EFFECT OF GENETIC MANIPULATION AND TRADITIONAL PROCESSING METHODS ON THE NUTRITIONAL QUALITY AND CONSUMER ACCEPTABILITY OF WHITE LOW PHYTIC ACID MAIZE

BY

THULISIWE BRILLIANT MYENI

BSc Biochemistry & Microbiology (UNIZULU), PGDip Food Security (UKZN)

Thesis submitted in fulfilment of the academic requirements for the degree of

MASTER OF AGRICULTURE (FOOD SECURITY)

School of Agricultural, Earth & Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal,

Pietermaritzburg

SOUTH AFRICA

DECEMBER 2015

ABSTRACT

Malnutrition is still a challenge among the majority of households in sub-Saharan Africa. This is largely due to consumption of foods that are low in nutritional value, such as cereal-based diets. Cereal based diets are high in phytate, which binds minerals and thereby limiting their absorption. Phytic acid content in maize may be reduced by biotechnological manipulation through either conventional breeding methods or recombinant DNA techniques. Further, phytic acid in white maize grain may be reduced by different traditional processing methods. However, there is limited information on the traditional methods of processing maize in KwaZulu-Natal.

The first objective of the current study was to evaluate the produced maize hybrids with low levels of phytate in their grains by conventional breeding. Low phytic acid (LPA) lines were developed over eight cycles of selection. The levels of grain phytate were determined using a colorimetric method. There was variation among the 61 maize inbred lines in terms of grain phytate (1.7 to 115 mg g^{-1}). A total of 20 progeny lines qualified as low phytate (LPA) varieties.

The second objective was to evaluate the effect of the selected traditional processing methods and conventional breeding on phytic acid and nutrient content, especially iron and zinc, of white maize. Raw whole grains of a high phytic acid (HPA) white maize variety (control) and food products, which were processed from the HPA maize, using traditional methods (milling, fermentation and boiling) commonly used by rural communities in KwaZulu-Natal, South Africa, were analyzed for their phytate and nutrient content according to the AOAC methods. The traditional processing methods caused decreases (41%–74% w/w) in the phytate content of the maize food products. Milling maize grain reduced phytic acid content, probably due to loss of the phytic acid in the bran. The boiled samp and whole grain samples, respectively, had less mineral content relative to the control, which might be due to leaching. However, according to the RDA (Recommended Dietary Requirement) for these minerals, these samples would contribute significantly to iron and zinc intake. The fermented sample had the highest increase in iron content (14% w/w) and therefore fermentation was the best of the traditional processing methods studied.

The acceptability of the traditionally processed maize products prepared with LPA maize was evaluated by a consumer panel (n=57) on a 5-point facial hedonic scale. About 24% of the participants disliked the texture of LPA un-fermented porridge. Fermented porridge made with either LPA maize or HPA maize was acceptable to the consumers. The taste of LPA samp and HPA samp was acceptable to 75% and 77% of the consumer panel, respectively.

These findings indicate that the phytic acid content of white maize can be reduced by conventional breeding and traditional processing. The study findings suggest that LPA maize can be used as a substitute of HPA maize in traditional maize food products. Thus, LPA maize has a potential to alleviate mineral deficiency in developing countries and could be used as a complementary strategy to combat hidden hunger in sub-Saharan Africa where maize is a staple.

PREFACE

The work described in this dissertation was carried out in the School of Agricultural, Earth and Environmental Sciences, at the University of KwaZulu-Natal, from February 2015 to February 2016, under the supervision of Dr Mthulisi Siwela and Dr Unathi Kolanisi.

Signed:________________ Date:________________

Thulisiwe Myeni (Candidate)

As supervisors of the candidate, we agree to the submission of this dissertation.

Signed:________________ Date:________________

Dr Mthulisi Siwela (Supervisor)

Signed:________________ Date:________________

Dr Unathi Kolanisi (Co-supervisor)

DECLARATION

I, Thulisiwe B Myeni, declare that:

(i) The research reported in this thesis, except where otherwise indicated, and is my own original research.

(ii) This thesis has not been submitted for any degree or examination at any other university.

(iii) This thesis does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those people.

(iv) This thesis does not contain other authors writing, unless specifically acknowledged as being sourced out from authors. Where other written sources have been quoted, then:

a) Their words have been re-written sources but the general information attributed to them has been referenced;

b) Where their exact words have been used their writing has been placed inside quotation marks, and referenced.

(v) This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, with the source(s) being detailed in the thesis and in the references section.

Signed: …………………………………….. Date……………………………….

Thulisiwe B Myeni

ACKNOWLEDGEMENTS

I acknowledge the following people and institutions for providing assistance during this study. Without their assistance, this work would have not been possible.

- DST-NRF Centre in Indigenous Knowledge System (IKS) for funding this study.
- My supervisor, Dr Muthulisi Siwela for his support and guidance throughout the study
- My co-supervisor, Dr Unathi Kolanisi for her constructive comments, guidance, and patience.
- My mentor Prof J. Derera for believing in my capabilities.
- Ntambanana community members for participating in this study
- Debbie Davis (Animal and Poultry Science, UKZN) for assistance with freeze drying of the traditional maize samples.
- CEDARA College of Agriculture for assistance with the nutritional analysis of the traditional maize samples.
- Nozipho Zungu, Ghirmay Zein, Bafana Mzimela and Ruth Olusanye for their assistance with data collection during the survey and sensory evaluation.
- My Father, my brothers and sisters for their support since my first year of study at university

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

FAO	: Food and Agricultural Organization
LPA	: Low phytic acid
HPA	: High phytic acid
RRA	: Rapid rural appraisal
PRA	: Participatory rural appraisal
RDA	: Recommended dietary allowance
TPs	: Traditional practices
rDNA	: Recombinant deoxyribonucleic acid
MAS	: Marker assisted selection
MDG	: Millennium Development Goals
GMO	: Genetically modified organisms

1 CHAPTER: GENERAL INTRODUCTION

1.1 Background

According to Muthayya (2013), 11% of all global deaths under the age of five are caused by hidden hunger. Malnutrition is thus still a major public health problem in developing countries. About two billion people in developing countriesare still faced with mineral deficiency (hidden hunger), with children and pregnant women being the most vulnerable groups. In South Africa, cereal grains, such as maize and wheat, contribute about 40% of total energy intake (McCann 2005; Labadarios et al., 2000). In sub-Saharan Africa, maize is the most consumed crop, because of various reasons. It requires relatively lower levels of labour than other crops, it is relatively less expensive, and it is more widely available throughout the year and can be consumed by all household members (Olembo, 2010). Though maize grain has high mineral content, phytic acid in the grain limits their absorption (Velu et al., 2014). Interventions such as supplementation, fortification, and diet diversification have been introduced to combat mineral deficiencies; however, none of these strategies has had satisfactory nutritional outcomes in developing countries due to several reasons, including unstable government policies, poor infrastructure and lack of continued adequate investment (Mishra, 2011). Moreover, these interventions (dietary diversification, fortification, and supplementation) have proven too costly for developing countries (Pambo et al., 2014). Moreover, rural households are generally resource poor; the majority of them produce their own maize, which they consume as staple to supply them with energy and nutrients. The maize grain is processed into several food types using indigenous or traditional methods, among them are milling, boiling, roasting and fermentation. There are a number of reports suggesting that the indigenous and/or traditional methods of processing maize grain is nutritionally beneficial, as they tend to increase nutrient availability, including minerals (Hurrell, 2004; Coulibaly et al., 2011a). The suggested mechanisms of increasing nutrient availability include reduction of phytic acid in the maize grain (Hurrell, 2004).

Breeding for low phytic acid maize is a new intervention that uses markers to identify and select genes of interest. Marker Assisted Selection (MAS) has been used to select crops with genes of interest and it enables the breeder to eliminate crops, which do not have the desired genes. The advantage of MAS is that no fertilizer, space, and labour are wasted on unwanted crops (Gu et al., 2005). Genetic engineering is done to develop crop varieties with increased amounts of essential minerals especially in cereals in what is referred to as biofortification (Srinivasan, 2001). Biofortification involves breeding staple crops for increased mineral and/or vitamin content using conventional breeding methods and/or modern recombinant DNA (rDNA) technology. There has been a great need of new innovative strategies to combat hidden hunger and other forms of malnutrition.Low phytic acid maize could be one of such innovations to assist in lowering malnutrition rates in rural households and help alleviate nutrition-related diseases. Nevertheless, consumer acceptance of food is fundamental to the receptivity of this intervention. Consumer acceptance entails psychological perceptions and sensorial reactions to the properties of food. Regarding biofortification of maize, studies show that yellow maize is less acceptable to consumers compared to white maize (Pretorious, 2011). In spite of this preference for white maize, some consumers are skeptical about white maize due to its genetic modification. Fortified white maize is perceived as having chemicals added to it (Khumalo et al., 2011). Lowering phytic acid has no effect on the colour of the grain, but the same may not be the case with its other sensory properties.

1.2 Problem Statement

Micronutrient deficiency (hidden hunger) among rural households in sub-Saharan African countries, including South Africa, is a silent threat to food and nutrition security. Hidden hunger is largely caused by following monotonous, lowly diverse diets that have low micronutrient content; amongst the diets cereal grain foods are one of the leading. In addition to low micronutrient content, anti-nutritional factors interfere with the bioavailability of the micronutrients.

In South Africa (SA), maize is a leading staple and as such is key to food and nutrition security. Resource poor communities, especially rural households, generally follow monotonous diets comprised mainly of maize and vegetables. Consequently, the SA government encourages rural smallholder farmers to plant maize and vegetables to improve the availability of food in the household (Mavengahama et al., 2013). Government even supplies farmers with seeds free. Despite these efforts, hidden hunger persists partly due to the limited bioavailability of the micronutrients. Maize has limited nutritional value- it has limited mineral content and their bioavailability is restricted by phytic acid. On the other hand, some traditional food processing methods (practices) have been reported to improve nutrient (including micronutrient) bioavailability through various mechanisms, including destruction of anti-nutritional factors and breakdown of nutrient/anti-nutritional factor complexes. However, there is limited evidence of whether the benefits of using these practices are known by the rural households. Indeed, Walingo (2009) notes a trend of abandoning these traditional processing methods by the nutritionally vulnerable rural communities due to changing lifestyles that influence the production, preparation, and preservation of food. There is a need to identify traditional methods that reduce phytic acid in maize grain and thereby increase the potential of maize grain to contribute to combating mineral deficiency. Further, the phytic acid content of maize grain may be reduced biotechnologically; this could be achieved by selectively breeding for low phytic acid maize and/or through rDNA technology. It would be also necessary to evaluate the potential for adoption of the proposed methods of reducing phytic acid in maize grain by the target communities through assessment of consumer acceptance of popular maize- dishes made with low phytic acid maize. The information obtained would be used to devise strategies for promoting the adoption of technologies that reduce phytic acid in maize grain.

1.3 Main objective

This study aims to determine the effect of traditional processing methods and conventional breeding on the phytic acid content of white maize as well as consumer acceptance of the maize-based dishes thereof.

Specific objectives:

- To evaluate the LPA white maize lines for low grain phytic acid content by genetic manipulation
- To investigate the effect of selected traditional processing and conventional breeding methods on the phytic acid and nutrient content of maize-based dishes.
- To determine the effect of selected traditional processing and conventional breeding methods on consumer acceptance of maize-based dishes.

1.4 Hypothesis

The phytic acid content of normal white maize grain may be reduced by specific traditional methods of processing maize and conventional breeding, which would increase the availability of the minerals for absorption by humans.

1.5 Definition of terms

Bioavailability

Means a nutrient can be absorbed by into the human blood and then delivered to the tissues (Anand et al., 2007).

Phytic acid:

This is an anti-oxidant, which stores phosphorus. It is found in legumes and cereals. It reacts with minerals through complex formation (Repo-Carrasco-Valencia et al., 2009).

Hidden hunger:

Hinder hunger is micronutrient deficiency. This deficiency leads to vulnerability to infectious diseases, and physical and mental impairment. It hampers growth in children and induces impaired functioning in adults (Tanumihardjo et al., 2008).

Marker Assisted Selection:

A selection process in which a gene of interest is selected not based on the gene itself but on the marker linked to it (Collard & Mackill, 2008).

Triangulation

The combination of methodologies in the study of the same phenomenon

1.6 Study limitations

The study was only limited to households in Ntambanana community namely Buchanana and Luwamba, who were planting and consuming maize. As a result, the findings of the study may not be applicable to other areas other than Ntambanana

1.7 References

Anand, P., Kunnumakkara, A.B., Newman, R.A. and Aggarwal, B.B., 2007. Bioavailability of Curcumin: problems and promises. *Molecular Pharmaceutics*, *4*(6), pp.807-818.

Collard, B. C. and D. J. Mackill 2008. "Marker-assisted selection: an approach for precision plant breeding in the twenty-first century." *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**(1491), pp. 557-572

Coulibaly, A., Kouakou, B. and Chen, J., 2011. Phytic acid in cereal grains: structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *American Journal of Plant Nutrition and Fertilization Technology*, *1*(1), pp.1-22.

Gu, W.K., Weeden, N.F., Yu, J. and Wallace, D.H., 1995. Large-scale, cost-effective Screening *and Applied*of PCR products in marker-assisted selection applications. *Theoretical Genetics*, *91*(3), pp.465-470.

Khumalo, T.P., Schönfeldt, H.C. and Vermeulen, H., 2011. Consumer acceptability and perceptions of maize meal in Giyani, South Africa. *Development Southern Africa*, *28*(2), pp.271-281.

McCann, J., 2005. *Maize and grace*. Harvard University Press.

Mavengahama S, McLachlan M, and De Clercq, W., 2013. The role of wild vegetable species In household food security in maize based subsistence cropping systems. *Food Security*; 5 (2), pp 227-33.

Mishra, A.P.A., 2011, June. Impact of Child Poverty on Adult Poverty in Developing Countries. In *International Symposium on Poverty Alleviation Strategies* (p. 503).

Muthayya, S., Rah, J.H., Sugimoto, J.D., Roos, F.F., Kraemer, K. and Black, R.E., 2013. The Global hidden hunger indices and maps: an advocacy tool for action. *PLoS One*, *8*(6), p.e67860.

Olembo, N., M'mboyi, F. and Oyugi, K., 2010. Success Stories in Crop Improvement in Africa. In *The Case of Rice in Sub-Saharan Africa. African Biotechnology Stakeholders Forum (ABSF). Nairobi*.

Pambo, K.O., Otieno, D.J. and Okello, J.J., 2014. Consumer awareness of food fortification in Kenya: The case of vitamin-A-fortified sugar. *International Food and Agribusiness Management Association*.

Pretorius, B., 2011. *Vitamin A content and bio-availability of South African maize meal (as purchased and consumed)* (Doctoral dissertation).

Repo-Carrasco-Valencia, R., Peña, J., Kallio, H. and Salminen, S., 2009. Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (Amaranthus caudatus). *Journal of Cereal Science*, *49*(2), pp.219-224.

Tanumihardjo, S.A., Bouis, H., Hotz, C., Meenakshi, J.V. and McClafferty, B., 2008. Biofortification of staple crops: an emerging strategy to combat hidden hunger. *Comp Rev Food Science, Food Safety*, *7*, pp.329-34.

Velu, G., Ortiz-Monasterio, I., Cakmak, I., Hao, Y. and Singh, R.P., 2014. Biofortification strategies to increase grain zinc and iron concentrations in wheat. *Journal of Cereal Science*, *59*(3), pp.365-372.

Walingo, M. K., 2009. "Indigenous food processing methods that improve zinc absorption and bioavailability of plant diets consumed by the Kenyan population." *African Journal of Food, Agriculture, Nutrition and Development* 9(1): 523-535

2 CHAPTER: GENERAL LITERATURE REVIEW

2.1 The challenges of malnutrition with special focus on micro-nutrient deficiencies-hidden hunger

Eleven million people are undernourished in developing countries with sub-Saharan Africa being one of the most nutritionally insecure regions of the World (FAO, 2014). South Africa, which is located in the sub-Saharan region, reveals similar trends. The average incidence of severe acute malnutrition in children under five years was 4.4 cases per 1 000 children in 2012/2013 (Govender et al., 2015). Malnutrition amongst adults is also a challenge. Iron and zinc deficiency are prevalent in sub-Saharan Africa, particularly because of monotonous diets, which are predominantly cereal grain-based (Gómez & Ricketts, 2013).

Hidden hunger is a deficiency of micronutrients, vitamins and minerals in the human body (Humanosphere, 2013). Hidden hunger is a silent disease; it has limited visible symptoms that pose a major challenge beyond the Millennium Developmental Goals (MDGs). Under food security and hunger, the newly introduced Sustainable Development Goals (SDGs), which have similar goals to those of the MDGs envisages: "Ending hunger and achieving long-term food security, including better nutrition, based on sustainable agriculture and fisheries production, distribution and consumption systems, (including efforts geared at) sustainable water security". Greater attention is still required on nutrition security as most developing countries rely heavily on agriculture and often have the most population groups that are vulnerable to nutrition insecurity, including hidden hunger. Hidden hunger is largely due to limited dietary diversification, which severely limits the achievement of a balanced diet.

2.1.1 Prevalence of malnutrition in sub-Saharan African countries, with a special focus on South Africa

Nearly 2.3 million children in South Africa are said to be undernourished, 21% -48% of whom are stunted; 8%-15% are underweight and 3.7% are wasted (WHO, 2010 & Giatau, 2010). As stated earlier, consumption of monotonous diets that are predominantly based on starchy staples contribute significantly to hidden hunger in developing countries, especially in sub-Saharan African countries (Keatinge et al., 2010).

Figure 2.1 depicts how the cycle of food and nutrition insecurity tends to persist among resource-poor households. When a malnourished woman is pregnant, there is a high probability that she will give birth to an underweight baby who most probably could suffer from stunting if their malnourishment is not attended timeously. Stunting causes brain impairment, which then affects normal cognitive function. A major implication of a compromised nutritional status is reduced mental capacity in a child. If malnutrition is not mitigated, it leads to stunting during adolescence with potential negative socio-economic impacts related to the costs of caring for a malnourished individual and the lack of productivity of that individual (de Pee et al., 2015). Growing with a frail body increases susceptibility to several health conditions at a later stage (Okubo et al., 2015). More so, micronutrient deficiencies in adults can bring about the onset of non–communicable diseases with detrimental impacts on life expectancy.

