to leaching. The effect of heat on phytic acid reduction during cooking may be explained partly by the formation of insoluble complexes between phytate and other components such as phytate-mineral, phytate-protein and phytate-protein-mineral complexes (Siddhuraju & Becker 2001). Another possible reaction which could have resulted in the reduction of phytic acid content of cooked grains include hydrolysis of inositol hexaphosphate to penta- and tetraphosphates (Siddhuraju & Becker 2001). Similarly, tannin-protein, tannin-polysaccharide and tannin-mineral complexes could have formed resulting in reduction of the proportion of assayable tannins.

The antioxidant activities of raw and cooked grains of two BGN landraces and the reference dry bean using DPPH and ABTS assays are presented in Figure 6.3 and Figure 6.4, respectively.

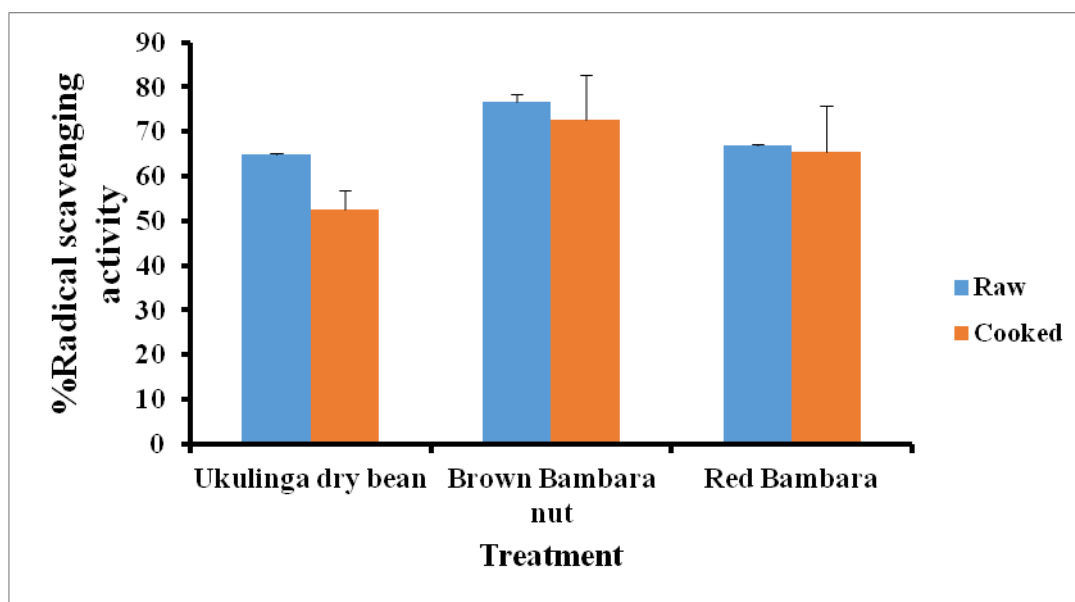


Figure 6.3 Effect of cooking on % DPPH radical scavenging activity of the legumes

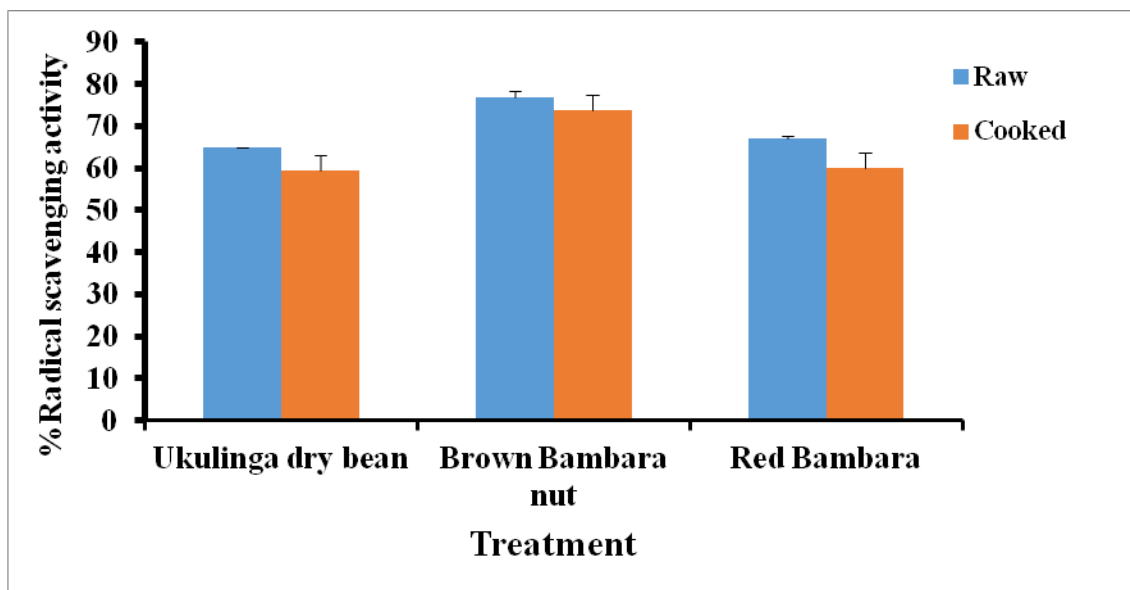


Figure 6.4 Effect of cooking on ABTS radical scavenging activity of legume samples

Raw brown bambara exhibited significantly ($p \leq 0.05$) higher ability to scavenge free radicals with DPPH values of 76.71% compared to the red bambara (67.03%) and the control bean (64.86%). High antioxidative potential of leguminous grains is generally associated with their TPC (Boateng, Verghese, Walker & Ogutu 2008; Han & Baik 2008; Siddhuraju & Becker 2007; Amarowicz, Naczki & Shahidi 2000). Han & Baik (2008) studied changes in the antioxidant activity and phenolic content of different legumes in comparison with an oil seed (soybean) during processing. These authors found that the TPC of the grains showed a significant positive correlation ($r \geq 0.7$) with the antioxidant activity, for both raw and the cooked grains. However, in the current study the TPC did not influence the antioxidant activity of the grains. Red bambara had slightly higher TPC (0.22%) than brown bambara (0.19%) and the control bean (0.13%) (Table 6.6). Thus, brown bambara possibly has a greater amount of higher molecular weight tannins than the red bambara and the control bean. Previous studies indicated that high molecular weight tannins exhibited relatively higher antioxidant activity than simple phenols (Hagerman, Riedl, Jones, Sovik, Ritchard, Hartzfeld & Riechel 1998).

After cooking, the DPPH values of the grains decreased by approximately 4, 9 and 10% for brown bambara, control bean and red bambara, respectively, whilst ABTS values of the cooked grains decreased by about 2, 5 and 19% for red bambara, brown bambara and the control bean,

respectively. Before cooking, the grains were soaked in water to facilitate softening of the grains and to reduce cooking time. The decrease in antioxidant activity could be attributed partly to soluble antioxidants that could be lost during soaking and the effect of heat during cooking (Xu & Chang 2008). The seed coat of legume grains has been reported to influence the extent and rate of leaching of polyphenols into water when soaked. Several other researchers have reported substantial reduction in antioxidant activity after pressure cooking (Rocha-Guzmán *et al* 2007; Siddhuraju & Becker 2007), boiling or steaming (Han & Baik 2008; Xu & Chang 2008).

Han & Baik (2008) reported an approximate 20% reduction in the antioxidant activity of lentil pardina cultivar, 18% reduction for soya bean and little or no changes for yellow pea, chick pea and green pea after cooking. Other factors which could account for the differences in antioxidant activity of the cooked grains include the initial tannin content (Table 6.6) of the respective grains, the distribution of the polyphenols between the seed coat and the cotyledon. Furthermore, polyphenols may also diffuse at different rates into the cotyledon from the seed coat during soaking, hence influencing the antioxidant activity of the cooked grains. For example, Rocha-Guzmán *et al* (2007) found that some varieties of common bean (*Phaseolus vulgaris* L.) showed higher TPC in the cotyledon than in the seed coat. Foods rich in antioxidants have been reported to be associated with a low risk of degenerative conditions, including cancer and heart disease (WHO 1998). These foods also help in the maintenance of exogenous antioxidants at or near optimal levels thus reducing the risk of tissue damage. Although there were reductions in the antioxidant activity of the bambara puree, the reductions were very minimal. The result suggests that bambara puree may play a role in reducing the incidence of these degenerative diseases. BGN could therefore be potentially useful in this regard.