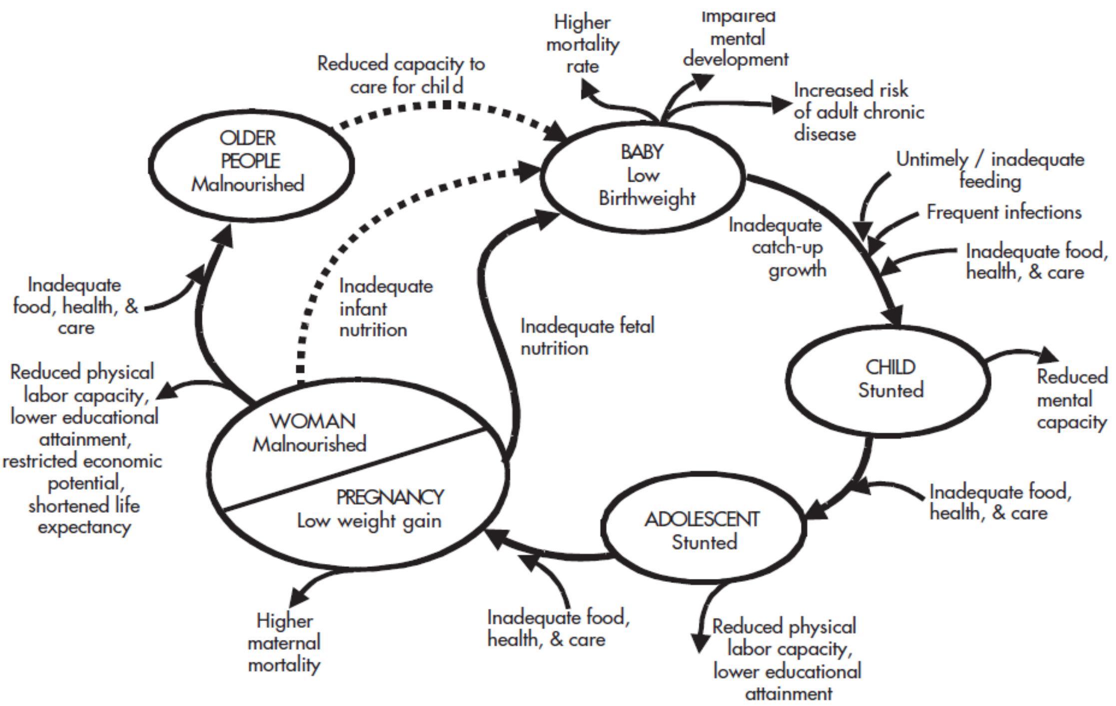

Figure 2.1: Malnutrition in the life cycle. Source: From ACC/SCN (2000)

Reducing the phytic acid content of maize could be a cost-effective and easily accessible intervention that is agriculture-based- it may have the potential to address micronutrient deficiencies (hidden hunger) thereby enhancing both food and nutrition security. The next section further elaborates on how lowering phytic acid in maize grain could improve food and nutrition security.

2.2 Current intervention strategies to address malnutrition in South Africa

Nutrition based interventions were introduced to assist in decreasing the rate of mineral deficiency (hidden hunger). Mineral deficiency is often overlooked because it is not directly visible to the eye (Biesalski, 2013). Yet, it affects the internal functioning and development of a human. Some 2 billion people in developing counties are affected. Current public health interventions to address micronutrient malnutrition include fortification, diet diversification, and supplementation.

2.2.1 Supplementation

These are products made to boost the nutritional content of people's diet. Supplements include vitamins, minerals, herbs, natural food supplements, meal replacements, and sports nutrition products. These products can be water-dispensable, crushable tablets, and even granular powders which are sprinkled onto complementary foods (Flynn et al., 2009). Many of these supplements are given to people to treat a special severe case Iron supplements, for example, are essential in providing components of haemoglobin and myoglobin which are required for enzyme based biochemical processes. Often, they are given to pregnant woman to prevent preterm infants, and prevent delayed motor functioning in infants. But, people tend to overlook this deficiency in teens and adults. Even in that age group, mental functioning can be adversely affected coupled with protracted fatigue that impairs the ability of adults to do physical work (Whittaker, 2011). Moreover, due to poor infrastructure, developing countries face the challenge of receiving supplements, which aggravates the prevalence of malnutrition in those countries.

2.2.2 Fortification

This intervention involves enriching or adding micronutrients (essential trace elements and vitamins) (Meenakshi et al., 2010). Fortification of food is being intended for low-income households, which is why fortification begins with identifying staple foods, which then are used as a vehicle for micronutrition. Unsurprisingly, maize is among cereal grain foods, which are recommended as food vehicles for fortification.

The fortification of two staple foods, namely maize meal and wheat flour with iron, zinc, thiamine, vitamin A, niacin, pyridoxine, folate, and riboflavin was legislated in South Africa in October 2003 (Steyn et al., 2008). However, fortification targets mostly the urban population because of the costs of processing as well as the location of industries with the expertise to ensure sound quality control and efficient distribution (Pambo et al., 2014). As such, Steyn (2008) calls attention to the unlikelihood of food fortification to make up for dietary deficiencies for children who are unable to consume large portions of fortified staple foods. The success of food fortification programme is also uncertain given the deterioration in the iron and zinc status of children and also and pregnant woman (Pambo et al., 2014)

2.2.3 Dietary diversification

Promotion of indigenous foods, introduction of new crops and diversification of crops are some of the approaches that can be used to address micronutrient malnutrition. Dietary diversification involves following a diet comprised of variety of food types that to ensures adequate intake of essential nutrients that promote good health (Galhena, 2013). Hence, it tends to be limited by crop seasonality as well as the inability of poor people to afford a diversified diet.

This intervention Dietary diversification is a long-term strategy that can be achieved through horticultural approaches such improvements in home food preparation, home gardens and crop preservation after harvest (Galhena, 2013). Promotion of indigenous foods, introduction of new crops and diversification of crops are some of the approaches that can be used to address micronutrient malnutrition.

2.2.4 Other strategies for combating nutrient deficiencies: Genetic manipulation through conventional breeding and rDNA technology

The genetic factors that determine the nutrient content of crops can be manipulated such that the factors that code for nutrient-rich (biofortified) crop types are deliberately selected for genetic manipulation of cereal grains with special focus on maize is reviewed in detail in Section 2.8. However, there can be challenges with consumer acceptance of genetically manipulated crops (Pillay et al., 2011; Muzhingi et al., 2008).

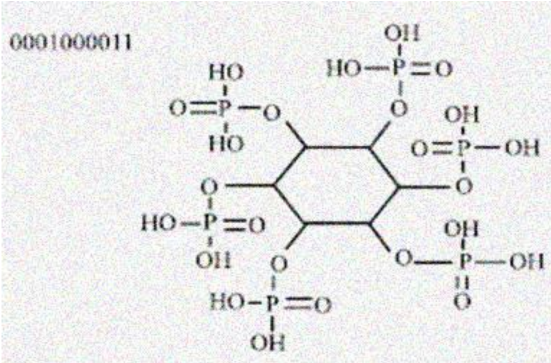

Figure 2.2: Structure of phytic acid

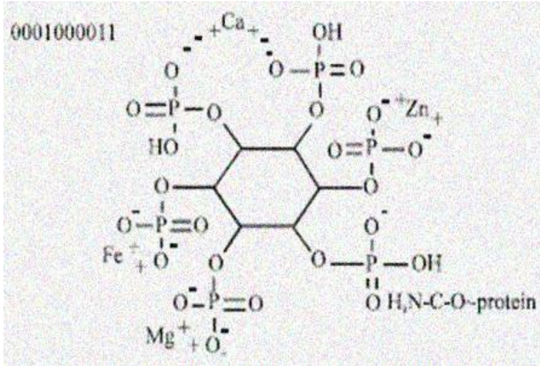

Figure 2.3: Phytic acid complexed with minerals and proteins

Source: Coulibaly et al., (2011)

2.3 Phytic acid, the anti-nutritional factor and health-promoting compound

Phytic acid whose chemical structure is shown in Figure 2.2 is also called 'myo-inositol hexophosphate; makes up 1-3% of most plants seeds and is found in the germ (Coulibaly et al., 2011). Phytic acid acts as a chelating agent of minerals such as zinc, calcium, magnesium, iron, and proteins. After binding minerals, a salt form of the phytate is formed and it is evacuated from the body through defecation (Soetan et al., 2010). Not only does phytate binds minerals but also proteins. In the absence of cation at low pH, phytic acid binds itself to protonated basic residues, while at high pH and in the presence of cation, it forms protein-metal-phytate complexes (Volynsky & Libardian, 2013). The complex structure reduces the ability of proteins to perform their physiological functions.

Monogastric animals, including humans lack the ability to hydrolyze phytate. Thus, numerous studies aim to find an effective way to decrease phytic acid content in plant food sources, including maize grain. Further to this goal, Troesch (2013) confirms that practices performed at the household level may lower the phytic acid content of maize based food.

2.4 Interaction of phytic acid with minerals

Phytic acid is best at binding multivalent cations which are Fe^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+} and Ca^{2+}present in cereal food. Minerals such as Iron and Zinc in maize-based diets are not absorbed during human digestion because of complex phytate interaction (Erdman & Poneros-Schneier, 2013). Iron and Zn are also tightly bound to low and high molecular weight molecules in seeds that interact in numerous ways with other components of ingested food during the passage through the human digestive system. This interaction leads to iron and zinc being less available for absorption in cereal based food. Currently, the estimates of iron and zinc absorption in the human gut in a cereal dominated diet ranges around 5% for iron and 25% for zinc (Bouis, 2010). A high phytate diet results in mineral deficiencies. The minerals deficiencies result in several health problems- calcium and phosphorus deficiencies result in poor bone growth, short stature, rickets, and narrow jaws and tooth decay. Iron and zinc deficiency result in anemia and mental retardation, respectively (Khan & Bhutta, 2010).

While, phytic acid is considered an anti-nutritional component in cereals and seeds, it possesses health-promoting properties. It has been found to have anti-cancer effects due to its antioxidant properties (Fardet, 2010). Phytic acid is also beneficial in that it reduces blood glucose levels in diabetic patients through decreasing the rate of starch digestion (Kumar et al., 2010). Several studies have emphasized the importance of knowing the nutritional status of the community in order to better harness positive nutritional role of phytic acid especially as an anti-oxidant and an anti-cancer agent (Landoni et al., 2013).

2.4.1 Methods of estimating mineral availability in foods

There are three methods used to estimate the mineral availability in food which are *in vivo, in vitro* and measuring the mineral-binding substances e.g. phytic acid and tannins. *In vivo* is a Latin word which means within the living. It is the experiment or observations done on the living tissue of the whole living organism in a controlled environment. *In vitro*, on the other hand, is a Latin word that means within the glass; meaning the experiment or observations are done on the tissue outside of the living organism in a controlled environment, usually using Petri dishes and test tubes (Emelda, 2011). The in vivo method various animals are used to test for mineral absorption whereby studies measure the difference between minerals, which were fed and excreted (Fern andez-Garcıa et al. 2009). With the in vivo method not only minerals absorption is tested but also carbohydrates, vitamins, phytochemicals: with this Baker 2008 emphasizes on the important of choosing the right animal on prediction of what could happen in the human system. The in vitro method involves biological molecules which are studied outside the normal biological context such as minerals, which examined in a solution or in a culture medium. In vitro mimics the digestive process, and it is enhanced or inhibited by the medium placed in to. One of the methods used in an in vitro method to determining mineral content is atomic absorption analysis, which measure minerals through flaming them into particles, which produces the wavelength of mineral present in a sample. In vitro methods have proved to be in prediction of many inhibitory/enhancing dietary factors, and have been used to inspect the influence of processing on mineral bioavailability from food. However, it is important to stress that the dialysability is a relative rather than an absolute estimate of mineral absorbability (Luten et.al, 1996). The invitro method have been successfully used in screening variation in bioavailable iron among maize varieties. The method used Caco-2 cells that simulated the digestive tract. The ground maize was digested using solution that had pepsin, pancreatic and bile extract. The harvested monolayers of the cell are assayed for ferritin and protein (Oikeh et al., 2003).

2.5 Phytic acid in maize grain

Maize produces approximately 51 million metric tons of phytic acid ($C_6H_{18}O_{24}P_6$) annually around the globe (Nuss & Tanumihardjo, 2010). The phytic acid stores phosphorus in an insoluble form in grains. Phytic acid is also thought to of agronomic advantage as it contributes to stress resistance through antioxidant and antimicrobial activity. Phytic acid in cereals vary in percentages, depending on growing conditions, harvesting techniques, processing methods and the age of the seed (Bouis, 2010). Phytic acid is higher in maize grain because high levels of application phosphate fertilizers in modern agriculture (Coulibaly et al., 2011). The high content of phytic acid in maize highlights its huge negative impact on nutrient availability, especially minerals, and the resultant risk of food and nutrition insecurity among the resource-poor population groups that follow monotonous diets that a largely based on maize.

Several approaches have been tried to reduce the phytic acid levels in maize grain. Genetic manipulation (biotechnology) has been proposed as one of the best approaches for reducing phytic acid in maize grain. However, research findings also show that several traditional methods of processing maize reduce its phytic acid content. The next sections review the literature of reduction of phytic acid in maize through traditional processing methods and genetic manipulation.

2.6 Effects of different processing methods on mineral bioavailability in maize

Indigenous knowledge can be a key tool to assist in new agricultural interventions. In less developed countries with lower resource levels, indigenous knowledge could provide food nutrition security for rural households' by promoting maize processing methods, which enhance mineral availability through removing about 40% of phytic acid. The processing of maize could increase the bioavailability of minerals for absorption. Bioavailability of micronutrients is defined as the amount of nutrients in a meal that can be absorbed and utilized for metabolic processes in the body (Welch & Graham, 2004). Currently the estimates of iron and zinc absorption in human gut in a cereal dominated diet ranges at 5% of iron and 25% of zinc (Bouis, 2010). Therefore, it is important for plant breeder to inspect the concentration of the available and bioavailable mineral after creating a new cultivar. This in turn could help decrease malnutrition in rural households. Moreover, these processing methods are sustainable (Hotz & Gibson, 2007).

2.7 Traditional methods of processing maize

Several methods have been tried to improve the nutritional quality of maize grain. These include fermentation, germination, soaking, milling, roasting and genetic manipulation. These methods reduce phytic acid in maize grain through several mechanisms, for by increasing the activity of naturally present phytate-degrading enzymes found in microorganisms and the grain.

2.7.1 Fermentation

Fermentation is the breakdown of organic substances, usually carbohydrates, by microorganisms in the absence of oxygen to release energy and various substances as by-products. Some of the fermentative microorganisms, particularly lactic acid bacteria have health-promoting effects and are thus called probiotic microorganisms or simply probiotics. Consuming food with these probiotic microorganisms can: i) improve intestinal tract health, ii) enhance the bioavailability of nutrients, iii) enhance the immunity system, iv) decrease symptoms of lactose intolerance and v) reduce risks of certain cancers. Fermentation has been found to be beneficial through destroying undesirable components in maize, e.g. ant-nutritional factors (including phytic acid) and enhancing the nutritional quality of the food, reducing cooking time thereby conserving energy and improving taste. At the household level, fermentation is enhanced by adding sugar, usually when yeast cells are present. The sugar provides energy for the yeast cell, which, are then enhanced to produce the fermentation enzymes (boosting “the catalytic effect”) (Reale et al., 2007). The microbial cells also produce phytase, enzyme that hydrolyses phytic acid in maize and thereby releasing minerals for absorption. Fermentation has the additional benefit of preserving the food, which is especially critical for households that do not have access to refrigeration as a storage and preservation technology. As already mentioned, fermentation also improves flavour as well as the digestibility of food.

2.7.2 Soaking

Soaking is a simple technological treatment that is often used to prepare foods such as legume and cereal grains. Soaking can be done for a short period of time (15 to 20 minutes) or a very long period (12 to 16 hours) (Hambridge et al., 2005). Prolonged soaking actually facilitates dehulling or swelling of the grain releasing the phytic acid embedded on the outer layer of the seed (Zijp et al., 2000). Cereal and legume grains are soaked in water at room temperature overnight. Soaking activates indigenous microorganisms, which degrade phytate. Temperature and pH have a huge effect on the hydrolysis of phytate during soaking: specifically, phytic acid is hydrolyzed at temperatures between 45^0C and 65^0C at pH levels 5 and 6 (do Santos et al., 2010). Soft porridge (*idokwe* in isiZulu) is prepared by soaking milled maize. *Idokwe* is mostly eaten by or fed to children.

2.7.3 Thermal processing

Thermal processing methods include roasting, boiling, and pasteurization. These methods vary in terms of processing temperature and whether water is added (moist heat) or not (dry heat) technologies. Thermal processing affects anti-nutritional factors and the availability of minerals. The heat treatment does not destroy minerals because they are heat stable, however, wet heating can result in leaching and thus affect the mineral content. Roasting results in the removal of the grain outer layer pericarp/fruit coat and through this processing method up to 40% of phytic acid in legumes has been reported to be reduced (Hemalatha et al., 2007a; Rehman & Shah, 2005). The advantage with roasting is that it can also reduce mycotoxins, including aflatoxins produced by as the fungus *Aspergillus flavus*. When the grain is roasted, the colour of the outer layer changes to golden brown and black. The aim of roasting is to improve taste, destroy unwanted enzymes, and improve nutritional quality as well preserve the grain (Hemaltha & Srinivansan, 2007). However, Malik (2002) reported that some of the minerals were lost during roasting.

2.7.4 Germination

Germination of cereals is used to increase nutritional value and palatability. During germination, phytate is degraded by the action of phytase, which provides the growing seedling with phosphate, mineral cations and myo-inositol. Apart from its storage function, phytate has also been assumed to play an important role in P homeostasis by buffering cellular P levels (Karp, 2013). During this process, maize seeds are soaked in water to initiate an enzymatic reaction. Treatment of seeds with ethanol, formaldehyde 0.2% and sodium hypochlorite 1% chlorine can help prevent mold growth. In addition, environmental factors such as light, temperature, oxygen, and water play an important role in decreasing phytate content (Ahn et al., 2010).

The enzymatic reactions thus assist in decreasing the level of anti-nutritional factors thereby releasing minerals. Germination is coupled with soaking because both of these reactions occur in a humid and warm environment. Afify (2011) reported that maize grain germination removed 23.9% of phytic acid after 72 hours at 25^{0}C. Thus, there is potential for traditional maize processing methods to remove phytic acid content in maize grain and there by contribute to alleviating mineral deficiencies.

2.8 Development of low phytic acid maize types by genetic manipulation

2.8.1 Breeding for low phytic acid maize

Breeding uses genetic resources in crop improvement. This is done by transferring desired genes from an un-adapted germplasm to a locally adapted line. Breeding aims at developing improved crop varieties that can benefit both the farmers and end-uses (consumers). Breeding is used to improve the traits of the crop, including yield, resistance to stress and nutritional quality.

The nutritional quality of crops can be improved by genetic manipulation in terms increasing nutrient content (biofortification) and/or improving nutrient availability. Biofortification is a process by which staple crops are purposefully developed to have higher nutritional density (Fraser & Bramley, 2004). Recombinant DNA (rDNA) technology and conventional breeding can be used singly or in combination in biofortication. Provitamin A-biofortified maize types have been successfully developed by genetic manipulation using conventional breeding and/or rDNA technology approaches (Chassy et al., 2008). Biofortification is probably the most feasible and sustainable strategy for reducing nutrient deficiencies (Miller & Welch, 2013).

Mineral availability in maize grain could be achieved through genetic manipulation of germplasm of normal (wild) maize grain types to produce low phytic acid maize grain types. Conventional breeding and/or rDNA technology can do this. Currently, there is vibrant research on the development of low phytic acid maize by conventional breeding (Aluru et al., 2011). rDNA is being used in breeding for low phytic acid maize- this is being achieved through a method of Marker Assisted Selection (MAS), which facilitates the identification of traits of interest or of a useful gene. Markers can identify a gene, which can then be transferred into an elite line in order to improve its traits. This helps reduce the normally long turnaround time of conventional breeding (Jiang, 2013). Although genetically modified crops tend to be affordable, locally adaptable, and a long-term solution to diet nutrient deficiencies, their acceptance by consumers may be low. This may be particularly true with those crops for which the rDNA-modified types (transgenic/GMOs) are the only alternative to boost nutrient content (Lipkie et al., 2013). Consumers are generally wary of the safety of GMO foods and have a notion that the production of GMO crops has a negative effect on the agro-ecological environment (Scholderer & Verbeke, 2012). Further, the sensory properties of biofortified crops may be less acceptable compared to the conventional crops- this has been well demonstrated in provitamin A-biofortified maize (Govender et al., 2015). The consumer acceptance of genetically modified maize is reviewed in detail in Section 2.9.

2.9 Consumer acceptance of genetically modified maize

Consumer acceptance refers to attitudes, consumer awareness of concepts and their choices of food products. The theory of accepting or rejecting food is multi-dimensional; it involves an individual’s chemical stimuli concentration, physiological perception and the consumer’s reaction (Costell et al., 2010). Not only does sensory perception determine the food acceptance; it also looks at previous experience and the information one gathered about the product (Rodbotten et al., 2009). Consumer acceptance grades the degree of satisfaction that the product is able to provide, meaning it is an interaction between food and man at a certain moment (Heldman, 2004). Apart from sensory attributes, perception (socio-economic and socio-cultural) factors such as price, fashion, family, cultural habits, religion and education can have an impact on a consumer’s decision to accept or reject the product.

2.9.1 Sensory acceptability of genetically modified maize

Genetic modification of maize does affect its sensory acceptability, especially provitamin A-biofortified maize (De Groote et al, 2010). Provitamin A-biofortified maize is a maize type that has been genetically modified with the primary aim of increasing grain provitamin A content. This is achieved either by conventional breeding methods and/or by rDNA technology. However, this modification results in changes in the sensory properties of the maize, mainly in changes in grain colour from white to yellow/orange, as well as imparting a strong aroma and flavour in the grain. These new characteristics have caused low sensory acceptability of the provitamin A-biofortified maize (Pillay et al., 2011; Muzhingi et al., 2008).

Genetic modification of normal white maize to low phytic acid maize may result in changes in its sensory properties; this is due to the changes in chemical composition, including decreases in phytate content, which may occur. From the available literature, is seems the case that the sensory acceptability of low phytic acid maize has not yet been investigated.

2.9.2 Consumer perceptions about biofortified maize products

Consumers have a negative attitude towards biofortified maize due to their perceptions about it (Kiria et al 2010). It has been reported that consumers do not easily accept colour changes to maize. As shown by De Groote (2011), consumers from South Africa were willing to pay 40% to 50% higher prices for premium white maize to avoid purchasing yellow maize, which was perceived as animal feed. The vitamin A fortification of the maize grain changed its sensory properties thereby creating a negative sensory perception (Stevens & Winter-Nelson 2008; HarvestPlus Brief 2006).

Perceptions are caused by psychological factors, such as previous experiences, opinions, likes and dislikes, attitudes, values and beliefs. Perceptions lead to a positive or negative attitude to a product. However, the negative attitudes can be changed if the consumer is well informed about the value of a product (Roininen & Tuorila, 1999, Connor & Douglas, 2001; von Alvensleben, 2001; Pearson, 2002).

In South Africa, white maize meal is a dominant staple food. Urban and rural households purchase commercially produced maize meal, thereby making it more acceptable to both commercial and small-scale farmers. Moreover, rural areas still grow white maize on small scale to be milled at nearby hammer mills (Khumalo, 2011). In Africa, white maize constitutes more than 90% total crop production and about 33% of world's white maize. White maize is widely consumed and preferred to the yellow maize. Yellow maize is perceived negatively; it is generally regard as feed rather than food. This is despite of the fact that yellow maize produced in sub-Saharan Africa has higher nutritional value than white maize because a significant number of its varieties contain pro-vitamin A (Nuss et al., 2011).

2.10 Conclusion

Previous research shows that, normal white maize white is key to food and nutrition security in sub-Saharan African countries, including South Africa. Yet, when consumed as it is, maize does not contribute significantly to combating micronutrient deficiencies (hidden hunger), especially mineral deficiencies due to the binding of a nutritionally significant proportion of some of the minerals by phytic acid. Reduction of phytic acid in maize grain by genetic manipulation and traditional methods of processing maize could increase the contribution of maize to combating hidden hunger, and ultimately enhancing food and nutrition security as depicted in the conceptual framework Figure 3.2.

2.11 References

Afify, A.E.M.M., El-Beltagi, H. S., El-Salam, S. M. A., and Omran, A. A., 2011. Bioavailability of iron, zinc, phytate and phytase activity during soaking and germination of white sorghum varieties. *Plos one*, 6(10), e25512

Aluru, M.R., Rodermel, S.R. and Reddy, M.B., 2011. Genetic modification of low phytic acid 1-1 maize to enhance iron content and bioavailability. *Journal of Agricultural and Food Chemistry*, 59(24), pp.12954-12962.

Biesalski, H.K., 2013. *Hidden Hunger* (pp. 25-50). Springer Berlin Heidelberg.

Bouis, H.E. and Welch, R.M., 2010. Biofortification—a sustainable agricultural strategy for Reducing micronutrient malnutrition in the global south. *Crop Science*, *50*(Supplement_1), pp.S-20.

Chassy, B., Egnin, M., Gao, Y., Kleter, G.A., Nestel, P., Newell-McGloughlin, M., Phipps, R.H. and Shillito, R., 2008. Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology. *Comprehensive Reviews in Food Science and Food Safety*, *7*(1), pp.50-113.

Costell, E., Tárrega, A. and Bayarri, S., 2010. Food acceptance: the role of consumer perception and attitudes. *Chemosensory Perception*, *3*(1), pp.42-50.

Coulibaly, A., Kouakou, B. and Chen, J., 2011. Phytic acid in cereal grains: structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *American Journal of Plant Nutrition and Fertilization Technology*, *1*(1), pp.1-22.

De Groote H, Kimenju SC and Morawetz UB., 2010 Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. *Agriculture Economics* 41, pp1-16

De Pee, S., Grais, R., Fenn, B., Brown, R., Briend, A., Frize, J., Shoham, J. and Kiess, L., 2015. Prevention of acute malnutrition: distribution of special nutritious foods and cash, and whom, and addressing underlying causes—what to recommend when, where, for how. *Food and Nutrition Bulletin*, *36*(1 suppl1), pp.S24-S29.os Santos, I.C., Fontanétti, A. and Galvão, J.C.C., 2010. Importance of maize for local and regional food security. *Informe Agropecuario*, *31*(254), pp.36-45.

Erdman Jr, J.W. and Poneros-Schneier, A., 2013. Phytic Acid Interactions with Divalent Cations in Foods and. *Mineral Absorption in the Monogastric GI Tract*, *249*, pp.161.

Fardet, A., 2010. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre?. *Nutrition Research Reviews*,*23*(01), pp.65-134.

Flynn, A., Hirvonen, T., Mensink, G.B., OckÃÐ, M.C., Serra-Majem, L., Stos, K., Szponar, L., Tetens, I., Turrini, A., Fletcher, R. and Wildemann, T., 2009. Intake of selected nutrients from foods, from fortification and from supplements in various European countries. *Food & Nutrition Research*, *53*.

Galhena, D.H., Freed, R. and Maredia, K.M., 2013. Home gardens: a promising approach to enhance household food security and wellbeing. *Agriculture &Food Security*, *2*(1) ,p 1

Gómez, M. I. and Ricketts, K. D., 2013. Food value chain transformations in developing countries: Selected hypotheses on nutritional implications. *Food Policy*, 31(42), pp. 139-50.

Govender, L., Pillay, K., Derera, J. and Siwela, M., 2015. Acceptance of a complementary food prepared with yellow, provitamin A-biofortified maize by black caregivers in rural KwaZulu-Natal. *South African Journal of Clinical Nutrition*, 27(4), pp.217-221.

Health 24, 2013. Malnutrition. http://www.health24.com/Medical/Diseases/Malnutrition-Client-20120721. Viewed 21 May 2015.

Heldman, D.R., 2004. Identifying food science and technology research needs. *Food Technology*, *58*(12), pp.32-34.

Humanosphere, 2013. Covering Global health because we give a damn: Exposing hidden Hunger of micronutrient deficiency. http://www.humanosphere.org/basics/2013/06/exposing-the-hidden-hunger-of-micronutrient-deficiencies/. Viewed 21 May 2015.

Jiang, G.L., 2013. Molecular markers and marker-assisted breeding in plants. *Plant Breeding from Laboratories to Fields*, pp.45-83.

Kiria, C.G., Vermeulen, H. and De Groote, H., 2010, September. Sensory Evaluation and Consumers' Willingness to pay for quality protein maize (QPM) using experimental auctions in rural Tanzania. In *3rd International Conference of the African Association of Agricultural Economists, Cape Town, South Africa* (pp. 19-23).

Khan, Y. and Bhutta, Z.A., 2010. Nutritional deficiencies in the developing world: current status and opportunities for intervention. *Pediatric Clinics of North America*, *57*(6), pp.1409-1441.

Kumar, V., Sinha, A.K., Makkar, H.P. and Becker, K., 2010. Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, *120*(4), pp.945-959.

Lestienne, I., 2005. "Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents." *Food Chemistry* 89 (3), pp. 421-425.

FAO, I., 2014. WFP (2014). *The State of Food Insecurity in the World*, pp.12.

Fraser, P.D. and Bramley, P.M., 2004. The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research*, *43*(3), pp.228-265.

Hambidge, K.M., Krebs, N.F., Westcott, J.L., Sian, L., Miller, L.V., Peterson, K.L. and Raboy, V., 2005. Absorption of calcium from tortilla meals prepared from low-phytate maize. *The American Journal of Clinical Nutrition*, *82*(1), pp.84-87.

Hemalatha, S., Platel, K. and Srinivasan, K., 2007. Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains. *European Journal of Clinical Nutrition*, *61*(3), pp.342-348.

Hotz, C. and Gibson, R.S., 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *The Journal of Nutrition*, *137*(4), pp.1097-1100.

Karp, H. 2013. *Dietary phosphorus sources and their acute effects on mineral metabolism in healthy women*. PhD Thesis. University of Helsinki.

Keatinge, J.D., Waliyar, F., Jamnadas, R.H., Moustafa, A., Andrade, M., Drechsel, P., Hughes, J.D.A., Kadirvel, P. and Luther, K., 2010. Relearning old lessons for the future of food—by bread alone no longer: diversifying diets with fruit and vegetables. *Crop Science*, *50*(Supplement_1), pp.S-51.Khumalo, T.P., Schönfeldt, H.C. and Vermeulen, H., 2011. Consumer acceptability and perceptionsof maize meal in Giyani, South Africa. *Development Southern Africa*, *28*(2), pp.271-281.

Landoni, M., Cerino Badone, F., Haman, N., Schiraldi, A., Fessas, D., Cesari, V., Toschi, I., Cremona, R., Delogu, C., Villa, D. and Cassani, E., 2013. Low phytic acid 1 mutation in maize modifies density, starch properties, cations, and fiber contents in the seed. *Journal of Agricultural and Food Chemistry*, *61*(19), pp.4622-4630.

Lipkie, T.E., De Moura, F.F., Zhao, Z.Y., Albertsen, M.C., Che, P., Glassman, K. and Ferruzzi, M.G., 2013. Bioaccessibility of carotenoids from transgenic provitamin A biofortified sorghum. *Journal of Agricultural and Food Chemistry*, 61(24), pp.5764-5771.

Meenakshi, J.V., Johnson, N.L., Manyong, V.M., DeGroote, H., Javelosa, J., Yanggen, D.R., Naher, F., Gonzalez, C., Garcia, J. and Meng, E., 2010. How cost-effective is biofortification in combating micronutrient malnutrition? An ex ante assessment. *World Development*, *38*(1), pp.64-75.

Muzhingi, T., Langyintuo, A. S., Malaba, L. C., and Banziger, M., 2008. Consumer acceptability of yellow maize products in Zimbabwe. *Food Policy*, 33(4), pp. 352-361.

Nuss, E.T. and Tanumihardjo, S.A., 2011. Quality protein maize for Africa: closing the protein inadequacy gap in vulnerable populations. *Advances in Nutrition: An International Review Journal*, *2*(3), pp.217-224.

Oikeh, S.O., Menkir, A., Maziya-Dixon, B., Welch, R. and Glahn, R.P., 2003. Assessment of concentrations of iron and zinc and bioavailable iron in grains of early-maturing tropical maize varieties. *Journal of Agricultural and Food Chemistry*, *51*(12), pp.3688-3694.

Okubo, H., Robinson, S.M. and Godfrey, K.M., 2015. 6 Maternal Nutrition and Its Influence on the Health of the Next Generation. *Nutrition, Fertility, and Human Reproductive Function*, p.107.

Pambo, K.O., Otieno, D.J. and Okello, J.J., 2014. Consumer awareness of food fortification in Kenya: The case of vitamin-A-fortified sugar. *International Food and Agribusiness Management Association*.

Parida A, and Rajalakshmi, S., 2014. Addressing Iron deficiency through Biofortification. *Bulletin of the Nutrition Foundation of India*. 35(4).

Pillay, K., Derera, J., Siwela, M. and Veldman, F.J., 2011. Consumer acceptance of yellow, provitamin A-biofortified maize in KwaZulu-Natal.*South African Journal of Clinical Nutrition*, *24*(4), pp.186-191.

Reale, A., 2007. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. *Journal of Agricultural and Food Chemistry* 55(8), pp.2993-29972

Rubey L., Ward, R.W., and Tschirley, D., 1997. Maize research priorities. The role of Consumer preferences. In: Byerlee, D., Eicher, C.K.(Eds.), *Africa's Emerging Maize Revolution*.Lynne Rienner Publishers, Boulder , CO , pp. 145–155.

Scholderer, J. and Verbeke, W., 2012. *Genetically modified crop production*: Social sciences, agricultural economics, and costs and benefits of coexistence. Hochschulverlag AG.

Soetan, K.O., Olaiya, C.O. and Oyewole, O.E., 2010. The importance of mineral elements for humans, domestic animals and plants-A review. *African Journal of Food Science*, *4*(5), pp.200-222.

Steyn, N.P., Nel, J. and Labadarios, D., 2008. Will fortification of staple foods make a difference to the dietary intake of South African children?. *South African Journal of Clinical Nutrition*, *21*(1), pp.22-26.

Tako, E., Hoekenga, O.A., Kochian, L.V. and Glahn, R.P., 2013. High bioavailablilty iron maize (Zea mays L.) developed through molecular breeding provides more absorbable iron in vitro (Caco-2 model) and in vivo (Gallus gallus). *Nutrition Journal*, *12*(1), p.1.

Volynsky, Y.M. and Libardian, V., Cosmeceutical Research Center, 2013.*Peeling Compositions*. U.S. Patent 8, pp.460, 687.

Whittaker, C. 2011. Iron supplementation in the pharmacy: review. *SA Pharmaceutical Journal*, 78**,** pp. 34-36.

World Health Organization, 2010. *World Health Statistics 2010*. World Health Organization.

Zijp, I.M., Korver, O. and Tijburg, L.B., 2000. Effect of tea and other dietary factors on iron absorption. *Critical Reviews in Food Science and Nutrition*, *40*(5), pp.371-398.

3 CHAPTER: RESEARCH DESIGN AND METHODOLOGY

3.1 Introduction

This chapter gives an outlined description and explanation of the study area; research design and methodology. This study adopted a combination of qualitative and quantitative research methods.

3.2 Description of a study area

The study was conducted in Ntambanana local municipality, within the uThungulu District municipality, in KwaZulu-Natal province of South Africa. The UThungulu District Municipality is approximately 160 km north of Durban and can be accessed via the R34 from Empangeni. The municipality covers an area of 1,083 km^2, has 12,826 households and a total population of 74,336, made of 54% females and 46% males (Ntambanana Municipality IDP, 2010/2011).

This municipality is located between two rivers, namely Mfolozi River to its north and UMhlathuze River to its south. Ntambanana has eight ward counsellors with 80 ward committee members. There are four rural nodes, which are service centers namely Buchanana, Mambuka, Luwamba and Heatonville as seen in Figure 5. Ntambanana municipality's integrated development plan (2011/2012) states that small scale and subsistence farming activities related to poultry, vegetable and cotton are common agricultural projects in this area. These projects are funded by the Department of Agriculture and they are aimed at poverty alleviation and enhancing food security. Ingonyama Trust owns 85% of the land in Ntambanana, while 15% of the land belongs to commercial farmers. This municipality is rated as one of the poorest in uThungulu (DC28). According to Ntambanana Municipality IDP, 2010/2011, the Ntambanana municipality was only able to collect 2% of total property rates from farmers in 2007/8, with a slight increase (6%) in 2008/9.