6.3.6 Functional properties of freeze-dried bambara puree

6.3.6.1 Water and oil absorption capacity

The water absorption capacity (WAC) of dried bambara puree flour is presented in Figure 6.5. Cooking significantly ($p \leq 0.05$) increased the WAC of all the samples to varying levels. Increased exposure of polar amino acids due to changes in protein conformation resulting from cooking could be responsible for the observed increase (Xu, Barbaro, Reese, Langaigne, Rutto & Kering 2015). Also, starch gelatinisation during the thermal treatment may also be a plausible

reason for this. A similar trend was observed in cooked soybean and BGN (Xu *et al* 2015; Adegunwa, Adebowale, Bakare & Kalejaiye 2014). A higher increase in WAC was observed in the reference dry bean samples. As previously mentioned, the higher WAC observed in the reference dry bean may be due to its higher protein content.

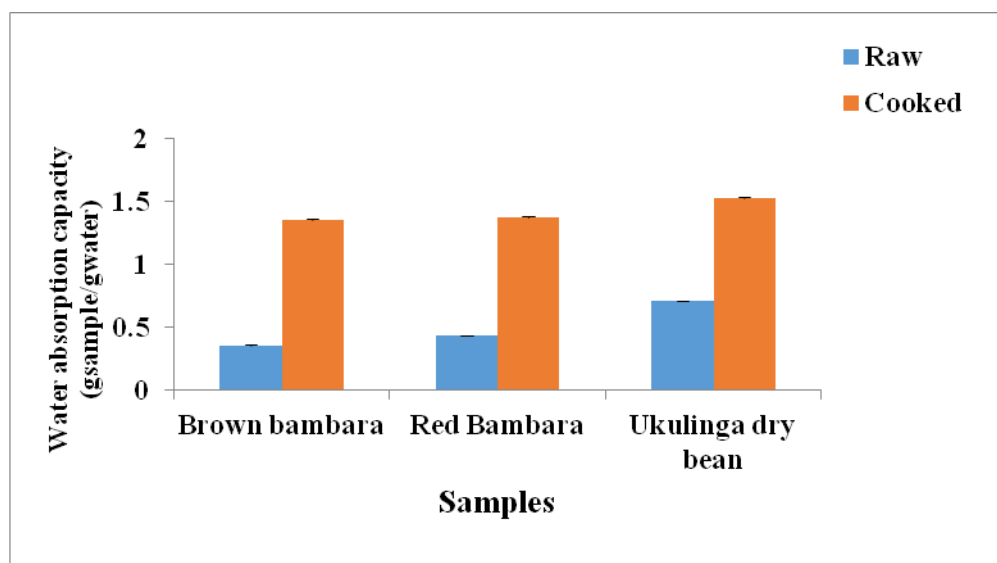


Figure 6.5 Effect of cooking on the water absorption capacity of dried bambara groundnut puree

The oil absorption capacity of the dried bambara puree is presented in Figure 6.6.

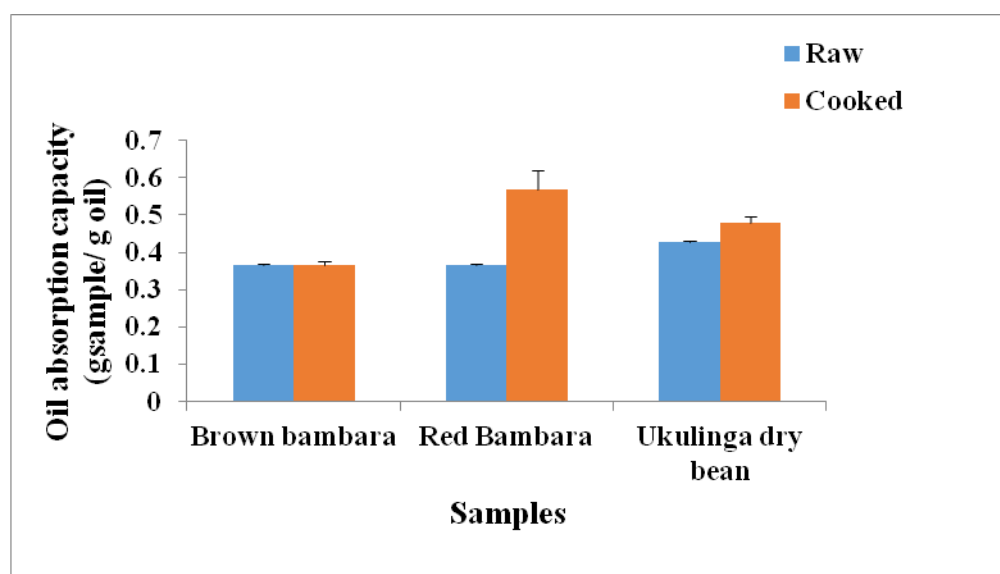


Figure 6.6 Effect of cooking on the oil absorption capacity of dried bambara groundnut puree

Cooking increased the OAC of the reference dry bean and that of the red bambara but had no effect on the OAC of the brown bambara sample. OAC is basically due to physical entrapment of oil with starch structure and non-polar side chains of proteins (Falade, Semon, Fadairo, Oladunjoye & Orou 2014). The observed differences in the OAC of puree samples may be as a result of changes in protein and starch conformation after processing. Brown bambara puree may therefore have the ability to retain fat-soluble nutrients, flavour and mouth feel in food formulations (Kaur, Kaushal & Sandhu 2013).

6.3.6.2 Rheological properties

The apparent viscosities of cooked grains (puree) are presented in Figure 6.7.

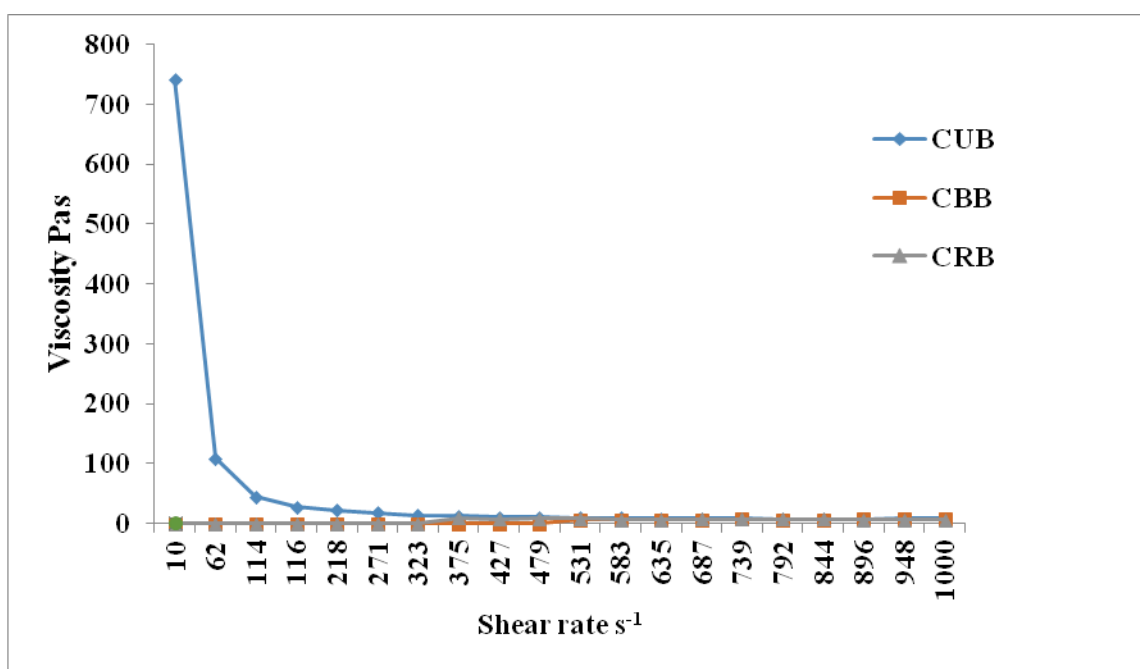


Figure 6.7 Apparent viscosity of dried bambara groundnut and reference bean puree CUB - Cooked dry bean flour; CBB - Cooked brown bambara flour; CRB - Cooked red bambara flour

Pureed, mashed or semi-solid foods are suitable complementary foods for children after 6 months (Dewey 2001). Apparent viscosities observed for bambara puree in this study were similar to those reported by complementary food produced from combination of rice, millet and maize (Mouquet, Greffeuille & Treche 2006) and also fermented and non-fermented millet and malted sorghum (Thaoge, Adams, Sibara, Watson, Taylor & Goyvaerts 2003). Cooked grains have

denatured proteins and gelatinised starch in the system and may possibly have minimal ability to absorb water and swell during viscosity measurement. The reference dry bean puree had the highest viscosity of about 742 Pa.s, which could be attributed to differences in the starch component compared to the bambara grains and possibly the higher protein content of the dry bean (Table 6.1a). According to Farno, Baudez, Parthasarathy & Eshtiaghi (2014), protein denaturation enhances the viscosity of a protein system, either through the formation of protein aggregates which occlude water or when there is no aggregation. The flow properties of pureed samples followed the Power-Law model (Table 6.7) with a fit of $r^2 \geq 0.5$.