About of 37% pensioners, meaning that most households are dependent on government grants for survival (Ralston et al., 2015), head South African households. Poverty associated with unemployment is one of the contributing factors to high illiteracy since many learners drop out of school to seek work in bigger cities like Durban and Johannesburg. Arts and informal sales are generally disregarded as economic activities, this creates the impression that rural communities are without a source of income and survive on subsistence production. Sixty-four percent of the population in the ethnic group areas receives no formal income (Ntambanana Municipality IDP, 2010/2011)

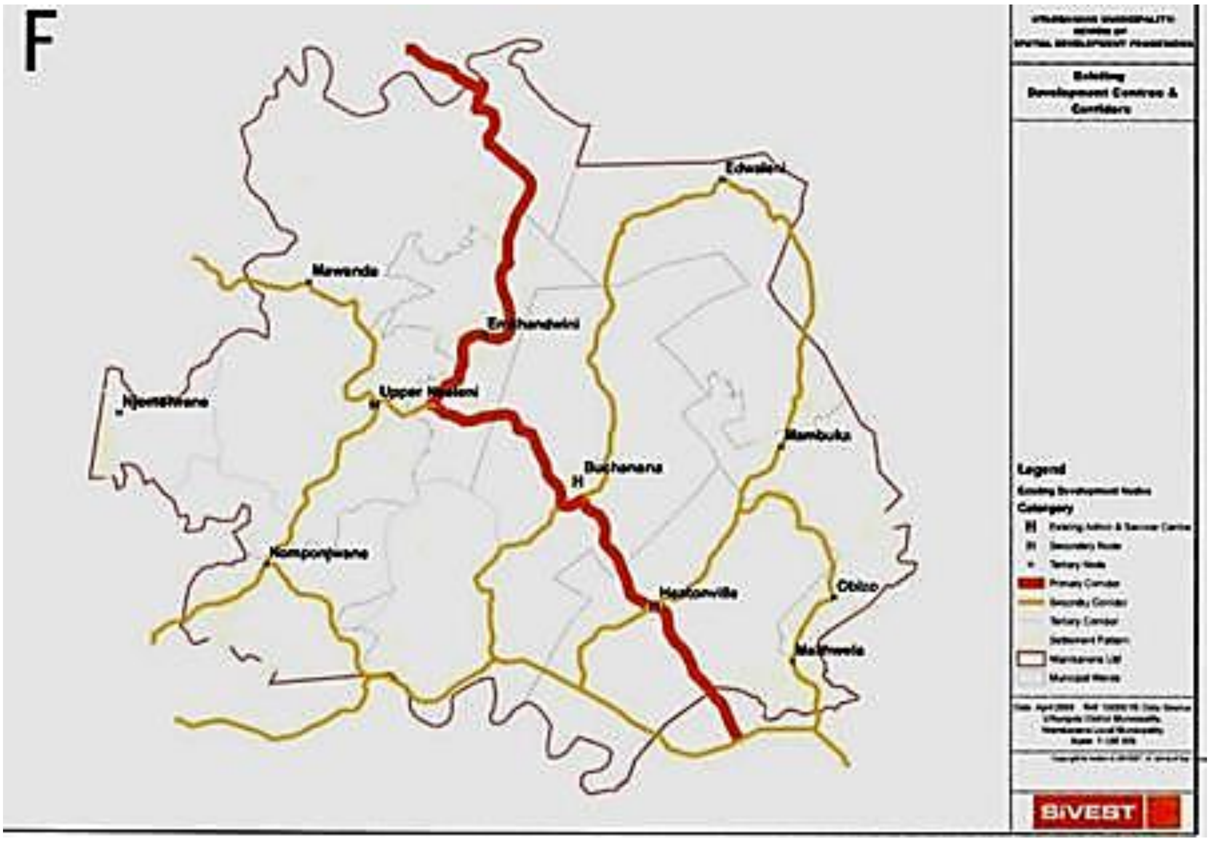

Figure 3.1: Map of Ntambanana Municipality

Source: IDP [Ntambanana Municipality Integrated Development Plan]. 2010/2011 review. Ntambanana Municipal offices Bhucanana

The Figure 3.2 represents the conceptual framework of the study. The decline utilization of the maize traditional processing methods could contribute to mineral deficiency. Thereby leading to finding and alternative solution. The plant breeders developed a maize line that had low phytic acid.

Prior to the current study, the low phytic acid lines were derived from an F2 population in prior studies at the University of KwaZulu-Natal, during 2006 to 2012. According to a study by Naidoo et al. (2012), the two inbred lines CM32 and LP16 were crossed to make the F1 population. The CM32 was the donor for the lpa1-1 gene to a locally adapted germplasm, LP16. The normal (wild-type) tropical locally adapted line P 16 and the temperate *lpa1-1* source CM 32 were used in this study The normal line was crossed with the LPA line to produce the F1 generation. The F1 was backcrossed to the recurrent parent (P 16) to produce the BC1F1 generation. The BC1F1 generation was planted in pots in the greenhouse and backcrossed to the recurrent parent, with no selection for the *lpa1-1*gene, to generate the BC2F1 generation. Conventional pedigree selection was applied to generate F8 lines out of the population. These lines were evaluated for phytic acid content. The top 10 lines were intermated to develop a low phytic acid synthetic in Table 4.3, while the bottom 10 in the variation of phytic acid Figure 4.1 were intimated to form the high phytic acid synthetic population. Grain from these synthetic bulks were used in this study.

3.3 Methodology

The integrated research method used in this study collected both quantitative and qualitative data (Creswell, 2003). The experimental and exploratory research designs were applied. The data collected were used for triangulation (Creswell & Clark 2007). Quantitative methods were used to assess the chemical composition (phytic acid and nutrient content) of grains of low phytic acid (LPA) maize lines and the white type (control) (white maize which had not been genetically manipulated by breeding). Similarly, the chemical composition of maize food processed by traditional methods and corresponding controls was quantitatively measured.On the other hand, quantitative methods were to collect narrative and non–ratio data, including utilization patterns, and consumer perceptions and acceptance of LPA The three traditional methods for processing were selected in this study. The selection was based on several authors reporting on the benefits of using these methods (Osman& Gassem, 2013; Pant et al., 2015 & Stahl, 2014). The three most commonly used methods for maize processing were identified through the Rapid Rural Appraisal (RRA).

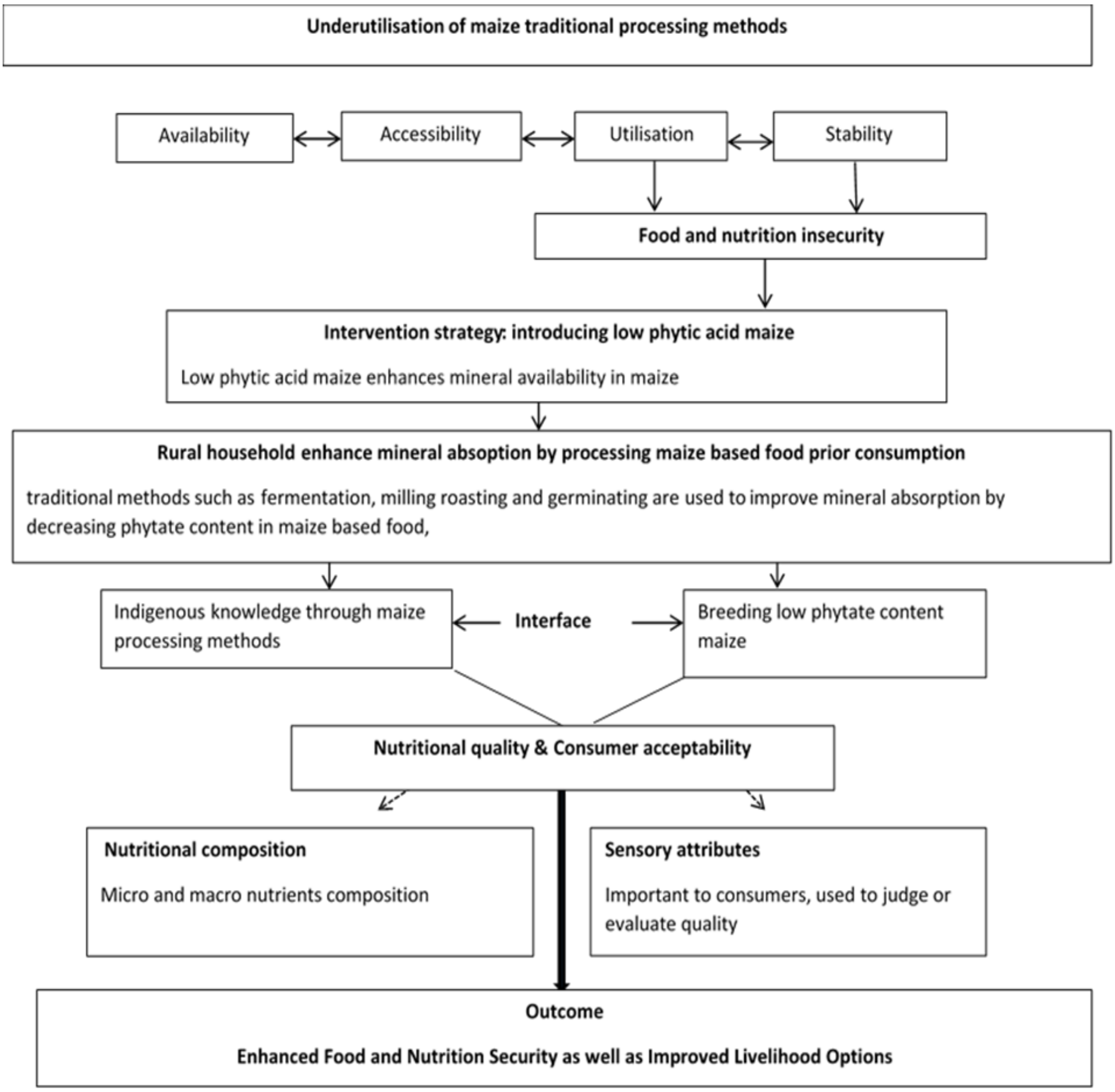

Figure 3.2: Conceptual framework

3.4 References

Creswell, J.W., 2003. A framework for design. *Research design: Qualitative, quantitative, and mixed methods approaches*, pp. 9-11.

FAO. 2010. The State of Food Insecurity in the World: Addressing food insecurity in protractedcrises."Viewed11October2015.http://www.fao.org/docrep/013/i1683e/i1683e.pdf.

IDP 2011. *Integrated Development Plan final report. Ntambanana Municipality*. Viewed 11October,2015.http://mfma.treasury.gov.za/Documents/01.%20Integrated%20Development%20Plans/201213/02.%20Local%20municipalities/KZN283%20Ntambanana/KZN283 %20Ntambanana%20IDP%20Final%202012-2013.pdf.

Heymann, H., 1995. Three-day advanced sensory analysis workshop. *Agricultural Research Council (ARC). Irene, Pretoria*.

Osman, M.A. and Gassem, M., 2013. Effects of domestic processing on trypsin inhibitor, phytic, acid, tannins and in vitro protein digestibility of three Sorghum varieties. *Inter J Agric Tech*, *9*, pp.1187-1198.

Pant, S., Kumar, A., Kumar, R., and Singh, M., 2016. Effect of Processing on Phytic PhosphorusContent in Maize. *The Indian Journal of Nutrition and Dietetics*, *53*(1), pp. 99-104.

Obinna-Echem, P. C., Beal, J., and Kuri, V., 2015. Effect of processing method on the mineral content of Nigerian fermented maize infant complementary food–Akamu. *LWT-Food Science and Technology*, *61*(1), pp.145-151.

Ralston, M., Schatz, E., Menken, J., Gómez-Olivé, F.X. and Tollman, S., 2015. Who Benefits—Or Does not—From South Africa's Old Age Pension? Evidence from Characteristics of Rural Pensioners and Non-Pensioners.*International journal of environmental research and public health*, *13*(1), p.85.

Stahl, A., 2011. Plant-food processing: implications for dietary quality. *Foraging and Farming: The evolution of plant exploitation*, *31*, pp. 171.

Tongco, M.D.C., 2007. Purposive sampling as a tool for informant selection.

4 CHAPTER

THE POTENTIAL OF LOW PHYTIC ACID MAIZE TO REDUCE HIDDEN HUNGER: EXPLORING MAIZE UTILISATION PATTERNS AND TRADITIONAL MAIZE PROCESSING METHODS AMONG RURAL HOUSEHOLDS OF NTAMBANANA

4.1 Abstract

Food and nutrition insecurity is a constant challenge despite the fact that around 70% of African households are engaged in agricultural production. Maize is a staple food for many rural households but, it provides limited nutritional value to the diet. This is mainly due to anti-nutritional factors such as phytic acid that limit the availability of micronutrients in maize. The aim of this study was to explore maize utilization patterns and traditional maize processing methods among rural households of Ntambanana, and to develop a new maize variety with lower levels of phytic acid content.

A series of seven focus group discussions were conducted to explore the utilization patterns of white maize and traditional methods used to process the maize. It was thought that some or all of the traditional methods used to process the maize could reduce the phytic acid content of the food products thereof. The focus group discussions were complemented by an experiment of crossbreeding maize varieties with low maize grain phytic acid genes (from the US-temperate line CM32) with a tropically adapted maize inbred line, LP16 (with high grain phytate) to produce a low phytic acid maize variety. The results indicated that maize was a leading staple food for all the households. Predominantly, the households produced maize for own consumption and preferred it to commercial maize, but after their own produced maize had been exhausted; they bought the maize from the markets. The results indicated that a number of traditional methods were used to process maize into food products- the most commonly used traditional methods were milling (14%), fermentation (44%) and making composite foods. The traditional processing methods were used mainly to improve the sensory properties of the maize-based foods rather than for nutritional benefits. Further, the focus group discussions that the traditional processing methods were disappearing due to modernization. A maize grain with reduced phytic acid levels of 1.7-16.2 mg/g compared to 26.2 mg/g phytic acid level in the positive control, CM32, seemed to have the potential to deliver available minerals to target communities that tend to follow monotonous maize-based diets.

4.2 Introduction

Globally, over one billion individuals suffer from hunger and undernourishment, and 265 million of them live in Africa (FAO, 2009). One in four undernourished people are found in Africa (FAO, 2014), an indication that food insecurity remains a serious challenge beyond the Millennium Developmental Goals (MDGs) aimed at combating malnutrition and food insecurity especially among poor rural households. Maize is classified as the third most important crop in the world (Hoisington & Melchinger, 2005) and in Africa; it is ranked second after cassava. In Africa, maize is the most consumed crop because it requires less labour, it is less costly and relatively more available throughout the year as it can be processed, prepared, and consumed in different forms.

On the other hand, micronutrient deficiencies (hidden hunger) continue to pose a threat to food and nutrition security in Africa. Collard and Mackill (2008) state that in Africa hidden hunger does not only lie on inadequate food intake or limited food availability; rather, hidden hunger has more to do with the nutritional quality of the available food. Most rural households in Africa rely on maize as a staple food source, but maize has limited nutritional quality. For example, the availability of micronutrients in maize is limited by anti-nutritional factors such as phytic acid. The phytic acid found in maize grain is known to bind the micronutrients, especially divalent metal ions, and thereby reducing their bioavailability (Tanumihardjo et al., 2008; Bouis & Welch 2010).

Thus, Malnutrition in the form of hidden hunger in most rural sub-Saharan African households is significantly partly due to use of predominantly maize-based diets, which have high levels of phytic acid (Hotz & Gibson, 2007). The problem of hidden is worsened by the fact that the diets of these households are of little diversity largely because the majority of these households are of low economic status. The low-income rural households that are very vulnerable to hidden hunger, especially children under six and pregnant women. Because hidden hunger is invisible or intangibility, affected communities are less easily alerted to take remedial action. Hidden hunger is thus a threat to food and nutrition security beyond the Millennium Developmental Goals (MDGs).

The literature shows that while agriculture has been focusing on making food available (quantity), the matter of the nutritional value (quality) of the food being produced has been overlooked (Drewnowski & Specter, 2004). While the nutrient-enhancer interventions such as supplementation and fortification have made a significant positive nutritional impact, poor rural households still suffer from hidden hunger. Research shows that these interventions are not cost-effective and usually do not reach rural households. Generally, rural households rely mainly on their own agricultural production for food and nutrition security. Scientific methods of combating hidden hunger, such a genetic biofortification, which is the breeding of staple food crops for higher micronutrient contents, and marker-assisted selection (Repo-Carrasco-Valencia et al., 2009) have been rigorously conducted. However, there are still gaps in the bioavailability of minerals, including zinc (Zn) and iron (Fe), in maize grain. Thus, this study investigated the effect of genetic manipulation by conventional breeding on the phytic acid content of the white maize grain.

Furthermore, it is documented in the literature that various traditional maize processing methods used by rural households enhance micronutrient availability and decrease the anti-nutritional factors, such as phytic acid (Raes et al., 2014). These traditional methods of processing maize include thermal and mechanical processing, fermentation, soaking and germination (Smil, 2000). Nevertheless, there is no clear evidence from the literature whether these methods are still used by the rural households, and if they are intentionally practiced to lower phytic acid in maize-based foods. Therefore, it is also the aim of this study to investigate whether consumers in the study area still use the said traditional methods of maize processing and to assess whether they are aware that the traditional methods could be enhancing mineral bioavailability.

4.3 Research method

4.3.1 Description of the study area

This study was conducted in Ntambanana local municipality that is one of the six local municipalities in UMkhanyakude District of KwaZulu-Natal province in South Africa. According to Ntambanana IDP 2013/14, Ntambanana community is dominated by small scale and subsistence farming activities with 36.7% of the households being headed by the pensioners depending on governmental social grant for survival (Ntambanana Municipality IDP, 2010/2011).

4.3.2 Research design, and materials and methods

An integrated research method was applied using explorative and experimental research designs. Qualitative data were collected through explorative methods, participatory rural appraisal (PRA) and Focus Group Discussions (FGDs), whilst quantitative data were collected largely by the scientific method of experimentation.

A participatory rural appraisal (PRA) research approach was used as an explorative research to explore maize utilization patterns and traditional methods used to process maize. Seven focus groups composed of maize producers and consumers participated in-depth discussions about maize utilization patterns and traditional methods used to process maize. A Focus Group Discussion (FGD) Guide was used to gain insight into sources of maize grain for household consumption, how often and in what food forms the maize was consumed. The FGDs also probed the participating household members on the types of traditional methods used to process maize grain as well as the reasons for their use.