Table 6.7 Flow property of pureed samples as indicated by Power law coefficients

Sample	n	k	r ²
Cooked brown bambara	0.056 ^a ± 0.003	1.80 ^b ± 0.01	0.83 ^a ± 0.02
Cooked red bambara	0.0539 ^a ± 0.000	1.57 ^b ± 0.33	0.84 ^a ± 0.03
Cooked Ukulinga dry bean	0.006 ^b ± 0.00	4.74 ^a ± 0.71	0.36 ^b ± 0.05

Mean ± SD

Means with different superscripts in a column are significantly different ($p \leq 0.05$)

SD: Standard deviation

The samples showed strong pseudoplastic behaviour with flow behaviour index (n) < 1 (Table 6.7). Consistency coefficient (k) indicative of viscous properties was higher in the reference dry bean puree than the bambara purees. This result is in agreement with the viscosity of the reference sample which also showed higher values than the bambara samples. Hence, the low viscosity of the bambara samples may be an advantage over the reference dry bean in complementary food formulations. The WHO recommended that good quality complementary foods must have low viscosity (WHO 2003).

6.3.6.3 Fourier transform infrared spectroscopy (FTIR) for raw and cooked samples

FTIR is a technique that adopts vibrational spectroscopy to analyse for structural changes in substances and is particularly suitable for denatured or aggregated proteins (Mune & Sogi 2016). This technique is important in the study of structural changes in proteins because of its high sensitivity to changes in hydrogen bonding (Ellepola, Choi & Ma 2005). FTIR was used in this study to assess the effect of cooking on the conformation of bambara groundnut protein

concentrates. FTIR bands were observed in the region of 4000-450 cm^{-1} in all the samples (Figures 6.8a, b & c).

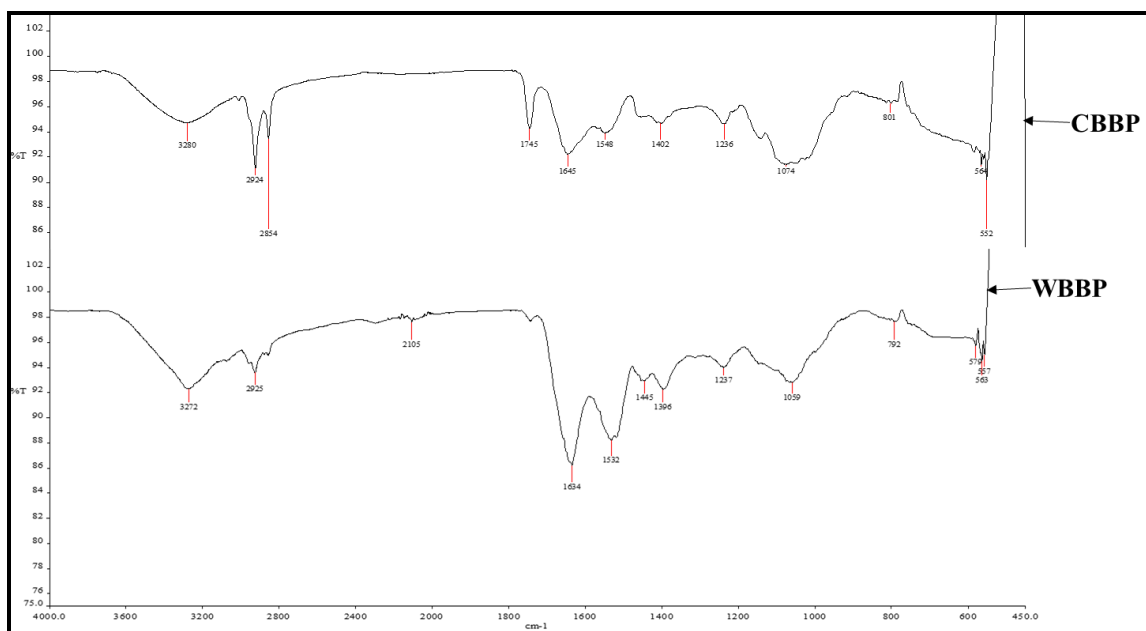


Figure 6. 8a Fourier transform infrared spectroscopy spectra of raw and pureed brown bambara groundnut protein concentrate
CBBP - Brown bambara groundnut puree protein; WBBP - raw bambara brown groundnut protein

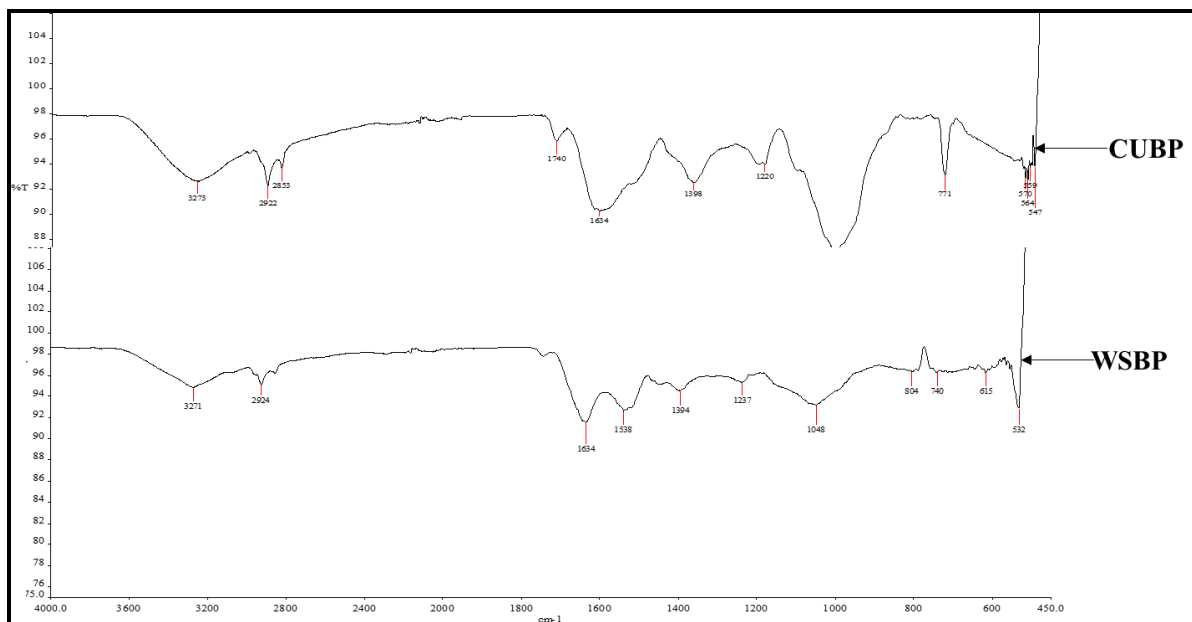


Figure 6.8b Fourier transform infrared spectroscopy spectra of raw and pureed reference dry bean protein concentrate CUBP - reference dry bean puree protein; WSBP - raw reference dry bean protein

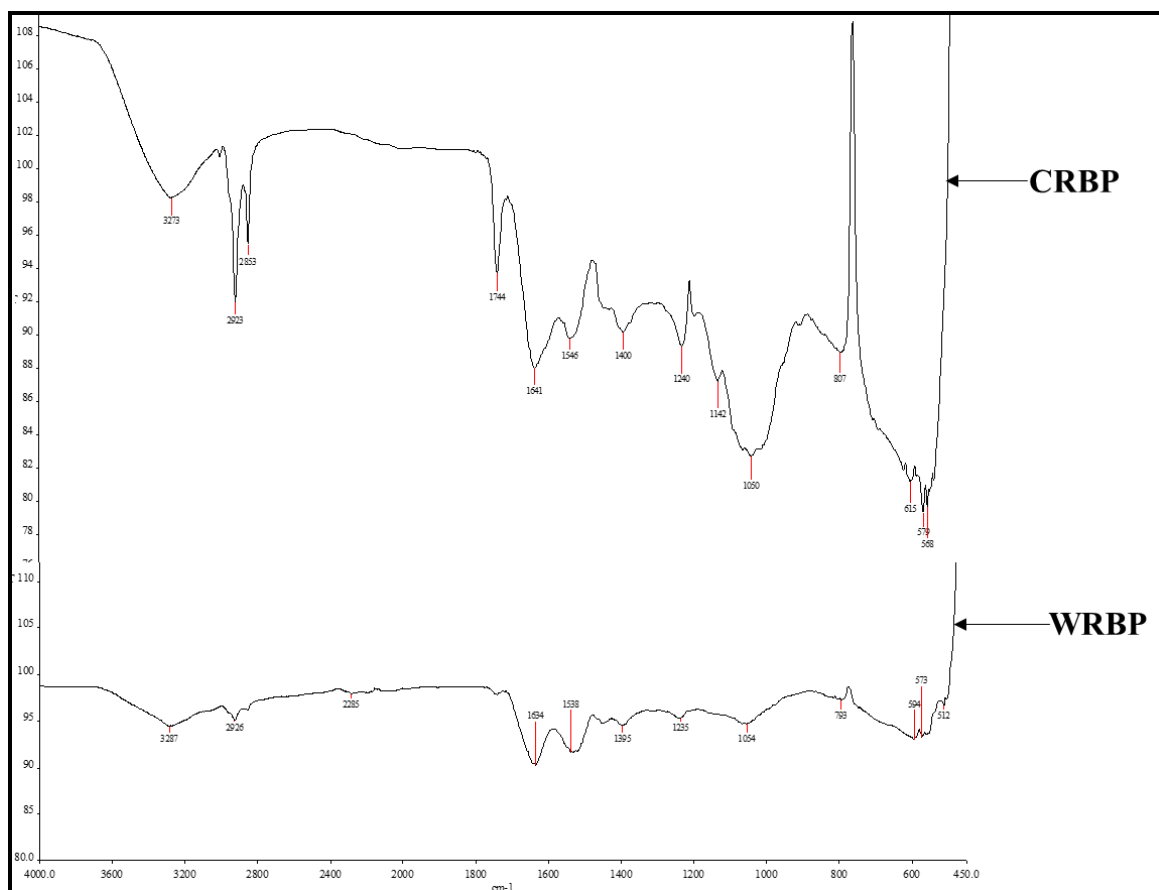


Figure 6.8c Fourier transform infrared spectroscopy spectra of raw and pureed red bambara groundnut protein concentrate

CRBP - red bambara groundnut puree protein; WRBP - raw bambara red groundnut protein

Multiple peaks were seen in the range of $1011\text{--}1745\text{ cm}^{-1}$. The peaks around 1550 and 1640 cm^{-1} are related to $\text{N}=\text{O}$ and $\text{C}=\text{O}$ bonds respectively. The presence of $\text{C}=\text{O}$ bonds combined with wavenumber around $3200\text{--}3300\text{ cm}^{-1}$ for N-H bonds indicates the presence of amides in the samples. Kaptso, Njintang, Nguemtchouin, Scher, Hounhouigan & Mbofung (2015) also suggested that peaks obtained around $3000\text{--}3500\text{ cm}^{-1}$ may be asymmetric vibrations of proteins with CO-NH AND CO-NH_2 bands. Bands around 1640 cm^{-1} have been said to be amide vibration mode which is mainly a $\text{C}=\text{O}$ weakly coupled with in-plane NH bending and CN stretching (Kudre, Benjakul & Kishimura 2013). Peaks around 1400 may be an indication of the presence of organophosphorus compounds (P-C bonds) which may arise from interactions between phytic acid and the organic components present in the legumes. Slight variations in the wavenumber of protein concentrates may be due to their amino acid composition, functional

group and interactions with other components (Kudre *et al* 2013). Food proteins undergo racemisation of L-amino acids to D-isomers and simultaneous formation of cross linked amino acids as most amino acids naturally occur as L-isomers (Friedman & Levin 2012). This greatly impacts the properties of these proteins and as a result affects digestibility and nutritional quality.

Cooking decreased the intensity of the bands at 3280, 2924 and 1645-1074 cm^{-1} in the cooked brown bambara puree. However, fewer bands were observed in the raw red bambara landrace and the reference dry bean than in their cooked counterpart. The observed decrease in band intensity around 1400 cm^{-1} in the brown bambara puree could be linked to the reduced antinutrient content (Table 6.6) and may explain the increased IVPD after cooking (Table 6.5). There was a general increase in band intensities in the amide II region 1650-1500 cm^{-1} (Figure 6.8b & c). Ellepola *et al* (2005) observed a similar trend in rice globulin subjected to thermal processing. This may indicate changes in secondary structures due to heat processing. The present data reveal marked changes in protein conformation as a result of heat processing which may influence digestibility of these proteins.

6.4 Conclusions

This study showed that BGN could be a relatively good source of macro- and micronutrients. bambara grains had much lower protein contents and substantially higher antinutrient contents than the reference dry bean. The amino acid content of the BGN puree was comparable to the WHO/FAO/UN reference although limiting in the sulfur-containing amino acid methionine. Most of the nutrients analysed were better retained in the BGN puree than the reference dry bean puree. Unsoaked BGN had longer cooking time compared to the soaked grains. Soaking the grains prior to cooking is therefore required to reduce cooking time and energy expenditure. Thermal processing greatly influenced the *in vitro* digestibility of BGN. Starch digestibility increased by approximately 16.11% and protein by approximately 5.34% upon cooking. This method of cooking could therefore be a promising way of processing this grain to retain nutrients and reduce antinutrients. BGN puree could be combined with cereals to improve the amino acid profile of the combined foods, particularly to improve levels of methionine, which are limited in bambara grain. However, improved bambara landraces with better nutrient profile, low antinutrients and better cooking properties may improve utilisation. It is recommended that

breeding programmes should aim to produce BGN varieties that have a higher nutrient content and require a short cooking time.

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Complementary feeding using commercially available foods may be expensive in rural settings, where people receive low income. Hence, utilising available traditional crops as low-cost alternatives to the conventional complementary foods could help meet the demands of the poor, while ensuring that the dietary requirements of infants are met. Bambara groundnut is a legume that could play a role in this regard since it is rich in nutrients and can also withstand extreme drought conditions, compared to other crops such as corn and peanuts used in complementary food formulation. Bambara groundnut has been used in combination with many other traditional crops in formulating different types of complementary foods. However, studies using bambara groundnut alone in addressing PEM, is scarce. Hence, the aim of this study was to evaluate the potential of BGN, an underutilised legume for use in complementary foods to address PEM which is prevalent especially among children in resource-poor communities of most of the countries, including South Africa, in the sub-Saharan Africa region. The objectives of this study were to: (i) determine the physico-chemical, functional and nutritional properties of BGN grain (ii) assess consumer awareness of bambara groundnut as a food source and formulate a complementary food from bambara grains and determine the consumer acceptance of the formulated complementary food in rural KwaZulu-Natal, South Africa and (iii) assess the functional and nutritional properties of the formulated complementary food. Ukulinga dry bean was included as a reference sample since it is widely consumed in the KwaZulu-Natal province.

7.1 Physico-chemical, functional and nutritional properties of bambara groundnut landraces

Bambara groundnut landraces showed different physical, nutritional and functional properties. The grains of bambara were characterised by significantly ($p \leq 0.05$) higher sphericity (approx. 0.833) than the reference dry bean (0.585). In terms of grain composition, the protein content of brown bambara and red bambara were significantly ($p \leq 0.05$) lower than the reference dry bean. Different traditional processing methods had varying effects on the nutritional and antioxidant properties of the grains. However, all the processing methods significantly ($p \leq 0.05$) reduced antinutrient content of the grains with a corresponding increase in *in vitro* protein digestibility.

The inclusion of BGN grain in the diets of populations that are vulnerable to PEM may help to improve their protein and energy intake and their overall nutritional status.

7.2 Consumer awareness of bambara groundnut as a food source in KwaZulu-Natal and acceptability of a bambara-based complementary food

The survey on the consumer awareness of BGN as a food source revealed that BGN was not a popular legume in uMshwathi Municipality, KwaZulu-Natal province, South Africa. This was mainly due to the unavailability of the grains and inadequate knowledge on its use. However, the survey participants were willing to use BGN to prepare complementary foods if it was accessible, affordable and beneficial to health. Most of the caregivers indicated that they would prepare a complementary food by cooking the grain alone and then prepare a puree. However, consuming bambara alone does not supply all the essential nutrients, especially essential amino acids in adequate quantities. Combining BGN with other foods like cereals may provide the limiting essential amino acids. These findings, which seem to be the first obtained from a nutritionally vulnerable rural community in South Africa, are encouraging as they suggest there is high probability that this community and other communities elsewhere in the sub-Saharan Africa in the same socio-economic, health and nutritional circumstances would adopt BGN grain for complementary feeding.