Naidoo et al., (2012) to develop a maize grain with reduced phytic acid content, did a prior experiment. New varieties with low phytic acid genes (from the US-temperate line CM32) were bred into a tropically adapted maize inbred line, LP16 (with high grain phytate). Molecular marker-assisted selection was used to identify recombinant F5 lines with lpa1-1 genes after four cycles of conventional pedigree selection to enhance adaptive traits. Plant tissues were sampled from this set, and the parents' DNA was extracted. To detect the lpa1-1 gene, the Rotor-Gene 6000 real time rotary analyzer was used as the wild type standard (homozygous dominant) and CM32 as the lpa1-1 homozygous recessive standard genotype. The F5 lines and their testcrosses were evaluated for yield and agronomic traits at two sites. Progeny lines were tested for phytate levels using a colorimetric method.

4.4 Results

4.4.1 Demographic characteristics of the study participants

The participants' ages ranged between 20 and 80 years with an average of 5 years' experience in farming maize. All the participants at least owned a backyard garden with a size varying between (0.00072m^2 to 2 hectares) and/or fields (1 hectare to 5 hectares). About 50%-75% of both gardens and fields land was dedicated to maize, a quarter to *Colocasia esculenta* (amadumbe) and other vegetables. The backyard gardens were mainly used as the household's source of food while the fields were used to generate income through the corporative groups.

4.4.2 Maize utilization patterns

Maize was the staple food for all the households. It was planted twice in a year, in September and December. Most households relied on their own grown maize from December to May. From June and July, the households mixed their own grown maize with the commercial maize (fortified maize) even though they mainly purchased commercial maize meal around September to November (Table 4.1).

Table 4.1: The maize calendar

	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec**
Planting									X			X
Harvesting			X									X
Consumption	X	X	X	X	X	X	X					X
Maize Purchasing						X	X	X	X	X	X	

The focus group discussions highlighted that the participants preferred their own grown maize to the fortified commercial maize- the fortified maize was mainly used as a *'supplement'* during pre-harvest periods. It was noteworthy that there was a difference in opinion between the older and younger household members about the use of own grown maize and commercial maize. The younger generation mentioned that the own maize was labour-intensive as it required pre-processing by the household, including milling, before preparing food.

'ukugaya izinto zogogo' (meaning, *milling its backward*)

And,

'Makunendlela engcono yini pho singasebenzisi yona' (meaning *if there is a better way why not use it*)

Although younger generation less preferred consumption of own grown maize, there was consensus on some issues, including that the own grown maize was highly sensorially acceptable across all generations and all participants perceived it to be a cost-effective food source. Own grown maize was said to be 'more filling'. Additionally, it was seen as healthier than the fortified commercial maize; the own grown maize as believed to be *chemical free.*

4.4.3 Traditional methods of processing maize

The participants used several traditional methods for processing and preparing maize maize-based foods/dishes. However, the main reasons for using these methods were to improve the sensory attributes of the maize-based dishes and to preserve cultural heritage.

The study participants said,

"*Yisiko lethu esalishiyelwa ngobabomkhulu* (it is our tradition shared with us by our grandparents)*; kubamnandi kunambitheke futhi kudleka ngezindlela eziningi ezahlukene* (it improves the flavour, taste and give variety)*"*.

The participatory rural appraisal (PRA) showed that the most commonly used traditional processing methods used for processing and preparation of maize-based foods were milling, fermentation and making composite foods. The focus group discussions revealed that the older generation household members, particularly women (Table 4.2), mainly used the traditional methods for processing and preparing maize-based foods.

Table 4.2: Traditional methods for processing and preparation of maize-based foods

TPs	Maize-based food	Most used method	Assumed benefits	Key benefit
Soaking	Incwancwa Stambu (Samp)	**	*'The sour taste is nice and it does not spoil faster'* *'Soaking samp makes it to cooks faster but we do this only if we are going to cook it using a stove, cooking on fire it does not need soaking'*	*Aerobacteria*, *Pseudomonas* and also *Bacillus* are activated, they contain phytase which degrades PA
Fermentation	Isinkwa sombila (Corn bread) Amahewu (Fermented maize beverage) Incwancwa	*****	*'The process is good for maturing the food items'*	Activate the phytase enzyme and also *Lactobacillus* which brings down PA level
Milling	Incumbe (Sour maize porridge) Stambu	****	*'It gives maize meal, samp or even maize flour to make different maize dishes'*	The pounding breaks the germ releasing it to be hydrolyzed
Roasting	Izinkobe (Steamed maize kernels)	**	*'Gives a different flavour to the maize kernels'*	Releases the pericarp, and gem is easily accessible
Use as composite foods	Amahiyoyo (beans mixed with maize) Incumbe	****	*'It's enjoyable when these foods are mixed'*	Help enhance the activation of the enzymes that breaks down PA

fairly often ** very often *** always

Table 4.2 represents the maize based food, which are traditionally processed. The community identified the mostly used methods and the assumed benefits. However according to the literature, the column key benefit shows the reactions, which leads to the degradation of phytic acid in traditionally, processed maize.

4.4.4 Maize progeny lines produced by genetic manipulation

The maize samples had 61 progeny lines resulting from crossing CM32 (exotic) and P16 (locally adapted) lines. These lines represented generation 5 (F5). The progenies took both genes from their parental lines; there were both low and high phytic acid lines with a variation in phytic acid level ranging from 0.07mg/g - 115 mg/g.

Table 4.3: Progeny lines containing low phytic acid

Inbred	Phytate (mg/g) = %	Rank
CM-32 (US, temperate)	**27.2 = 0.3**	**Positive Control**
DLPA-1	1.7 = 0.17	1
DLPA-2	7.1= 0.71	2
DLPA-3	7.1= 0.71	3
DLPA-4	10.4= 1.04	4
DLPA-7	11.6= 1.16	5
DLPA-5	11.6= 1.16	6
DLPA-6	11.6=1.16	7
DLPA-8	12.1= 1.21	8
DLPA-9	15.8= 1.58	9
DLPA-10	16.2= 1.62	10
P16 (ZW, tropical)	**62.8= 6.28**	**Negative Control**

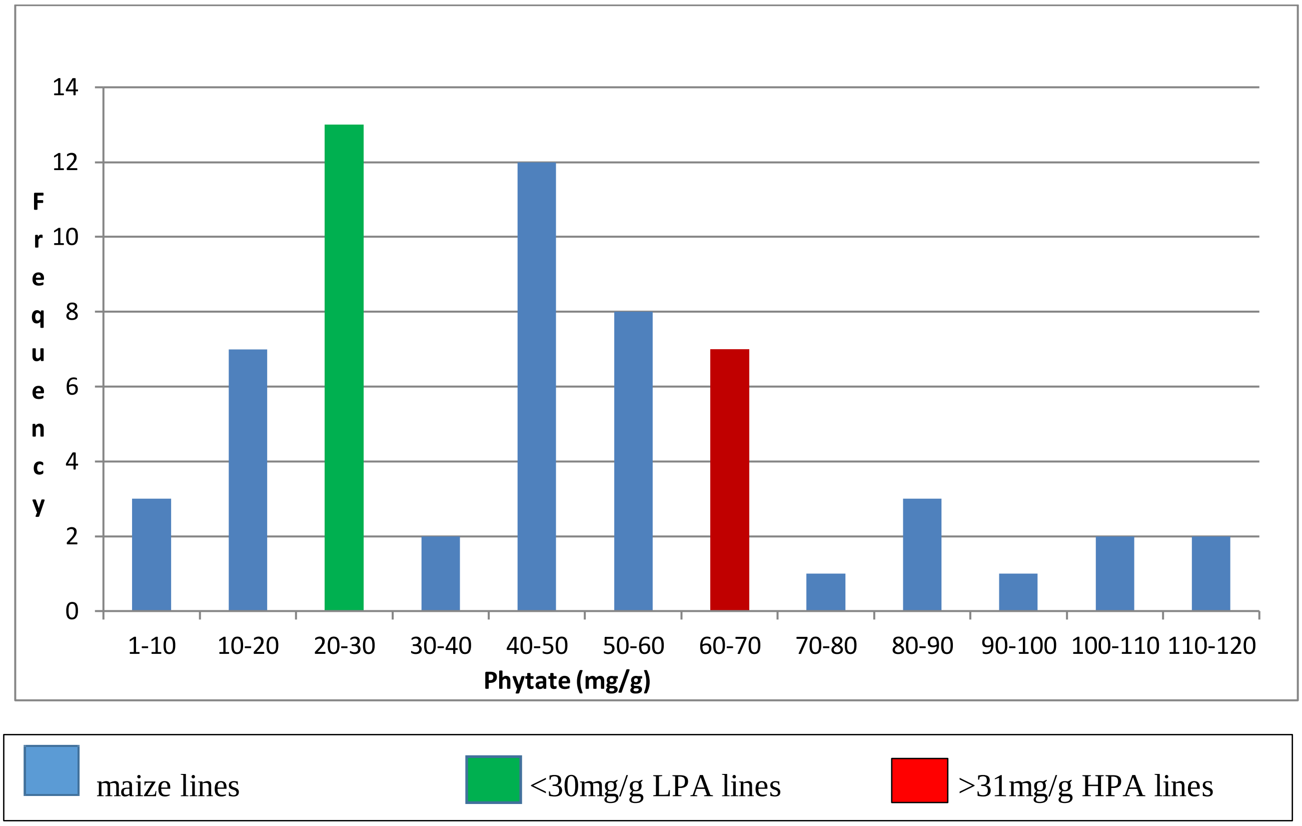

Figure 4.1: Variation in phytic acid (phytate) level across progeny lines

4.4.5 Progeny lines with low phytic acid

Table 4 present the top10 progeny lines with low phytic acid. The phytic acid content ranged from 1.7-16.2 mg/g, which was much lower than the control CM32 (21.7 mg/g).

The LPA with positive progenies ranging between DLPA-2 to DLPA-10 had a reduced phytic acid content showing the potential to supply available micronutrients in the maize grain. The phytate level in a grain should be at a range between 10 mg/g to 25 mg/10 g so that minerals can be available.

4.5 Discussion

All the households planted 50-75% of their gardens and/or fields with maize for use as a staple food. As documented in the literature, 70% of African households engage in agricultural production and the most produced crop is maize (Hoisington and Melchinger, 2005; United Nations Environmental programme, 2007; FAOSTAT, 2010).

Burchi (2011), observed that hidden hunger was most prevalent in communities that were involved in agriculture. As mentioned by Collard and Makill (2008), the problem is not inadequate food intake or limited food availability but the nutritional quality of the food available. The limited understanding and existing perception that maize has a high nutritional value of own grown maize is misleading, especially if the maize is not accompanied by other nutritious foods such as vegetables and meat.

The study participant s had limited knowledge about the nutritional value of processed maize, see Table 4.2. As indicated earlier, traditional methods for processing and preparing maize-based foods were used mainly to improve the sensory attributes of the foods rather than their nutritional value. The participants did not know that the various traditional methods they used, such as fermentation (e.g. *idokwe*) and soaking (e.g. samp) activated the lactic acid bacteria, which contributed to the degradation of phytic acid by providing a suitable condition for the activity of endogenous phytase enzyme (Bilyeu et al., 2008). The limited knowledge about the benefits of using these traditional methods requires attention. Awareness needs to be created involving education in order to promote and enhance the use of these methods. However, it was found that these practices were disappearing. Looking into the future beyond the MDGs, better quality (including low phytic acid) maize varieties should be developed and made available and accessible for better food and nutrition security. The study findings confirm Walingo's (2009) concern about two fundamental factors which continue to be a threat to vulnerable groups even beyond MDGs: firstly, the changing lifestyles which influence production, food preparation, and preservation of food. Secondly, the collapse of indigenous knowledge transfer systems resulting in a decline in- and limited transfer of indigenous knowledge systems (IKS) and traditional practices across generations.

The maize lines with low phytic acid levels developed in this investigation have a potential to be a better food and nutrition security alternative intervention as compared to other interventions. There is a need to replace conventional maize hybrids and varieties grown by smallholder farmers with significantly more nutritious varieties. This is imperative especially for households who mainly rely on agriculture for household food and nutrition security in which the crop nutritional quality could enhance the nutritional plate leading to more active lives and better overall wellbeing. Low phytic acid maize holds a better promise in alleviating hidden hunger and life style diseases. The low phytic acid in maize variety neither affects colour nor the nutritional quality of the seed.

However, agronomic traits and sensory properties still need to be investigated for better adoption and the maximum utilization of the low phytic maize varieties. There is a need to educate the smallholder farmers about suitable maize varieties, the importance of nutrition and fortified foods. Further studies should be done on the acceptance (agronomic traits, consumer perceptions & sensory evaluation) to evaluate their potential for adoption from both an agronomic and end-user (consumer) point of view. Also, consumer awareness and nutritional knowledge should be promoted to empower consumers to make optimal choices when making decisions about selecting and preparing nutritious diets.

4.6 Conclusion

This chapter showed that low phytic acid have been realized. African households still rely on maize for their well-being and with approximately 70% of households consuming maize, the low phytic acid maize can assist in alleviating mineral deficiency. Also the traditional maize processing method have greater benefit, since it is being practiced. This could be a cost-effective and sustainable intervention for improving the food and nutrition status of many rural households.

4.7 References

Beavers, A. W., 2014. *Recurrent selection to alter seed phytic acid content and iron bioavailability in maize.* Master's Thesis, Iowa State University

Bilyeu, K. D., Zeng, P., Coello, P., Zhang, Z. J., Krishnan, H. B., Bailey, A., Beuselinck, P. R. and Polacco, J. C., 2008. Quantitative conversion of phytate to inorganic phosphorus in soybean seeds expressing a bacterial phytase. *Plant Physiology,* 146**,** pp.468-477.

Burchi, F., Fanzo, J. and Frison, E., 2011. The role of food and nutrition system approaches in tackling hidden hunger. *International Journal of Environmental Research And Public Health*, *8*(2), pp.358-373.

Collard, B.C. and Mackill, D.J., 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*,*363*(1491), pp.557-572.

Drewnowski, A. and Specter, S.E., 2004. Poverty and obesity: the role of energy density and energy costs. *The American Journal of Clinical Nutrition*,*79*(1), pp.6-16.

Fardet, A., 2010. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre ?. *Nutrition Research Reviews*,*23*(01), pp.65-134.

Food and Agricultural Organisation. 2009. 1.02 billion people hungry. http://www.fao.org/news/story/en/item/20568/icode/. Viewed 12 November 2014

Food and Agriculture Organization. 2014. 2015 World Hunger and Poverty Facts and statistics. http://www.worldhunger.org/articles/Learn/world%20hunger%20facts%202002.htm . Viewed 27 May 2014.

Gibson, R. S., 2006. "Zinc: the missing link in combating micronutrient malnutrition in developing countries." *Proceedings of the Nutrition Society,* **65**(01), pp. 51-60.

Hoisington, D.A. and Melchinger, A.E., 2004. From theory to practice: Marker-assisted selection in maize. In *Molecular Marker Systems in Plant Breeding and Crop Improvement* pp. 335-352. Springer Berlin Heidelberg.

Hotz, C, and Gibson, R. S., 2007. "Traditional Food-Processing and Preparation Practices to Enhance the Bioavailability of Micronutrients in Plant-Based Diets." *The Journal of Nutrition* 137(4),pp. 1097-1100.

IDP 2011. *Integrated development plan final report. Ntambanana Municipality*. Viewed 11 October,2015.http://mfma.treasury.gov.za/Documents/01.%20Integrated%20Development%20Plans/201213/02.%20Local%20municipalities/KZN283%20Ntambanana/KZN283 %20Ntambanana%20IDP%20Final%202012-2013.pdf.

Tanumihardjo, S.A., Bouis, H., Hotz, C., Meenakshi, J.V. and McClafferty, B., 2008. Biofortification of staple crops: an emerging strategy to combat hidden hunger. *Comp Rev Food Science Food Safety, 7*, pp.329-34.

Smil, V., 2000. Phosphorus in the environment: natural flows and human interferences. *Annual Review of Energy and the Environment*, *25*(1), pp.53-88.

Repo-Carrasco-Valencia, R., Peña, J., Kallio, H. and Salminen, S., 2009. Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (Amaranthus caudatus). *Journal of Cereal Science*, *49*(2), pp.219-224.

United Nation Environmental Programme, 2007. *Food Agricultural and Resource Economics Sustainable Development*. Viewed 28 May 2014. www.eoearth.org

Walingo, M.K., 2009. Indigenous food processing methods that improve zinc absorption and bioavailability of plant diets consumed by the Kenyan population. *African Journal of Food, Agriculture, Nutrition and Development*,*9*(1), pp.523-535.

5 CHAPTER

IMPACT OF SELECTED TRADITIONAL PROCESSING METHODS ON THE PHYTIC ACID CONTENT AND NUTRITIONAL COMPOSITION OF MAIZE

5.1 Abstract

Minerals play an important role in the metabolism and physiological activities of the human body. In the developing world, over three billion people are still malnourished and deficient in minerals. Increasing the contribution of staple foods, including maize, towards mineral intake by humans can be one of the sustainable and cost effective approaches for alleviating mineral deficiencies, a form of hidden hunger. Some traditional processing methods have been reported to reduce the phytic acid content of cereal grains and hence increase their mineral availability. This study assessed the impact of selected traditional processing methods on the phytic acid content and nutritional composition of maize. The results showed that milling maize and fermentation resulted in a decrease in phytic acid content. Fibre content decreased by 49% upon milling the maize grain, which can be attributed to reductions of the germ and seed coat material that have a high content of protein. The results also indicated that boiling samp and whole maize kernels resulted decreases in mineral content, probably due to leaching. However, according to the recommended daily allowance (RDA), the retained minerals would meet the human daily requirements. Therefore, traditional processing methods can work as alternatives in combating iron and zinc deficiency. Moreover, low phytic acid maize cultivar has potential in availing minerals to improve health in rural households.

5.2 Introduction

About 67% of maize is produced in low and middle-income countries where it serves as the main livelihood option for most farmers. The majority of rural communities in sub-Saharan Africa produce and process their own maize. Unlike commercial maize products, for example maize meal (flour), which are fortified with available nutrients, the maize produced and consumed by the rural communities is generally not fortified during processing, except by compositing with other ingredients, especially legumes. Of more concern, is the limited availability of nutrients in the maize due to the effects of anti-nutritional factors, for example, mineral availability is limited by the binding effects of phytic acid. Thus, subsistence farmers, especially in the sub-Saharan region, still have a high rate of mineral deficiencies otherwise known as hidden hunger, a condition to which maize has partial influence because of its high phytic acid content (Kumar et al., 2010). Phytic acid content in unprocessed cereal grains ranges from 0.1% to 2.2%; 0.2% to 2.9% in legumes; and 1% to 5% in oilseeds (Chen, 2004; Lestienne et al., 2005).