7.3 Functional and nutritional properties of a bambara grain complementary food (puree)

Brown bambara puree had comparable protein content to that of the reference dry bean puree. However, the red bambara landrace puree had significantly ($p \leq 0.05$) lower protein content. Potassium, phosphorus and calcium were the major minerals present in the puree. The brown bambara variety had higher concentrations of amino acids than the red variety. An increase in the amino acid concentration of the bambara landraces was observed after cooking for most of the amino acids tested. However, methionine contents of the brown bambara, red bambara and the reference bean decreased by 100, 80 and 20% respectively. All the macronutrients in the raw bambara grains were well retained after processing into puree. FTIR spectra of the puree samples indicated that several changes occurred in the protein conformations; generally, the conformational changes suggested improved protein digestibility. Cooking of BGN into a puree

reduced the antinutrients to relatively low levels thereby likely resulting in improved nutrient bioavailability. These findings indicate that bambara can be processed into puree of high nutritional value, especially quality starch and protein, by processing it through soaking followed by wet cooking.

Overall, the findings of this study indicate that there is a good potential utilisation of BGN grain in the processing of nutritious complementary foods to, such as puree, to contribute to addressing malnutrition, especially PEM, which is particularly rampant among children in resource-poor population groups in developing regions, especially sub-Saharan Africa. BGN would be more affordable and accessible compared to most other food sources, especially animal food sources. However, the adoption and appreciable utilisation of BGN as a food source, and in complementary food in particular, in developing region needs holistic facilitation processes, including consumer education, supportive policies, and development of BGN varieties with desirable agronomic traits and grains with superior end-user (consumer) properties, including storage, processing, sensory and nutritional quality attributes

7.4 Implications of findings and recommendations

- 7.4.1 This study only focused on two BGN landraces and a reference dry bean. Future studies should be done on other landraces not studied in this work.
- 7.4.2 Agricultural extension programmes to encourage cultivation of BGN and improve market availability coupled with nutrition programmes to highlight the nutritional benefits of bambara grain are required to improve its utilisation.
- 7.4.3 The effect of storage conditions, agricultural practices and environmental factors on the nutritional composition of BGN should be determined in future studies, as these may influence nutritional composition and cooking properties.
- 7.4.4 The cost of preparing the BGN puree was not calculated for this study. This should be done to determine if BGN is feasible for use by communities of low socio-economic status.

- 7.4.5 Other nutritious food products should be developed using BGN and other locally available ingredients like fortified white maize, rice, margarine and provitamin A-biofortified maize (in areas where commercially fortified maize are not available) to improve the nutritional status of infants and children vulnerable to malnutrition.
- 7.4.6 Efforts should be made to develop easy to cook BGN varieties.

7.5 Study critique

- 7.5.1 Proximate amino acid composition of all the processed samples were not analysed due to high costs. Analysing other processed samples for the nutrients could give a better understanding of the influence of processing on the nutrient composition of these samples.
- 7.5.2 The consumer acceptability studies were carried out in the KwaZulu-Natal province only on the BGN puree, which was popular in the survey area. This food may not be the most popular in other provinces. Hence, the result of these studies is limited to the province in which the study was conducted.
- 7.5.3 The consumer acceptability study was restricted to black African caregivers caring for children between the ages of six months and three years at the time of the study. Future studies could include caregivers from other racial groups to identify other complementary foods that could be prepared from BGN and to determine their perception of BGN as a protein source.

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APPENDICES

APPENDIX A: ETHICS APPROVAL FROM THE HUMANITIES AND SOCIAL SCIENCES ETHICS COMMITTEE, UNIVERSITY OF KWAZULU-NATAL



29 January 2016

Mrs Adewumi Toyin Oyeyinka 215041660
School of Agricultural, Earth and Environmental Sciences
Pietermaritzburg Campus

Dear Mrs Oyeyinka

Protocol reference number: HSS/0740/015D

Project title: Functional, Nutritional and Sensory Properties of a Maize (Zea mays) Bambara grounded (vigna subterraneas) Complementary Food

Full Approval – Expedited Application

In response to your application received 11 June 2015, 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 Kirthee Pillay & Dr Muthulisi Siwela
Cc Academic Leader Research: Professor Onesimo Mutanga
Cc School Administrator: Ms Marsha Manjoo

Humanities & Social Sciences Research Ethics Committee

Dr Shenuka Singh (Chair)

Westville Campus, Govan Mbeki Building

Postal Address: Private Bag X54001, Durban 4000

Telephone: +27 (0) 31 260 3587/8350/4557 Facsimile: +27 (0) 31 260 4609 Email: ximbap@ukzn.ac.za / snvmanm@ukzn.ac.za / mohunp@ukzn.ac.za

Website: www.ukzn.ac.za



Founding Campuses: ■ Edgewood ■ Howard College ■ Medical School ■ Pietermaritzburg ■ Westville

**APPENDIX B: LETTER OF APPROVAL TO CONDUCT RESEARCH BY THE
UMGUNGUNDLOVU HEALTH DISTRICT OFFICE**



health

Department:
Health
PROVINCE OF KWAZULU-NATAL

UMGUNGUNDLOVU DISTRICT OFFICE

Physical Address: 171 Hoosen Haffajee, Pietermaritzburg, 3219
Postal Address: Private Bag X9124, Pietermaritzburg, 3200
Telephone: 897 1002 Fax: 033 897 1078 Email: Thule.kunene@kznhealth.gov.za
www.kznhealth.gov.za

DISTRICT MANAGERS OFFICE

Enquiries: Mrs. N. M. Zuma-Mkhonza
06 January 2016

**TO: MRS ADEWUMI TOYI OYEYINKA
SCHOOL OF AGRICULTURAL, EARTH AND ENVIRONMENTAL
SCIENCES
PIETERMARITZBURG CAMPUS**

Dear Mrs Oyeyinka

**RE: FUNCTIONAL , NUTRITIONAL AND SENSORY PROPERTIES OD A MAIZE (ZEA
MAYS) BAMBARA GROUNDED (VIGNA SUBTERRANEEES) COMPLEMENTARY
FOOD.**

I have pleasure in informing you that support and permission have been granted to you by the District Office to start research on Functional , Nutritional and Sensory Properties od a Maize (Zea mays) Bambara grounded (vigna subterranees) Complementary Food.

PLEASE NOTE THE FOLLOWING

1. Please ensure that you adhere to all policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. This research will only commence once this office has received the full ethics approval has been received and the confirmation from the Provincial Health Research Committee in the KZN Department.
3. Please ensure that this office is informed before you commence your research.
4. The District Office will not provide any resources for this research.
5. You will be expected to provide feedback on your findings to the District Office.

Thank you,

**MRS N.M. ZUMA - MKHONZA
DISTRICT MANAGER
UMGUNGUNDLOVU HEALTH DISTRICT**

**APPENDIX C: LETTER OF APPROVAL TO CONDUCT RESEARCH BY THE
DEPARTMENT OF HEALTH, KWAZULU-NATAL PROVINCE**



health
Department:
Health
PROVINCE OF KWAZULU-NATAL

330 Langalibalele street,
Private Bag X9051 PMB, 3200
Tel: 033 395 2805/3189/3123 Fax: 033 394 3782
Email: hkrm@kznhealth.gov.za
www.kznhealth.gov.za

DIRECTORATE:

Health Research & Knowledge
Management (HKRM)

Reference: HRKM008/16
KZ_2016RP2_819

19 January 2016

Dear Mrs A T Oyeyinka
(University of KwaZulu-Natal)

Subject: Approval of a Research Proposal

1. The research proposal titled 'Functional, Nutritional and Sensory Properties of a Maize (Zea mays)-Bambara groundnut (Vigna subterranea) Complementary Food' was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

The proposal is hereby **approved** for research to be undertaken at Gcumisa clinic.

2. You are requested to take note of the following:
 - a. Make the necessary arrangement with the identified facility before commencing with your research project.
 - b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.
3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hkrm@kznhealth.gov.za

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

Yours Sincerely

Dr E Lutge

Chairperson, Health Research Committee

Date: 21/01/16

APPENDIX D: SURVEY QUESTIONNAIRE IN ENGLISH

Instruction: Please answer the following questions on bambara groundnut by either ticking the appropriate boxes or writing your answers in the spaces provided

1. Have you seen this legume before? Yes No

2. Do you know what this legume is? Yes No

If yes, what do you call it? _____

If no, what legume is it similar to? _____

3. Have you used it before? Yes No

If yes, how do you use it? _____

If no, why have you not used it before? _____

4. Would you cultivate this legume if it was made available to you? Yes No

5. Would you purchase this legume if it has more nutrients than the other common grain legumes available at the supermarket? Yes No

6. Would you purchase this legume if it was cheaper than the other common grain legumes available at the supermarket? Yes No

7. Would you purchase this legume if it was more expensive than the other common grain legumes available at the supermarket? Yes No

8. Would you use it for family cooking? Yes No

9. What family foods would you use this legume to make? _____

10. Would you use this legume in preparing foods for infants? Yes No

11. What infant foods would you use this legume to make? _____

12. How would you prepare and serve these infant foods using bambara groundnut? _____

Thank you for your participation.

APPENDIX E: SURVEY QUESTIONNAIRE IN ISIZULU

Indlela yokwenza: siyacela uphendule lemibuzo elandelayo nge bambara ngokuthi ubhale ebhokisini noma uphendule ezikhaleni ozinikiwe

1. Wake wasibona lesitshalo phambilini? **Yebo** **Cha**

2. Uyasazi ukuthi siyini lesitshalo? **Yebo** **Cha**

Uma uyebo usibiza ngani? _____

Uma cha isiphi isitshalo esifana nayo? _____

3. Wake wasisebenzisa lesositshalo? **Yebo** **Cha**

Uma yebo usisebenzisa kanjani? _____

Uma cha yinindaba ungasisebenzisanga phambilini? _____

4. Ungasitshala lesitshalo uma ungakwazi ukusithola? **Yebo** **Cha**

5. Ungasithenga lesitshalo uma sinomsoco omningi kunezitshalo ezijwayelekile zezinhlamvu ezitholakala emasuphamakethe? **Yebo** **Cha**

6. Ungasithenga lesitshalo uma sishibhile kunezitshalo ezijwayelekile zezinhlamvu ezitholakala emasuphamakethe? **Yebo** **Cha**

7. Ungasithenga lesitshalo uma sibiza kunezitshalo ezijwayelekile zezinhlamvu ezitholakala emasuphamakethe? **Yebo** **Cha**

8. Ungasisebenzisa ukuphekela umndeni wakho? **Yebo** **Cha**

9. Ungenza hlobo luni lokudla komndeni ngalesitshalo _____

10. Ungasisebenzisa lesitshalo ukupheka ukudla komtwana? **Yebo** **Cha**

11. Ungenza hlobo luni lokudla komtwana? _____

12. Ungakulungiselela kanjani ukudla ozokunika umtwana usebenzisa loluhlobo lesitshalo? _____

Siyabonga ukuba ingenxe kwakho.

APPENDIX F: SURVEY CONSENT FORM IN ENGLISH

I am currently a student at the University of KwaZulu-Natal, doing my PhD in Human Nutrition. I will be preparing an infant food made from bambara groundnut (an underutilised legume) in order to try to reduce the prevalence of malnutrition and increase the consumption of underutilised legumes. I would like to find out whether consumers are aware of bambara groundnut, the possible reasons for it not being used and the best methods for preparing it.

- The researcher's name is Adewumi Toyin Oyeyinka who is from Dietetics and Human Nutrition at the University of KwaZulu-Natal. Contact details for the researcher are as follows 0849962782 or wumishaks@yahoo.ca

- For further information regarding the study, you may contact Dr Kirthee Pillay, who is the project supervisor. Contact details: 033-2605674 or pillayk@ukzn.ac.za. The Human and Social Sciences Ethics Research Office can be contacted on 033-2604557 or mohunp@ukzn.ac.za.

- All the data collected from this study will remain confidential and will only be used for the purpose of this research project. All participants will remain anonymous.

- Participation in this study is completely voluntary. All participants may leave the study at any time they wish, without any negative consequences.

- There are no potential benefits from participating in this study. No participants will receive any payments or financial reimbursements for participating in this research project.

- All data will be destroyed when it is no longer needed.

Declaration:

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

Signature

Date

APPENDIX G: SURVEY CONSENT FORM IN ISIZULU

Ngiwumfundi wasenyuvesi yakwazulu natal, ngifunda iPhD ngokudla okuwumsoco kubanti. Ngizobe ngenza ukudla kwabantwana okwenziwe ngamantongomane ibambara ukuzama ukunciphisa ukungondleki kwabantwana sikhulise ukudliwa kwebambara. Ngifuna ukwazi ukuthi abathengi bokudla banalo yini ulwazi ngohlobo lokudla ekuthiwa indlube, sikhona yini isizathu sokuthi ingasetshenziswa nokukwazi izindlela ezilula zokuwupheka.

-Igama lomcwani u Adewumi Toyin Oyeyinka owumfundi ngezokudla okunomsoco ebantwini ufunda enyuvesi yakwa zulu natal. Inamba yakhe yoking ithi 0849962782 noma angambhalela lapha wumishaks@yahoo.ca

-Ngolunye ulwazi oluthe xaxa mayelana nalesifundo ungathintana no dokotela Kirthee Pillay okunguyena ophethe lesifundo inamba yakhe 033-2605674 or pillayk@ukzn.ac.za. Ulwazi olubanzi ngehovisi kwezesayensi nocwani litholakala 033-2604557 or mohunp@ukzn.ac.za

-Konke esibhala kukho okuqoqiwe kuyogcinwa ngokunakekela futhi kuyosetshenziselwa lolucwani, bonke ababe inxeny amagama abo awayikwaziwa.

-Ukuba ingxeny kulolucwani kwenziwa ngokuthanda komuntu akuphoqwa muntu, bonke abayingxeny bangayeka noma inini mabengasathandi ngaphandle kokusongelwa.

-Akukho nzuzo kulolucwani, ngakho akukho muntu oyokhokhelwa ngaloma isinxephezelo ngokuba ingxeny kulolucwani.

-Inkulumo eqoshiwe etholakele ngesikhathi kudingidwa noma kuboniswa ngalesifundo kuyosetshenziselwa lesifundo bese luyagcinwa kahle.

-Emva kokusetshenziswa kwakho konke kuyobe sekuyahlwa makungadingeki.

Isifungo:

Mina _____ (igama eliphele nesibongo) ngiyavuma ukuthi lolucwani ebesixoxa ngalo siyiqembu ngichazeliwe ngalo kabanzi futhi ngiyaqonda inhloso lalolucwani nokuthi ulwazi luyohlangukani kanjani. Ngiyavuma ukuba ingxeny yalolucwani ngiyazi ukuthi lolucwani ngilwenza ngokuthanda ngingaphoqiwe futhi ngingayeka noma nini mangithanda.

Sayina

Usuku

APPENDIX H: SENSORY QUESTIONNAIRE IN ENGLISH

Participant number:
Sample number:
Date of birth (dd/mm/yy):

INSTRUCTIONS:

Please rinse your mouth with water before starting.

Please rinse your mouth with water after tasting each sample.

Please taste the samples of the food in the order presented, from left to right.

Please rate the taste, texture, aroma, colour and overall acceptability of the samples by putting a cross on the picture that best describes that sample.

You may re-taste the sample if you wish.

Example:

AROMA



Very bad Bad Neutral Good Very good

Please use the example above to assess the samples presented.

TASTE



Very bad Bad Neutral Good Very good

TEXTURE**Very bad****Bad****Average****Good****Very good****AROMA****Very bad****Bad****Average****Good****Very good****COLOUR****Very bad****Bad****Average****Good****Very good****OVERALL ACCEPTABILITY****Very bad****Bad****Average****Good****Very good****Thank you for your participation.**

APPENDIX I: SENSORY QUESTIONNAIRE IN ISIZULU

Inombolo yomhlanganyeli:

Inombolo yesampula lokudla:

Usuku lokuzalwa (dd/mm/yy):

INDLELA YOKWENZA:

Sicela uyakaze umlomo ngamanzi ngaphambi kokuqala

Sicela uyakaze umlomo ngamanzi emva kokuzwa ukudla ngakunye.

Sicela uzwe amasampula okudla ngendlela okuhlelwe ngayo kusukela esinxeleni kuya esidleni.

Sicela usho izinga lokunambitheka, iphunga, umbala nokho konke ukuzwakala ngokufaka isiphambano esithombe ngendlela obona ukuthi ichaza kancon.

Ungaphinde uzwe futhi amasampula uma ufuna.

Isibonelo:

IPHUNGA



**Kubi kakhulu Kubi kuphakathi Kumnandi Kumnandi kakhulu
nendawo**

Sicele usebenzise isibonelo ukuchaza isampula elilethiwe.