However, some traditional methods of processing maize grain, including fermentation, soaking and milling, have been reported to reduce the negative effects of phytic acid on mineral availability (Smil, 2000). Different mechanisms have been proposed for the reduction of the nutritionally negative effects of phytic acid- reduction in phytic content due to different mechanisms, such as leaching and enzymatic degradation have been often proposed. For example, a study done by Fredlund (1997) showed that traditional processing methods had the potential to decrease phytate in cereal grains such as wheat, barley, oats and rye- phytate content was decreased by 46%-77% upon traditional processing (milling and soaking) of wheat, rye and barley. Oats registered the least reduction of phytic acid, 8%-26% phytic acid after milling, but with an improvement of 72% and 77% from milling and soaking. However, due to variability in these traditional methods, there is a need to investigate case by case the supposed positive effects of the traditional methods on the mineral availability and/or phytic acid reduction in the cereal grains, including maize. Furthermore, the traditional processing methods may result in changes in the broader nutritional composition of the cereal grain, such as maize. Therefore, the aim of this study was to assess the impact of selected traditional processing methods, milling, fermentation, and boiling, on the phytic acid content and nutritional composition of maize.

5.3 Research methodology

5.3.1 Research technique and Sampling technique

A quantitative research method was used based experimental design. The criteria of choosing the way of processing maize in this study was obtained from the Rapid Rural Appraisal. Key informants helped identify households which had processing equipment and which still practiced the traditional processing methods of maize.

5.3.2 Preparation of processed maize sample

Grain of low and high phytic acid maize varieties were milled into mealie meal (maize flour) at the University of KwaZulu-Natal using a milling machine. For Samp preparation, the Ntambanana participants used to mill both LPA and HPA maize supplied a traditional wooden pounder. The milled products were used to prepare popular South African food products: Figure 5.3, porridge (idokwe) made from mealie meal, Figure 5.2, samp (isitambu) and Figure 5.1, boiled maize (izinkobe).

Figure 5.1: Boiled maize grains (izinkobe)

Figure 5.2: Cooked Samp

Figure 5.3: Maize product (idokwe) processed by fermentation

5.3.3 Determination of phytic acid content

Phytic acid of raw and cooked traditionally processed maize were determined using a procedure of Lucas and Markakas (1975). Exactly 2.0 g of the sample were weighed into a 250 mL conical flask. A volume of 100 mL of 2% concentrated HCl was used to soak the sample for 3 hours and then filtered with a Whatman No. 1 filter paper. Accurately, 30 ml of 0.3% ammonium thiocyanate solution were added into the solution as indicated and titrated with standard Iron II Chloride solution containing 0.00195 g iron/mL. The end point was observed to be yellow which persisted for 5 min. The percentage phytic acid was calculated using the formula given below.

% Phytic acid = y × 1.19 × 100; where, y = titre value × 0.00195 g of the filtrate

The nutritional analysis and the phytic acid determination of the maize samples was done in duplicate using standard methods to ensure quality control at various steps.

Ntambanana participants prepared four homogenized meals of different compositions. The samples were taken to the laboratory and freeze dried immediately

5.4 Nutritional analysis

Using standard methods, the maize samples were analyzed for their protein, fat, NDF (Neutral detergent fibre), total mineral (ash) and individual mineral contents. A brief description of how each of the nutrients was determined is given below:

5.4.1 Protein

The protein content was determined by Dumas combustion method (AOAC official method 968.06) (AOAC 2002)

5.4.2 Fat

The fat content was analyzed using Soxhlet procedure following AOAC official method 920.39 (AOAC, 2002).

5.4.3 Neutral detergent fibre (NDF)

The fibre content was determined as the neutral detergent fibre (NDF). The NDF was determined following AOAC official method 2002.04 (AOAC 2002).

5.4.4 Total mineral content

The ash was measured according to the AOAC official method 942.05 (AOAC, 2003).

5.4.5 Individual minerals

The calcium magnesium manganese, zinc, iron, sodium, potassium, copper and phosphorus were analyzed following the AOAC Method 6.1.2 (AOAC, 1984).

5.5 Data analysis

The statistical package for Social Science (IBM SPSS) version 23 was used to generate means and standard deviations that were calculated from the duplicate nutrient values. The Dunnet test was used to determine whether there was a significant difference in the nutritional composition of traditional maize sample with maize grain (landrace) as the reference. A p-value of <0.05 was regarded as being statistically significant.

5.6 Results and discussion

5.6.1 Effect of traditional processing methods on the nutritional composition of white maize

The commonly consumed maize-based foods were prepared with the traditional (landrace) white maize and were analyzed for nutritional composition. The nutritional composition of the processed maize products is shown in Tables 5.1.

Table 5.1: Effect of traditional processing methods on the macronutrient composition of white maize

Samples	Mean± Standard deviation Nutrient content on dry matter basis Protein	Fat	NDF
Raw maize grain (control)	10.57 ± 0.18	3.79 ± 0.76	14.48 ±1.00
Cooked samp	**9.57**± 0.11	**11.68** ± 0.52	**9.86** ± 0.007
Cooked fermented maize meal (fermented porridge)	**9.01** ± 0.28	**16.59** ± 0.52	**7.90** ± 0.09
Boiled maize grain	10.19 ± 0.24	**9.33** ± 0.47	14.29 ±0.94

Mean values in bold are significantly different from those of the control (Dunnett test p<0.05)

The protein content of the fermented porridge and the cooked samp was significantly low when compared with the control (raw maize grain). The boiled maize grain had a similar protein content to the control. The protein content decreased in the fermented porridge and the cooked samp probably partly due to utilization by the fermentative microorganisms (Taiwo 2009).

The cooked fermented maize porridge, cooked samp and boiled maize grain had higher fat content compared to the control. An apparent increase of fat content might be due to the cooking process which causes minerals to leach and fat compensate for mineral loss (Lestienne et al., 2005).

The fibre content of the reference maize grain was similar to that of boiled maize, however, the cooked samp and cooked fermented porridge had significantly lower fibre contents. The decrease of fibre might be due to the losses of seed coat during milling (Nuss & Tanumihardjo, 2010). The mineral content of maize grain (control) compared to traditional processed is shown in Table 5.2

Table 5.2: Effect of traditional processing methods on the mineral content of white maize

Samples	Mean ± Standard deviation mg/kg iron	zinc	Ash (g/100 g)
Maize meal (**control**)	21.00 ± 0.00	22.00 ± 1.41	1.35 ± 0.31
Cooked samp	**13.89** ± 1.61	**12.81** ± 0.08	1.47 ± 0.17
Cooked fermented maize meal (fermented porridge)	24.84 ± 1.62	**19.43** ± 0.07	1.12 ± 0.23
Boiled maize kernels	17.83 ± 1.49	**18.88** ± 0.01	**2.07** ± 0.07

Data reported as means of two replicates ± standard deviation.

Mean values in bold are significantly different from those of the control (Dunnett test $p<0.05$)

The iron content was higher in fermented porridge compared to the control. The zinc content was significantly low in all the sample but the cooked samp had the lowest iron and zinc content. This might be because soaking unrefined maize reduces iron by approximately 20% (Hotz & Gibson 2001). Although the iron and zinc decreased, it is still adequate according to the RDA (Recommended Dietary Requirement) for these minerals (Institute of Medicine 2001). The ash content in the boiled maize sample was significantly high compared to the control. The fermented porridge ash content was significantly low. This might be due to the decrease in relative moisture content in maize during drying that led to decrease of ash content (Carvalho et al., 2004)

5.6.2 Effect of traditional processing methods on the phytic acid content of maize

The traditionally processed samples were tested for phytic acid content in comparison with the low phytic acid grain, which had undergone conventional breeding.

The Table 5.3 and Figure 5.4 shows the multiple comparison of the treatments against the control

Table 5.3: Effect of traditional processing methods on the phytic acid content of white maize

Sample	**Mean** Phytic acid content (mg/g, dry basis)
LPA maize **(reference**)	0.600[a]
Boiled maize kernels	0.710[ab]
Uncooked fermented meal	0.785[ab]
Uncooked and non-fermented maize meal	0.805[ab]
Cooked porridge Cooked fermented maize meal (fermented porridge	0.930[bc]
Uncooked samp	1.010[bc]
Cooked samp	1.170[c]

Different letters within the same column denote that there was significant difference between the means of the samples. According to Tukey test where $p<0.05$

The phytic acid content was significantly different in all samples. The low phytic acid maize showed no significant difference in phytic acid content when compared with cooked fermented porridge and uncooked fermented maize meal. This might be because during fermentation the microorganism (*Bacillus. sp*) breaks produces the phytase enzyme which hydrolyses phytic acid (Greffeuille et al., 2011). The LPA maize sample was significantly different to the uncooked maize kernel. All the cooked samples showed no significant difference with each other was significantly different with low phytic acid reference sample. The milling showed no significant difference with reference sample, which might be due to the fact that milling can reduce phytic acid content (Hotz & Gibson 2007).

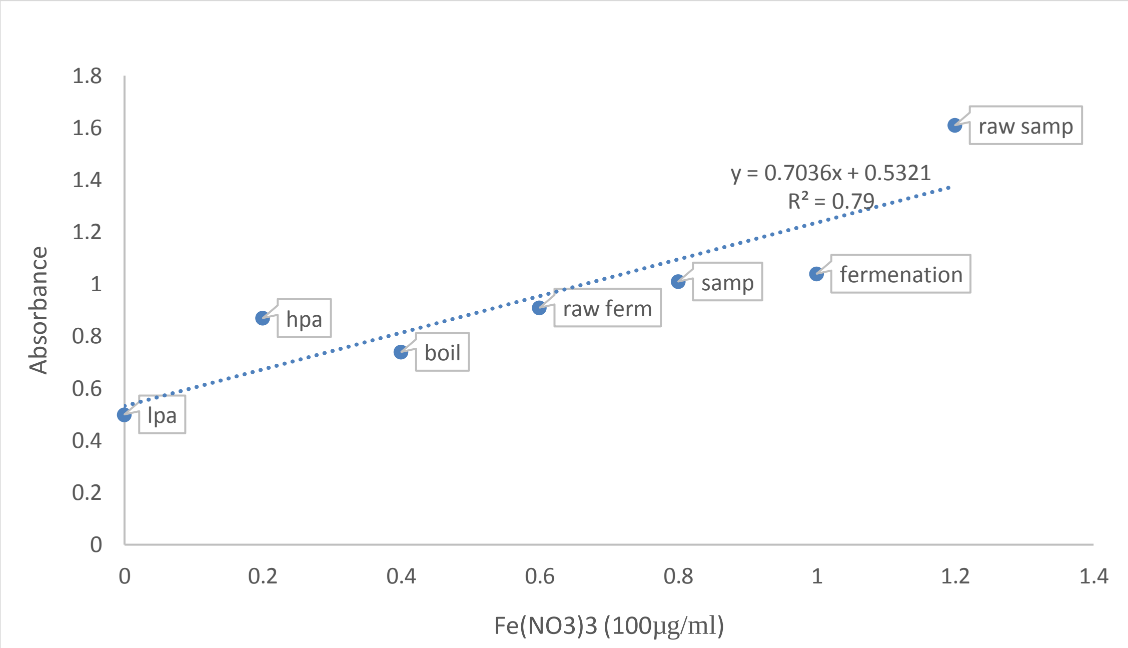

Figure 5.4 Traditionally processed maize samples compared with LPA maize meal (Control)

Low phytic acid content was significantly different among all the maize samples. The loss of phytate grain was between 41% - 74% in the samples processed by traditional methods. This was supported by Kumari (2015) who after soaking soybeans, found phytate to range from 46% - 65%, which increased the extractability of minerals

These results show the potential benefits of using traditional processing methods to reduce the phytic acid content. These methods can be recommended to improve food and nutrition security in rural households.

Iron increased by 14% in fermentation processes though zinc decreased. The significantly higher concentration of iron in the fermentation sample could be due to the hydrolytic activities of the various endogenous microbes such as *Lactococcus lactis* towards phytate (Greffeuille et al., 2011). Iron and zinc in samp and boiled samples decreased by 38% and 14% respectively: a phenomenon which may be due to the leaching effect. Moreover, higher decreases in zinc could be because zinc and iron are not located in the same place in the seeds and are not linked with the same molecules. Lestienne et al., 2005 similarly observed this after soaking grains iron and zinc leached, but zinc was higher in soaked maize because it bounds to enzymes and proteins for structural role. The fermentation process was therefore favorable for iron increases and could be recommended as a processing method for higher iron availability.

5.7 Conclusion

This study shows that various processing methods have potential in lowering phytic acid in maize towards improving the availability of minerals. Even though some of the processing methods decreased the level of minerals, nutritional levels still qualified as per the RDA of minerals. Encouraging these methods could thus assist developing countries for a different strategy to improve mineral availability. The samp and boiled maize were lower in iron compared to white maize meal however, fermentation increased iron by 14%. This means fermentation process can be recommended for improving iron availability in rural households.

5.8 References

Association of official Analytical Chemists (AOAC) 1984. *Official Methods of Analysis*, 14th ed. Association of Official Analytical Chemists. Arlington, Virginia.

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

Association of official Analytical Chemists (AOAC) International 2003. *Official Methods of Analysis AOAC International*, 17th ed. AOAC I international: Gaithersburg, M, USA.

Carvalho, D.C.D.O., Albino, L.F.T., Rostagno, H.S., Oliveira, J.E.D., Vargas Júnior, J.G.D., Toledo, R.S., Costa, C.H.R., Pinheiro, S.R.F. and Souza, R.M.D., 2004. Chemical and energy composition of corn sample under different drying temperature and storage periods. *Revista Brasileira de Zootecnia*, *33*(2), pp.358-364.

Chen, F., Dong, J., Wang, F., Wu, F., Zhang, G., Li, G., Chen, Z., Chen, J. and Wei, K., 2007. Identification of barley genotypes with low grain Cd accumulation and its interaction with four microelements. *Chemosphere*, *67*(10), pp.2082-2088.

Fredlund K., Asp N.G., Larsson M., Marklinder I., and Sandberg A.S. 1997. Phytate reduction in whole grains of wheat, rye, barley and oats after hydrothermal treatment. *Journal of Cereal Science*, 25(1), pp. 83-91.

Greffeuille V., Kayodé A.P., Icard-Vernière C., Gnimadi M., Rochette I., and Mouquet-Rivier C., 2011. Changes in iron, zinc and chelating agents during traditional African processing of maize: effect of iron contamination on bioaccessibility. *Food Chemistry*, 126(4), pp. 1800-7.

HarvestPlus Brief, 2006. HarvestPlus: Breeding Crops for Better Nutrition. Washington DC

Hotz, C. and Gibson, R.S., 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *The Journal of Nutrition*, *137*(4), pp.1097-1100.

Hotz, C. and Gibson, R.S., 2001. Assessment of home-based processing methods to reduce the phytate content and phytate/zinc molar ratio of white maize (Zea mays). *Journal of Agricultural and Food Chemistry*, *49*(2), pp.692-698.

Kumari, S., Krishnan, V., Jolly, M., and Sachdev, A., 2015. Reduction in phytate levels and HCl- extractability of divalent cations in soybean (Glycine max L.) during soaking and germination. *Indian Journal of Plant Physiology*, *20*(1), pp. 44-49.

Kumar, V., Sinha, A. K., Makkar, H. P., and Becker, K., 2010. Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, *120*(4), pp. 945-959.

Lestienne I, Icard-Vernière C, Mouquet C, Picq C, and Trèche, S., 2005. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chemistry*. 89(3), pp. 421-5.

Lucas GM, Markakas P., 1975 Phytic acid and other phosphorus compounds of bean (Phaseolus vulgaris) *Journal of Agriculture and Food Chemistry*.;23:13-5

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

Pinstrup-Andersen, P. (2013). Guiding food system policies for better nutrition. *Background paper for the State of Food and Agriculture*.

Shiferaw, B., Prasanna, B.M., Hellin, J. and Bänziger, M., 2011. Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, *3*(3), pp.307-327.

Singh, U., Praharaj, C. S., Chaturvedi, S. K., and Bohra, A., 2016. Biofortification: Introduction, Approaches, Limitations, and Challenges. In *Biofortification of Food Crops* (pp. 3-18). Springer India.

Smil, V., 2000. Phosphorus in the environment: natural flows and human interferences. *Annual Review of Energy and the Environment*, *25*(1), pp.53-88.

Stahl, A., 2014. 11 Plant-food processing: implications for dietary quality. *Foraging and Farming: The Evolution of Plant Exploitation*, *31*, p.171.

Taiwo, O., 2009. Physical and nutritive properties of fermented cereal foods. *African Journal of Food Science*, *3*(2), pp.023-027.

Weingärtner, L., 2005. The concept of food and nutrition security. *Achieving Food and Nutrition Security*, *3*, pp.21-52.

6 CHAPTER

FOOD SECURITY STATUS OF NTAMBANANA HOUSEHOLDS, THEIR CONSUMPTION OF TRADITIONALLY PROCESSED MAIZE AND ACCEPTANCE OF LOW PHYTIC ACID MAIZE

6.1 Abstract

Food and nutrition insecurity is huge challenge in the sub-Saharan African countries, including South Africa. The most affected population groups are the resource-poor rural communities who rely largely on small-scale agriculture and traditional agro-processing methods for their food and income High phytic acid (HPA) maize grain (normal white maize) is a leading staple with sub-Saharan Africa, yet, the minerals in the grain are less available due to the anti-nutritional activity of phytic acid. Low phytic acid maize (LPA) grain produced through conventional breeding may contribute towards alleviating mineral deficiencies, which are prevalent in most of the Sub-Saharan African countries, including South Africa. Previous studies have mostly reported on the yielding potential, drought tolerance, and shelf-life potential of agricultural produce. However, less effort has been given to the effects of the breeding process on the sensory properties of the produce.

A systematic random sample of 77 households completed a survey questionnaires administered to determine the household food security status (HFIAS) and utilization of traditionally processed maize-based foods. A consumer sample of 57 panelists composed of both males and females evaluated the acceptability of popular traditionally processed maize-based traditional foods (fermented porridge, samp and boiled maize kernels) prepared with low phytic acid maize compared with corresponding controls made with white maize.

The findings of the survey showed that 47.7% households were severely food insecure and 26.6% were moderately food insecure with women being the most affected. It was found that the households had limited dietary diversity. About 59% of the households preferred and consumed their own grown maize as opposed to buying the commercial maize. They processed the maize into different dishes using traditional methods, including boiling maize kernels, and fermenting maize and then cooking it into porridges and beverages. Sensory

evaluation results showed that the overall acceptability of all the food samples prepared with HPA and LPA maize, separately, was high and similar, although the samples made with LPA maize showed slightly higher overall acceptability. Overall, these results suggest that HPA maize can be replaced with LPA maize to make a significant contribution towards alleviating mineral deficiencies among rural households who predominantly consume maize as their leading staple food with little dietary diversification.

6.2 Introduction

Diets of most rural households in the sub-Saharan African countries have low diversification and are mostly white maize-based. Maize contributes to over 60% of energy, iron, zinc, riboflavin and half of total protein total intake when animal food sources are scarce (Ecker & Qaim 2011). However, the bioavailability of minerals in maize is low due to the presence of the anti-nutritional factor, phytic acid (Nuss & Tanumihardjo, 2010). Iron deficiency (which can lead to anemia) remains a problem affecting the health and productivity of adults, leading to cognitive impairment in infants and young children. Similarly, zinc deficiency leads to retarded skeletal development and immunodeficiency disorders (Rwegerera et al., 2015; Lachat et al., 2006). In South Africa, about 24.1% of pre-school children and 50% of pregnant women suffer from anaemia. This shows the need for an effective and easily accessible intervention that can alleviate micronutrient malnutrition in rural households (Sirdah et al., 2014).

South Africa has introduced interventions addressing micronutrient deficiencies through food fortification, supplementation and dietary diversification (Burchi et al., 2011). Information on the impact of these interventions is limited, especially on pregnant women and children (Darnton, Hill & Mkparu, 2015). In spite of the South African legislation on the fortification of maize meal since 2003, rural households continue to rely on own produced maize. This is in part, due to the poor access of rural households located in remote areas to fortified commercial maize. Introducing maize with low phytic acid content produced by conventional breeding could form part of a long-term sustainable intervention to address food and nutrition insecurity among rural households. Conventional breeding of staple crops with dense mineral content would provide the rural farmers with a chance of accessing high nutrient maize without relying on purchasing fortified food products (Nestel et al., 2006).

Biofortification of maize has been introduced by CIMMYT and Harvest Plus to increase levels of pro-vitamin A, zinc and iron to combat micronutrient deficiencies in rural areas. However, the consumer acceptability of provitamin A-biofortified maize staples has been found low (Meenkshi et al., 2010). For example, it was reported that consumers did not easily accept the resultant colour change in maize, and as shown by de Groote (2011), consumers from South Africa were willing to pay 40% to 50% in higher prices for premium white maize flour compared to the yellow/orange provitamin A-biofortified maize. Therefore, evaluation of the end-user quality of maize produced by conventional breeding should including assessment of its consumer acceptability.

Several studies indicate that traditional maize processing methods lower phytic acid (chapter 5). The most popularly recognized traditional processing methods for maize include fermentation, malting, milling, heat treatment, and germination. However, as reported previously (Chapter 5), the utilization of these methods is decreasing due to the limited knowledge of the benefits of these methods beyond food preservation and sensory enhancing properties. Apart from the primarily desired lactic fermentation (souring) of maize, several other biochemical processes occur, these include the release of the enzyme phytase by microorganisms, notably Bacillus spp. (Sokrab ,2012), which hydrolyze phytic acid in the maize grain and thereby release the minerals making them more bioavailable to the human body. Sokrab (2014) compared the mineral availability of high phytic and low phytic acid maize varieties. In the study, a low phytic acid maize sample was produced by prolonged (2-14 hours) germination and fermentation. The low phytic acid maize sample had high iron extraction capacity which increased bioavailability compared to the high phytic acid maize (control). Moreover, Sharma (2015) was able to extract 25%-36% of iron and zinc after soaking and germinating soya-beans, meaning that traditional practices do have the potential in availing minerals for absorption.

White maize grain is generally the grain of choice for the consumers in sub-Saharan Africa. However, with the introduction of the genetically modified maize, consumers have become increasingly alert to the different qualities in the maize product (De Groote & Kimenju, 2008). There is thus a need to assess if the genetic manipulation of maize through conventional breeding and/or rDNA technology affects the sensory properties of maize based food products.

Low phytic acid maize could be a cost-effective and a less labour intensive food and nutrition intervention to combat hidden hunger (a form of micronutrient deficiency), especially among the rural poor households. The aim of this assess the food security status of households in Ntambanana and evaluate consumer acceptability of popular traditional maize foods prepared with low phytic acid (LPA) produced by conventional breeding.

6.3 Research methodology

A mixed methodology was used for the study involving both quantitative and qualitative methods. A survey using questionnaires and sensory evaluation explored the research problem under study. The questionnaires explored the consumption and utilization of own produced maize and the utilization of traditional methods to process the maize into popular maize-based foods. The household food security status (HFIAS) score and the 24-hour recall were used to assess the food security status and food consumption pattern, respectively, of the households.

A five-point pictorial hedonic scale was used to evaluate the consumer acceptability of porridge(idokwe), samp (istambu) and boiled maize kernels (izinkobe) prepared with low phytic acid (LPA) maize compared to corresponding controls made with high phytic acid (HPA) maize. The pictorial hedonic scale of sensory evaluation questionnaire asked panelists to indicate whether they: disliked very much (score 1), disliked slightly (2), neither liked nor disliked (3), liked slightly (4) and liked very much (score 5) the colour, aroma, texture, taste and overall acceptability, respectively, of each sample. A pictorial hedonic scale was found to be appropriate because illiterate and semi-illiterate consumers compared to the traditional 9-point hedonic scale can understand it easier.

6.3.1 Sampling technique

A total of 77 household representatives from the two selected villages (Bhucanana and Luwamba) in Ntambanana participated in the survey. The two participating villages were purposively selected for the survey based on the information provided by an extension officer that they were the most active in maize production in the community of Ntambanana. A systematic random sampling (including every third household) was applied to select study participants within the two selected villages. In cases where the selected household did not plant maize, the next household was selected. A total of 57 adults, including males and females were recruited randomly to participate in sensory evaluation.

6.3.2 Research Procedure

Survey

A questionnaire in IsiZulu, the local vernacular language, was administered with the help of trained field workers who were members of the local community and spoke the local language. The field workers also assisted illiterate respondents to fill out the questionnaires.

6.3.3 Sensory evaluation

Sensory evaluation panelists

As already indicated, 57 panelists from Ntambanana participated in sensory evaluation. The sensory panelists were recruited using letters distributed at a school. The letters were sent selectively to maize-producing parents of the schoolchildren.

Maize varieties

The maize varieties used in this study were produced using backcrossing at Ukulinga farm. LP16 is an elite maize grain variety in Southern Africa, it acted as a recurrent plant. The low phytic acid (LPA) maize varieties were the test, whilst the high phytic (HPA) maize varieties acted as the control. The maize varieties were harvested manually and left to dry (±25^{0}C) for 20 days. The maize was then threshed manually; it was bulked and stored in a cold room (±4^{0}C) before milling. A grain sample of 3 kg each was milled and 1 kg was made for samp using a traditional pounder with 1 kg left over. In all 5 kg of both LPA and HPA, maize varieties were used to prepare food samples.

6.3.4 Preparation of samples of maize foods

Non-fermented porridge

The thin porridge was prepared by bringing 3000 ml of tap water to a boil and then adding 488g of maize meal to 1000ml of cold water to make a paste. This paste was then added into the boiling water and stirred until it was smooth. The porridge was cooked on medium heat for 40 minutes with the lid on and occasional stirring. After the porridge was cooked, it was cooled at room temperature and 20g of sugar was added.

Fermented porridge

The thin porridge was prepared by bringing 3000 ml of tap water to boil, and adding 488 g of maize meal to 1000 ml of cold water to make a paste, which was also added to the boiling water and stirred until it was smooth. The porridge was cooked on medium to high heat for 40 minutes with the lid on with occasional stirring. After the porridge was properly cooked, it was left cool at room temperature and 20 g of sugar added. The porridge it was placed in a closed container and left to stand for 48 hours in a dark storage at room temperature to ferment.

Samp

A total of 746 g of samp was soaked overnight in 2000 ml of boiled water. Two thousand ml of boiling water was then added to the pre-soaked samp and boiled for an additional 125 minutes, with the lid on. An additional part of 750 ml of water was added to the samp during the cooking period. Fat was heated in a different pot for frying onions, and then added to the samp with a pinch of salt after which it was stirred until it was distributed evenly. The cooking continued for 30 minutes with the lid on, which was occasionally opened for stirring, until it was ready to be served.

Boiled maize kernels

A total of 604 g of maize grain was placed in a pot of 1500 ml of boiling water. The maize grain was boiled for 3 hours, and an extra 500 ml of water every 45 minutes added until the kernels turned white in colour. This change signals that the kernels are cooked.

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

To reduce bias associated with the labelling of samples a Table of Random Permutations of nine to assign each sample a unique three-digit code was used. The samples were tasted from left to right.

To prevent panelists from influencing each other's responses, they were sitted back to back about an arm's length from each other. All participants were provided with a glass of water, serviette, small platter containing a single sample per platter and four sensory evaluation questionnaires written in IsiZulu and a pencil to write with (Appendix B).

6.4 Validity and reliability of method

A rapid rural appraisal and orientation of the area was conducted before the actual data collection and the questionnaires were piloted to a group of locals who did not form part of the actual study. All the questionnaires were first developed in English then translated into isiZulu. For further confirmation, the field workers reviewed the language of the questionnaires and lastly the pilot study was held with the locals in Ntambanana. Field workers were trained for 2 days. They also had experience with data collection from the pilot study and the actual survey.

6.4.1 Data analysis

Statistical analysis was done using a Statistical package for Social Sciences (SPSS) Version 23. Descriptive statistics were used to calculate means, standard deviation, replicate values and percentages of the data.

6.4.2 Results and discussion

6.4.3 Respondent demographic profile

The majority (77%) of the participants were mainly women, dominated by women over 50yrs of age. As shown in table 8, generally literacy levels were very low.

Table 6.1: Socio-Demographic Profile of Ntambanana Respondents

Gender		**Age**		**Level of education**		**Employment status**		**Household income (Rand/month)**	
Male (23%)	17	20-40 (31%)	23	No education (31%)	23	Unemployed (72%)	53	< R800 (21%)	16
Female (77%)	58	41-50 (21%)	16	Primary school (31%)	23	Part time (19%)	14	R801- R1500 (47%)	35
		>50 (48%)	36	Secondary school (34%)	26	Full time (9%)	7	R1501 - R3500 (23%)	17
				Tertiary education (4%)	3			> R3500 (9%)	7
N =								75`	

The domination of women was no surprise- similar to the observation made by Hovorka & de Zeeuw (2009), women play a key role in farming and in alleviating poverty, especially among rural households. An estimated 47% households received between R801 to R1500 per month with an average of 5 household members. According to Stats SA 2011/12, a person should have R 20.65 per day, which means that the households were not able to afford the food they preferred and they were vulnerable to food insecurity.

6.4.4 Food and nutritional security in Ntambanana households

As shown in Figure 6.1, only 8% of the households were food secure. These households were generally earning more than R3 500 per month with an average household size of five persons.

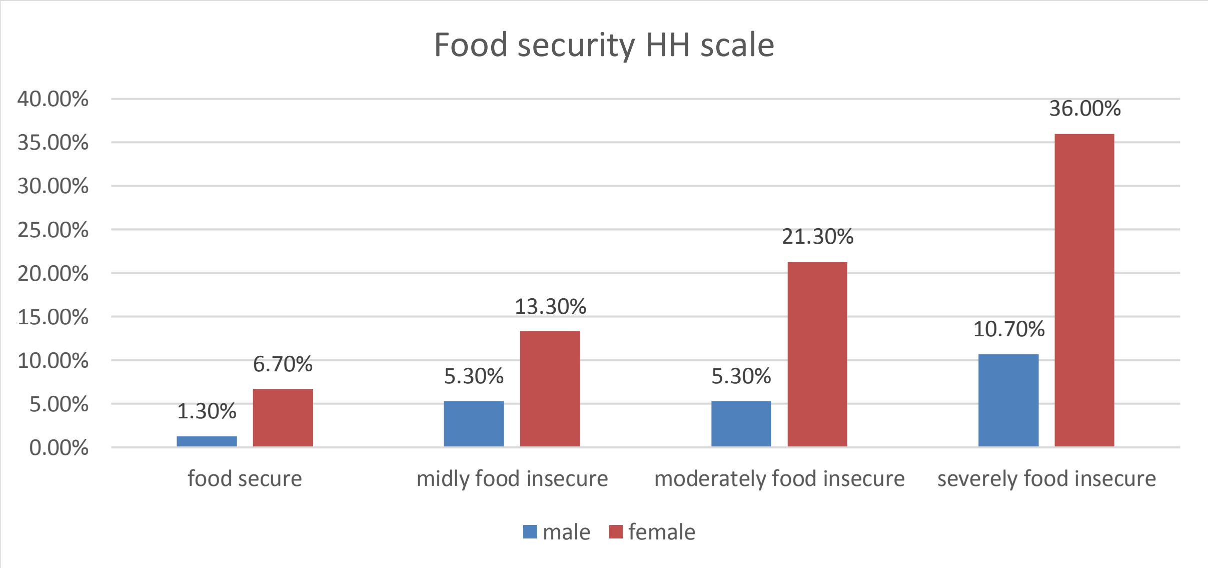

Figure 6.1: Household Food Security Scale for Rural Ntambanana

At household level food security refers to the availability of food in one's home which one has access to (du Toit, 2011). In this case, a household was regarded as food secure when the members of the family did not experience hunger or in fear of starvation. However, most participants were worried about the shortage of food. Although such households were involved in agricultural activities, crop diversity was very limited and agricultural production was seasonal.

The findings from the HFIAS score showed that women were the most vulnerable to food insecurity (36%). Most women reported giving up their meals for the children. Also, other participants reported that household heads were given food first, followed by children and last women who scrounged on left overs. Similar findings were obtained in a study done by FAO (2015) in which women were vulnerable to chronic under nutrition due to food discrimination.

The 24-hour recall showed that the most consumed foods were cereals (74%) (Figure 6.2), because they were less expensive than other food groups. This shows the importance of maize biofortification with iron and zinc. Maize with low phytic acid has a better capacity to reduce malnutrition in rural households. Dark, green and leafy vegetables and other vegetables such as tomatoes, onions and amaranth are common relishes. Onion is a mineral absorption enhancer when mixed with grains and cereals (Gautam et al., 2010).

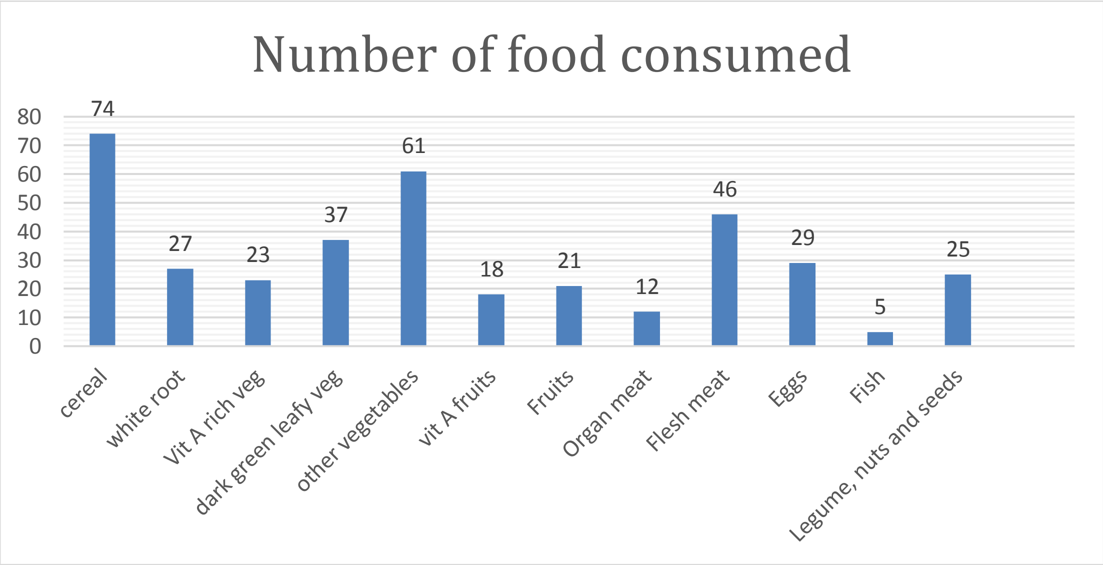

Figure 6.2: Food Consumed in 24- Hour Recall

A total of 96% participants consumed cereal grain foods in the past 24 hours and about 79% combined the cereal foods with vegetables and 60 % with meat. Generally, the maize-based foods are supposed to be accompanied by plant and animal food items to enhance mineral absorption. Hotz (2007) demonstrated the importance of consuming food from animal sources since they are rich in micro-nutrients and also stated that combining meat with maize-based diets increased mineral absorption. However, animal food products are generally costly and are as such omitted from the daily meals of most rural households. Nevertheless, green leafy vegetables, when combined with maize meal, increase iron and zinc in the diet (Kruger et al., 2015). These vegetables are cost-effective especially if they are locally grown.

As stated by Sibhatu (2015), most households involved in agriculture do not diversify their crops. Maize is always prioritized, and consequently such households do not achieve balanced diets (Bamji, 2007; Graham et al., 2007). Aliber (2009) brings this into focus by showing that South African households with income levels of less than R1 000 per month usually purchase less fruits and vegetables with meat only being consumed occasionally. Unfortunately, the socio-economic status of the most households and limited crop diversification in their agricultural production constrains the ability of the households to realize balanced maize-based dishes, let alone diets.

6.4.5 Production and consumption of own maize

The majority of the households owned both back yard gardens 0.00072 hectares and fields (2 ha). However, only 81% of the households cultivates both pieces of land due to water shortages. Maize was the dominant staple food among the households. As many as 59% of the households consumed maize meal every day as opposed to just 10% who rarely consumed maize. About 81% of the households grew their own maize and processed it themselves shown in Table 4.2. They purchased commercial maize off-season or when there was shortage of own produced maize. According to the study, maize was perceived to be the most significant food source due to its high nutritive value, abundant availability, accessibility and versatility in making various maize-based foods. All household members could also consume it at any time of the day see Table 4.2.