UKUNAMBITHEKA



Kubi kakhulu Kubi kuphakathi nendawo Kumnandi Kumnandi kakhulu

UKUZWAKALA**Kubi kakhulu****Kubi****Kuphakathi nendawo****Kumnandi****Kumnandikakhulu****IPHUNGA****Kubi kakhulu****Kubi****Kuphakathi nendawo****Kumnandi****Kumnandi kakhulu****UMBALA****Kubi kakhulu****Kubi****Kuphakathi nendawo****Kumnandi****Kumnandi kakhulu****MASUHLANGANISE KONKE****Kubi kakhulu
kakhulu****Kubi****Kuphakathi nendawo****kumnandi****Kumnandi****Siyabonga ngokuba ingxenywe kwakho.**

APPENDIX J: STANDARDISED RECIPE FOR PUREE PREPARATION**INGREDIENTS**

250g bambara grains

750 mL hot water

2500 mL tap water

1 mL salt

METHOD

Soak 250 g of bambara grains in 750 mL hot water for 18 hr

Drain soaked grains

Add 2500 mL water to grains and bring to boil on high heat (plate control setting 6) using a Defy Thermofan Stove (Model 731 MF)

Boil the grains with the pot lid on for 150 mins

Add salt and allow to simmer for 15 mins

Mash with a wooden spoon

APPENDIX K: CONSENT FORM FOR SENSORY EVALUATION IN ENGLISH

I am a student at the University of KwaZulu-Natal, doing my PhD in Human Nutrition. I will be preparing an infant food made from bambara groundnut (an underutilised legume) in order to try and reduce the prevalence of malnutrition and increase the consumption of underutilised legumes. I would like to find out if caregivers would find bambara groundnut acceptable to use as a complementary food. The participants will be required to taste three samples of the infant food and rate the samples using a simple picture scale. There will be no discomforts or hazards for participants who agree to participate in this study.

- The researcher's name is Adewumi Toyin Oyeyinka who is from Dietetics and Human Nutrition at the University of KwaZulu-Natal. Contact details for the researcher are as follows 0849962782 or wumishaks@yahoo.ca

- For further information regarding the study, you may contact Dr Kirthee Pillay, who is the project supervisor. Contact details: 033-2605674 or pillayk@ukzn.ac.za. The Human and Social Sciences Ethics Research Office can be contacted on 033-2604557 or mohunp@ukzn.ac.za.

- All the data collected from this study will remain confidential and will only be used for the purpose of this research project. All participants will remain anonymous.

- Participation in this study is completely voluntary. All participants may leave the study at any time they wish, without any negative consequences.

- There are no potential benefits from participating in this study. No participants will receive any payments or financial reimbursements for participating in this research project.

- All data will be destroyed when it is no longer needed.

Declaration:

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

Signature

Date

APPENDIX L: CONSENT FORM FOR SENSORY EVALUATION IN ISIZULU

Ngiwumfundi wasenyuvesi yakwazulu natal, ngifunda iPhD ngokudla okuwumsoco kubanti. Ngizobe ngenza ukudla kwabantwana okwenziwe ngamantongomane ibambara ukuzama ukunciphisa ukungondleki kwabantwana sikhulise ukudliwa kwebambara. Ngingathanda ukwazi ukuthi abanakekeli bezingane bangayamukela yini ibambara njengokudla okungasetshenzisa njalo.

Igama lomcwani u Adewumi Toyin Oyeyinka owumfundi ngezokudla okunomsoco ebantwini ufunda enyuvesi yakwa zulu natal. Inamba yakhe yoking ithi 0849962782 noma angambhalela lapha wumishaks@yahoo.ca

Ngolunye ulwazi oluthe xaxa mayelana nalesifundo ungathintana no dokotela Kirthee Pillay okunguyena ophethe lesifundo inamba yakhe 033-2605674 or pillayk@ukzn.ac.za. Ulwazi olubanzi ngehovisi kwezesayensi nocwaningo litholakala 033-2604557 or mohunp@ukzn.ac.za

Konke esibhala kukho okuqoqiwe kuyogcinwa ngokunakekela futhi kuyosetshenziselwa lolucwaningo, bonke ababe inxenye amagama abo awayikwaziwa.

Ukuba ingxenye kulolucwaningo kwenziwa ngokuthanda komuntu akuphoqwa muntu, bonke abayingxenye bangayeka noma inini mabengasathandi ngaphandle kokusongelwa

Akukho nzuzo kulolucwaningo, ngakho akukho munto oyokhokhelwa ngaloma isinxephezelo ngokuba ingxenye kulolucwaningo

Inkulumo eqoshiwe etholakele ngesikhathi kudingidwa noma kuboniswa ngalesifundo kuyosetshenziselwa lesifundo bese luyagcinwa kahle

Emva kokusetshenziswa kwakho konke kuyobe sekuyalahlwa makungasadingeki

Isifungo:

Mina _____ (igama eliphele nesibongo) ngiyavuma ukuthi lolucwaningo ebesixoxa ngalo siyiqembu ngichazeliwe ngalo kabanzi futhi ngiyaqonda inhloso lalolucwaningo nokuthi ulwazi luyohlenganiswa kanjani. Ngiyavuma ukuba ingxenye yalolucwaningo ngiyazi ukuthi lolucwaningo ngilwenza ngokuthanda ngingaphoqiwe futhi ngingayeka noma nini mangithanda.

Sayina

Usuku

APPENDIX M: FOCUS GROUP DISCUSSION QUESTIONS IN ENGLISH

1. Have you ever cooked bambara groundnut before? Please share your experience.
2. Is this the first time that you have tasted a food made with bambara groundnut? Please describe how you feel about its sensory attributes;
 - ❖ Taste
 - ❖ Smell
 - ❖ Texture
 - ❖ Colour
3. Is the bambara groundnut different from any other beans/legumes ever used? Elaborate on the difference or similarities if there any.
4. As a caregiver, would you feed your baby a complementary food made from bambara groundnut? Explain your reasons.
5. What other kind of infant foods would you prepare with bambara groundnut?
6. Is bambara groundnut available in your area? If yes, explain how you get it and if no explain why you think it is not available.
7. Would you buy bambara groundnut if it was cheaper than other beans?
8. Would you buy bambara groundnut if it has positive effects on your baby's health?

APPENDIX N: FOCUS GROUP DISCUSSION QUESTIONS IN ISIZULU

1. Wake wawapheka amantongomane ebambara ngaphambilini? watholani awusixoxele. .
2. Ngabe okokuqala uzwa ukudla okwenziwe ngalamntongomabe bambara? Awusho uzwe kanjani kulekhu okulandelayo;
 - ❖ ukunambitheka
 - ❖ iphunga
 - ❖ ukuzwakala
 - ❖ umbala
3. Nganbe amantongaomane ebambara ahlukile kwabanye obhontshisi /izitshalo ezake zasetshenziswa? Awunabe kabanzi kokufanayo uma kukhona.
4. Njengomnakekeli, ungayinika ingane ukudla okuhambisana okuvela kumbila namantongomane ebambara? Awusho izizathu.
5. Hloboluni likudla kwabantwana ongalenza ngamatongomane e bambara?
6. Ngabe amantongomane ebambara akhona ngakini, chaza uwathola kanjani uma ungawatholi chaza ukuthi ucabanga ukuthi yinindaba engatholakali.
7. Ungawathenga amantongomane ebambara uma eshibhile kunabanye obhontshisi?
8. Ungawathenga amantongomane ebambara uma enomphumela omuhle empilweni yengane?

APPENDIX O: CONSENT FORM FOR FOCUS GROUP DISCUSSION IN ENGLISH

I am a student at the University of KwaZulu-Natal, doing my PhD in Human Nutrition. I will be preparing an infant food made from bambara groundnut (an underutilised legume) to try to reduce malnutrition and increase the consumption of bambara. I would like to find out more about the bambara groundnut infant food by having a focus group discussion with the participants.

- The researcher's name is Adewumi Toyin Oyeyinka who is from Dietetics and Human Nutrition at the University of KwaZulu-Natal. Contact details for the researcher are as follows: 0849962782 or wumishaks@yahoo.ca

- For further information regarding the study, you may contact Dr Kirthee Pillay, who is the project supervisor. Contact details: 033-2605674 or pillayk@ukzn.ac.za. The Human and Social Sciences Ethics Research Office can be contacted on 033-2604557 or mohunp@ukzn.ac.za.

- All the data collected from this study will remain confidential and will only be used for the purpose of this research project. All participants will remain anonymous.

- Participation in this study is completely voluntary. All participants may leave the study at any time they wish, without any negative consequences.

- There are no potential benefits from participating in this study. No participants will receive any payments or financial reimbursements for participating in this research project.

- Audio recordings from the focus group discussions will be used for the purpose of this study and will be stored appropriately.

- All data will be destroyed when it is no longer needed.

Declaration:

I _____ (full name and surname) hereby confirm that the group discussion has been clearly explained to me and I understand the purpose of this research project and how the information will be collected. I consent to participating in the research project. I understand that participation is voluntary and I can leave the study if I desire.

Signature

Date

APPENDIX P: CONSENT FORM FOR FOCUS GROUP DISCUSSION IN ISIZULU

Ngiwumfundi wasenyuvesi yakwazulu natal, ngifunda iPhD ngokudla okuwumsoco kubanti. Ngizobe ngenza ukudla kwabantwana okwenziwe ngamantongomane ibambara ukuzama ukunciphisa ukungondleki kwabantwana sikhulise ukudliwa kwebambara. Ngingathanda ukwazi kancono ngendlubu esetshenziswa ekudleni kwabantwana abanncane ngokuhlanganisa abangeneleli bocwaningo ukuze bangicobelele ngolwazi abanalo.

Igama lomcwaning u Adewumi Toyin Oyeyinka owumfundi ngezokudla okunomsoco ebantwini ufunda enyuvesi yakwa zulu natal. Inamba yakhe yoking ithi 0849962782 noma angambhalela lapha wumishaks@yahoo.ca

Ngolunye ulwazi oluthe xaxa mayelana nalesifundo ungathintana no dokotela Kirthee Pillay okunguyena ophethe lesifundo inamba yakhe 033-2605674 or pillayk@ukzn.ac.za. Ulwazi olubanzi ngehovisi kwezesayensi nocwaningo litholakala 033-2604557 or mohunp@ukzn.ac.za

Konke esibhala kukho okuqoqiwe kuyogcinwa ngokunakekela futhi kuyosetshenziselwa lolucwaningo, bonke ababe inxenye amagama abo awayikwaziwa.

Ukuba ingxenye kulolucwaningo kwenziwa ngokuthanda komuntu akuphoqwa muntu, bonke abayingxenye bangayeka noma inini mabengasathandi ngaphandle kokusongelwa

Akukho nzuzo kulolucwaningo, ngakho akukho munto oyokhokhelwa ngalonomo isinxephezelo ngokuba ingxenye kulolucwaningo

Inkulumo eqoshiwe etholakele ngesikhathi kudingidwa noma kuboniswa ngalesifundo kuyosetshenziselwa lesifundo bese luyagcinwa kahle

Emva kokusetshenziswa kwakho konke kuyobe sekuyalahlwa makungasadingeki

Isifungo:

Mina _____ (igama eliphele nesibongo) ngiyavuma ukuthi lolucwaningo ebesixoxa ngalo siyiqembu ngichazeliwe ngalo kabanzi futhi ngiyaqonda inhloso lalolucwaningo nokuthi ulwazi luyohlangukiswa kanjani. Ngiyavuma ukuba ingxenye yalolucwaningo ngiyazi ukuthi lolucwaningo ngilwenza ngokuthanda ngingaphoqiwe futhi ngingayeka noma nini mangithanda.

Sayina

Usuku

**APPENDIX Q: CONSENT LETTER FOR FOCUS GROUP DISCUSSION
RECORDING IN ENGLISH**

Dietetics and Human Nutrition,
College of Agriculture, Engineering and Science,
University of KwaZulu-Natal,
Pietermaritzburg Campus.

Dear Participant

INFORMED CONSENT LETTER

My name is Adewumi Toyin Oyeyinka, a Human Nutrition Ph. D candidate studying at the University of KwaZulu-Natal, Pietermaritzburg campus, South Africa. I will be preparing an infant food made from bambara groundnut (an underutilised legume) to try to reduce malnutrition and increase the consumption of bambara. I would like to find out more about the bambara groundnut infant food by having a focus group discussion.

Please note that:

- Your confidentiality is guaranteed as your inputs will not be attributed to you in person, but reported only as a population member opinion.
- The interview may last for about 1 hour and may be split depending on your preference.
- Any information given by you cannot be used against you, and the collected data will be used for purposes of this research only.
- Data will be stored in secure storage and destroyed after 5 years.
- You have a choice to participate, not participate or stop participating in the research. You will not be penalized for taking such an action.
- Your involvement is purely for academic purposes only, and there are no financial benefits involved.
- If you are willing to be interviewed, please indicate (by ticking as applicable) whether or not you are willing to allow the interview to be recorded by the following equipment:

	willing	Not willing
Audio equipment		
Photographic equipment		
Video equipment		

I can be contacted at:

Email: wumishaks@yahoo.ca Cell: +27849962782.

My supervisor is Dr. Kirthee Pillay who is located at the School of Agricultural, Earth and Environmental Sciences, Pietermaritzburg campus of the University of KwaZulu-Natal.

Contact details: email: pillayk@ukzn.ac.za. Phone number: 033-2605674

The Human and Social Sciences Ethics Research Office can be contacted on 033-2604557 or mohunp@ukzn.ac.za.

Thank you for your contribution to this research.

DECLARATION

I..... (full names of participant) hereby confirm that I understand the contents of this document and the nature of the research project, and I consent to participating in the research project.

I understand that I am at liberty to withdraw from the project at any time, should I so desire.

.....
Signature of participant

.....
Date

**APPENDIX R: CONSENT LETTER FOR FOCUS GROUP DISCUSSION
RECORDING IN ISIZULU**

Dietetics and Human Nutrition,
College of Agriculture, Engineering and Science,
University of KwaZulu-Natal,
Pietermaritzburg Campus.

Mhlanganyeli,

INCWANDI YESIVUMELWANO

Igama lami ngingu Adewumi Toyin Oyeyinka, ngenza iPhD emayelana nokudla okunomsoco okudliwa ngabantu. Ngifunda esikhungweni semfundo ephakeme enyuvesi yakwa Zulu-Natali emgugundlovu lapha eningizimu Afrika. Ngizobe ngenza ukudla kwabantwana abancane ngisebenzisa ibambara, ukuzama ukwehlisa izinga lwezingane ezingenawo umsoco kanti nokwengeza izinga lokudla ukudla okunendlubu. Imbangela yokukwenza loluncwaningo ukuze sikwazi ukuzama ukwehlisa izinga lokoda kwezingane ezincane, nokukhuphula izinga lokusetshenziswa kwama ntongomane agxutshwe nombila omhlophe. ngingathanda ukwazi kabanzi ngaloluncwaningo lokwenza ukudla kwezingane olwenziwe ngamantongomane axhutshwe nombila omhlophe. Lokhu ngizokwenza ngokuthola abantu abazophawula ngovo lwabo ngokutshenziswa kwama ntongomane exutshwe nombila omhlophe.

Isixwayiso

- Imininingwane yakho uyaqinisekwa ngawo ukuthi izogcinwa njengokwazi kwakho, kepha ibikwe njengovu lwabaningi.
- Ingxoxo nkulumo ingathatha isikhathi esingange hora, futhi ingahlukaniswa ibe izinhlelo ezimbili
- Yonke imininingwane osinika yona emayelana nawe angeke isetshenziswe ukucindezela wena . yonke lemininingwane izosetshenziswa ukufeza injongo yaloluncwaningo kuphela.
- Lonke loluncwaningo luzogcinwa endaweni ephaphile bese luyalahlwa emva kweminyaka eyisihlanu.
- Kulilungelo lakho ukukhetha ukungabi ingxanye yaloluncwaningo, ukushiya phansi koncwano angeke kukulethele izinkinga.
- Ukubambisana kwakho kuloluncwaningo kuhlangene nemfundo kuphela, ngakho ke akukho mkomelo yezimali etholakalayo.
- Uma unaso isifiso sokuba umhlanganyeli kuloluncwaningo siyacela ukhombise ngokuloba uphawu lokuqoka/umaka we thiki.

	Ngiyavuma	Nginyanqaba
Umshini womsindo/wokulalela		
Umshini weokuthatha izithombe		
Umshini wokuqopha izithombe		

Nansi imininingwano yami engitholakala kuyona

i-imeyli: wumishaks@yahoo.ca

inombolo yocingo: 0849962782

umholi wami uDokotela Kirthee Pillay otholakala esikoleni se-Agricultural, Earth and Environmental Sciences eMgugundlovu.

Imininingwane atholakala kuyona i-imeyli: pillay@ukzn.ac.za .

Inombolo yocingo: 0332605674

Ngolwazi oluthe xaxa ungasheyela ihovisi le- The Human and Social Science Ethnic Research office.

Izinombolo zocingo: 0332604557 noma kwi imeyli ethi mohunp@ukzn.ac.za.

Siyabonga ngokuzibandakanyisa nalolu ncwaningo

Isivumo

**Ngingu (amagama onke nesibongo somlekeleli).
Ngiyavuma futhi ngiyaqonda imingomo yalemibhalo yocwaningo. Ngiyavuma ukuba ngumlekeleli kulolucwaningo. Ngiyaqonda ukuthi ngingahoxa nanoma yisiphi isikhathi phakathi nocwaningo mekungenzeka ukuthi angisathandi ukuqhubeka nocwaningo.**

.....

Isignesha yomlekeleli

.....

Usuku