Table 6.2: Production and Maize Consumption

Maize meal consumption		maize farming	
almost never	3 (4%)	no	14 (19%)
sometimes	7 (9%)	yes	61 (81%)
very often	21 (28%)		
always	44 (59%)		
Total	75 (100%)		

The households that purchased maize meal spent between R50 and R400 for 10 kg to 80 kg per month, respectively. The quantity depended on the number of people living in the household. On average, a household of six persons buying 80 kg of maize and earning around R1 500 would have already spent 27% of its income on one food item. It could be argued that relying on own grown maize is a cost-effective option especially in-season. The problem is that the maize is deficient in minerals and other nutrients; fortified commercial maize (maize meal) is nutritionally superior to the own grown maize. This then highlights an opportunity for the cost-effective agricultural-based intervention to address malnutrition, including mineral deficiencies (a form of hidden hunger) among rural communities such as those in Ntamabanana.

6.4.6 Traditional maize processing methods in Ntambanana

Fermentation was the most used maize processing method, 44% of the survey participants used it to process maize. According to Egli 2002, fermentation can remove about 40% of the phytic acid in maize. In this study, fermentation was mainly used in idokwe beverage making. Only 14 % of the study participants milled their maize, milling was done to produce (samp) isitambu, maize meal flour (impuphu), and incwancwa. A local milling company supplied the equipment for milling. As shown in Figure 15, some of the traditional methods of processing maize, such as milling and roasting were not often used.

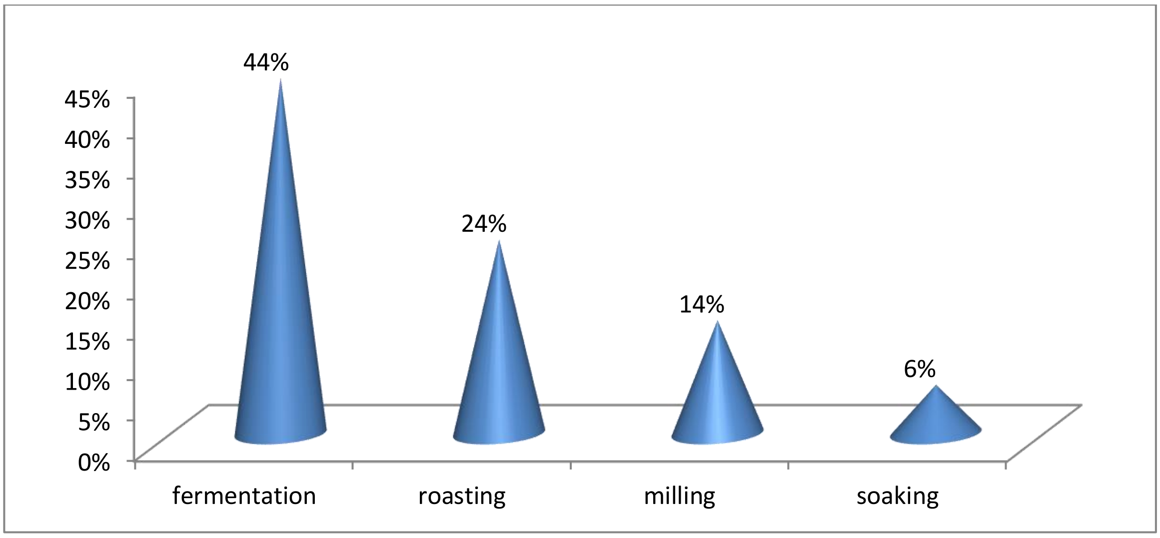

Figure 6.3: Maize Processing Frequency in Households

As reported earlier (Chapter 4), Ntambanana rural households processed maize by milling less than the other methods because them deemed it labour intensive. Samp usually requires soaking overnight to reduce its cooking time, but it also requires pounding the maize grain if using the local grown maize. Idokwe, was still regarded as the most preferred beverage because of its refreshing quality making it suitable for all household members, especially in summer. LPA acid maize could be adopted to further improve the mineral availability of this beverage.

6.4.7 Consumer acceptability of LPA maize-based traditional dishes

Table 6.3 shows a comparison of consumer acceptability of a traditionally fermented low phytic acid (LPA) maize with a traditionally fermented high phytic acid (HPA) maize.

Table 6.3: A comparison of consumer acceptability of a traditionally fermented low phytic acid (LPA) maize with a traditionally fermented high phytic acid (HPA) maize

Sensory attribute	Rating	HPA un fermented porridge (%)		LPA un-fermented porridge (%)		HPA fermented porridge (idokwe) (%)		LPA fermented porridge (idokwe) (%)	
Aroma	Very bad	4*	(7.0)**	3*	(5.3)**	4	(7.0)	3	(5.3)
	Bad	13	(22.8)	9	(15.8)	8	(14.0)	9	(15.8)
	Neutral	5	(8.8)	11	(19.3)	5	(8.8)	7	(12.3)
	Good	16	(28.1)	23	(40.4)	20	(35.1)	26	(45.6)
	Very good	19	(33.3)	11	(19.3)	20	(35.1)	12	(21.1)
Taste	Very bad	4	(7.0)	3	(5.3)	0		7	(12.3)
	Bad	7	(12.3)	8	(14.0)	11	(19.3)	2	(3.5)
	Neutral	1	(1.8)	7	(12.3)	1	(1.8)	13	(22.8)
	Good	32	(56.1)	33	(57.9)	28	(49.1)	28	(49.1)
	Very good	13	(22.8)	6	(10.5)	17	(29.8)	7	(12.3)
Colour	Very bad	2	(3.5)	3	(5.3)	1	(1.8)	2	(3.5)
	Bad	6	(10.5)	12	(21.1)	8	(14.0)	5	(8.8)
	Neutral	11	(19.3)	5	(8.8)	6	(10.5)	3	(5.3)
	Good	29	(50.9)	24	(42.1)	22	(38.6)	35	(61.4)
	Very good	9	(15.8)	13	(22.8)	20	(35.1)	12	(21.1)
Texture	Very bad	4	(7.0)	1	(1.8)	1	(1.8)	2	(3.5)
	Bad	7	(12.3)	14	(24.6)	8	(14.0)	8	(14.0)
	Neutral	10	(17.5)	8	(14.0)	6	(10.5)	4	(7.0)
	Good	17	(29.8)	22	(38.6)	20	(35.1)	33	(57.9)
	Very good	19	(33.3)	12	(21.1)	22	(38.6)	10	(17.5)
Overall acceptability	Very bad	6	(10.2)	3	(5.1)	3	(5.1)	1	(1.7)
	Bad	5	(8.5)	12	(20.3)	5	(8.5)	6	(10.2)
	Neutral	8	(13.6)	2	(3.4)	6	(10.2)	3	(5.1)
	Good	15	(25.4)	22	(37.3)	18	(30.5)	31	(52.5)
	Very good	23	(39.0)	18	(30.5)	25	(42.4)	16	(27.1)

**Number of respondents who gave the rating; ** % of the respondents who gave the rating*

About 59% of the panelists liked the aroma of the unfermented porridge prepared with low phytic (LPA) maize, but the percentage of panelist who liked the high phytic acid (HPA) maize porridge was higher by 2%. The overall acceptability of the unfermented porridges made with the two maize types was similar.

The aroma acceptability of the fermented porridges made with the two maize types showed a similar trend as the unfermented porridges 70% of the panelists liked the aroma of the high phytic acid maize porridge, which compared fairly well with 66% of the panelists liking the low phytic acid maize porridge.

The overall acceptability of the un-fermented HPA maize porridge and un-fermented LPA maize porridge was similar, although the LPA maize was liked by a slightly higher percentage of panelists (3% higher). Similarly, the overall acceptability of the fermented LPA maize porridge was (7% more liked) than that of the fermented HPA maize porridge.

The results indicate that the sensory properties of porridges (non-fermented and fermented) prepared with LPA and HPA maize are similar. The fermented LPA maize porridge would have an added advantage of further reducing the phytic acid content of the product through the action of the phytase enzyme, which is often reported to be produced during cereal grain lactic acid fermentation. Thus, LPA maize can be used in place of HPA maize to make popular traditional porridges in Ntambanana and thereby contribute to the alleviation of mineral deficiencies, a form of hidden hunger.

The results of evaluation of consumer acceptability of a traditional low phytic acid (LPA) maize samp compared to a traditional high phytic acid (HPA) maize samp are shown in Table 6.4.

Table 6.4: A comparison of consumer acceptability of a traditional low phytic acid (LPA) maize samp with a traditional high phytic acid (HPA) maize samp

Sensory attribute	Ratings	HPA samp (isitambu) (%)		LPA samp (%) (isitambu)		LPA boiled maize (izinkobe) (%)		HPA boiled (izinkobe) (%)	
Taste	Very bad	3	(5.3)	0		2	(3.5)	4	(7.0)
	Bad	7	(12.3)	7	(12.3)	1	(1.8)	9	(15.8)
	Neutral	3	(5.3)	7	(12.3)	8	(14.0)	8	(14.0)
	Good	19	(33.3)	20	(35.1)	29	(50.9)	16	(28.1)
	Very good	25	(43.9)	23	(40.4)	17	(29.8)	20	(35.1)
Aroma	Very bad	5	(8.8)	4	(7.0)	2	(3.5)	2	(3.5)
	Bad	4	(7.0)	1	(1.8)	1	(1.8)	9	(15.8)
	Neutral	0		6	(10.5)	2	(3.5)	7	(12.3)
	Good	28	(49.1)	26	(45.6)	27	(47.4)	18	(31.6)
	Very good	20	(35.1)	20	(35.1)	25	(43.9)	21	(36.8)
Colour	Very bad	5	(8.8)	2	(3.5)	2	(3.5)	2	(3.5)
	Bad	3	(5.3)	6	(10.5)	1	(1.8)	14	(24.6)
	Neutral	2	(3.5)	2	(3.5)	2	(3.5)	11	(19.3)
	Good	26	(45.6)	24	(42.1)	29	(50.9)	17	(29.8)
	Very good	21	(36.8)	23	(40.4)	23	(40.4)	13	(22.8)
Texture	Very bad	5	(8.8)	2	(3.5)	3	(5.3)	3	(5.3)
	Bad	0		3	(5.3)	1	(1.8)	8	(14.0)
	Neutral	5	(8.8)	2	(3.5)	1	(1.8)	4	(7.0)
	Good	27	(47.4)	31	(54.4)	30	(52.6)	27	(47.4)
	Very good	20	(35.1)	19	(33.3)	22	(38.6)	15	(26.3)
Overall acceptability	Very bad	5	(8.5)	2	(3.4)	2	(3.2)	3	(5.1)
	Bad	2	(3.4)	6	(10.2)	0	(0)	10	(16.9)
	Neutral	4	(6.8)	2	(3.4)	5	(8.5)	4	(6.8)
	Good	19	(32.2)	23	(39.0)	20	(33.9) 30	20	(33.9)
	Very good	27	(45.8)	24	(40.7)		(50.8)	20	(33.9)

*Number of respondents who gave the rating; ** % of the respondents who gave the rating

About 80% of the panelists liked the taste of the boiled maize kernels prepared with low phytic (LPA) maize, but the percentage of panelists who liked high phytic acid boiled kernels was lower by 17%. The overall acceptability of the boiled maize kernels, LPA boiled maize kernels was (17% more liked) than that of the HPA boiled maize kernel.

The texture acceptability of the cooked samp and boiled maize kernels made with LPA maize types was higher (5% more liked) than that of the HPA samp and boiled maize kernels. The overall acceptability of samp prepared with two maize types was similar. The results show that the sensory properties of samp and boiled maize kernels prepared with both LPA and HPA maize are similar, despite the 2% difference. The percentage of panelists who liked the taste of the samp samples made with LPA and High Phytic Acid (HPA) maize, separately, was similar, 75% and 77%, respectively.

6.5 Conclusion

The sensory evaluation results indicate that low phytic acid maize would be an acceptable replacement of high phytic acid maize in popular traditional maize-based dishes consumed in Ntambanana. Thus, low phytic acid maize has the potential to contribute in addressing the problem of mineral deficiencies among rural households in rural KwaZulu-Natal, and probably elsewhere in rural areas of sub-Saharan Africa. Since women are involved in food preparation, the importance of traditional maize processing should be emphasized. Additionally, there is a need to educate these rural households about the nutritional benefits of these traditional food processing methods since there was significance in the sensory attributes on low phytic maize.

6.6 References

Bamji, M.S., 2007. Nutrition-secure India-How do we get there? Nutrition conclave discusses the way forward. *Current Science-Bangalore-*,*93*(11), p.1473.

Burchi, F., Fanzo, J., and Frison, E., 2011. The role of food and nutrition system approaches in tackling hidden hunger. *International Journal of Environmental Research and Public Health*, *8*(2), pp.358-373.

Darnton-Hill, I., and Mkparu, U. C., 2015. Micronutrients in Pregnancy in Low-and Middle-Income Countries. *Nutrients*, *7*(3), pp. 1744-1768.

De Groote, H., and Kimenju, S. C., 2008. Comparing consumer preferences for color and nutritional quality in maize: Application of a semi-double-bound logistic model on urban consumers in Kenya. *Food Policy*, *33*(4), pp. 362-370.

De Groote, H., Kimenju, S. C., and Morawetz, U. B., 2011. Estimating consumer willingness to pay for food quality with experimental auctions: the case of yellow versus fortified maize meal in Kenya. *Agricultural Economics*, *42*(1), pp.1-16.

du Toit, D. C., 2011. Directorate Economic Services. http://www.nda.agric.za/docs/genreports/foodsecurity.pdf. Viewed 1December 2015.

Ecker, O. and Qaim, M., 2011. Analyzing nutritional impacts of policies: an empirical study for Malawi. *World Development*, *39*(3), pp.412-428.

FAO, 2015. Food security, Women play a decisive role in household food security, dietary diversity and children's health. http://www.fao.org/gender/gender-home/gender-programme/gender-food/en/. Viewed 1December 2015

Gautam, S., Platel, K., and Srinivasan, K., 2010. Higher bioaccessibility of iron and zinc from food grains in the presence of garlic and onion. *Journal of Agricultural and Food Chemistry*, *58*(14), pp. 8426-8429.

Graham R.D., Welch R.M., Saunders D.A., Ortiz-Monasterio I., Bouis H.E., Bonierbale M, DeHaan S, Burgos G, Thiele G, Liria R, Meisner C.A., 2007. *Nutritious subsistence foodsystems. Advances in Agronomy*. 92, pp. 1-74.

Hotz, C., and Gibson, R. S., 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *The Journal of Nutrition*, *137*(4), pp. 1097-1100.

Katz, S. H., and Weaver, W. W., 2003. *Encyclopedia of Food and Culture*. Scribner.

Kruger, J., Mongwaketse, T., Faber, M., van der Hoeven, M. and Smuts, C.M., 2015. Potential contribution of African green leafy vegetables and maize porridge composite meals to iron and zinc nutrition. *Nutrition*, *31*(9), pp.1117-1123.

Lachat, C. K., Van Camp, J. H., Mamiro, P. S., Wayua, F. O., Opsomer, A. S., Roberfroid, D. A., and Kolsteren, P. W., 2006. Processing of complementary food does not increase hair zinc levels and growth of infants in Kilosa district, rural Tanzania. *British Journal of Nutrition*, *95*(01), pp.174-180.

Meenakshi, J.V., Banerji, A., Manyong, V., Tomlins, K., Hamukwala, P., Zulu, R. and Mungoma, C., 2010. Consumer acceptance of provitamin A orange maize in rural Zambia (HarvestPlus Working Paper No. 4).

Nestel, P., Bouis, H. E., Meenakshi, J. V., and Pfeiffer, W., 2006. Biofortification of staple food crops. *The Journal of Nutrition*, *136*(4), pp. 1064-1067.

Nuss, E.T. and Tanumihardjo, S.A., 2010. Maize: a paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science and Food Safety*, *9*(4), pp.417-436.

Sharma, A., Kumari, S., Wongputtisin, P., Nout, M.J.R. and Sarkar, P.K., 2015. Optimization of soybean processing into kinema, a Bacillus-fermented alkaline food, with respect to a minimum level of antinutrients. *Journal of Applied Microbiology*, *119*(1), pp.162-176.

Sibhatu, K.T., Krishna, V.V. and Qaim, M., 2015. Production diversity and dietary diversity in smallholder farm households. *Proceedings of the National Academy of Sciences*, *112*(34), pp.10657-10662.

Sirdah, M.M., Yaghi, A. and Yaghi, A.R., 2014. Iron deficiency anemia among kindergarten children living in the marginalized areas of Gaza Strip, Palestine. *Revista brasileira de hematologia e hemoterapia*, *36*(2), pp.132-138.

Sokrab, A.M., Ahmed, I.A.M. and Babiker, E.E., 2014. Effect of fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes. *Journal of Food Science and Technology*, *51*(10), pp.2608-2615.

Rwegerera, G. M., Joel, D. R., Bakilana, C., and Maruza, M. P., 2015. The triad of Iron deficiency anemia, hepatosplenomegaly and growth retardation with normal serum zinc levels in a 14-year-old boy. *Nigerian Journal of Clinical Practice*, *18*(5), pp. 690-692.

7 CHAPTER: GENERAL CONCLUSION AND RECOMMENDATIONS

7.1 Introduction

This chapter will discuss the main conclusion and recommendation. The aim of this study was to determine the effect of indigenous processing methods on the bioavailability of minerals on maize and breeding on phytic acid content and consumer acceptance of white maize. The objectives of the study were: (i) To breed for maize with low phytic acid content; (ii) To investigate the effect of selected indigenous/traditional processing methods in Zulu (wild type) maize; (iii) To investigate people's perception on low phytic acid maize; (iv) To investigate the utilization of traditional processing methods of maize.

7.2 Conclusion

This study has provided useful baseline on nutritional and consumer acceptability of low phytic acid maize, an area in which data were lacking. Also the frequency of traditional maize processing methods in the households.

The results showed that there was little understanding of the nutritional benefit of traditional maize processing. The existing perception about the nutritional value of own grown maize was misleading, especially if the maize is not accompanied by other nutritious foods such as vegetables and meat. Maize meal was still overlooked because of the perception that it contains chemicals and it is not nutritious. Even though some sample prepared with the LPA maize were accepted but there is a need to educate people about the nutritional value of the biofortified maize product to change their perceptions. The participants were still using the maize traditional processing method and the mostly used being fermentation (idokwe), however these methods were used to improve sensory attribute of maize and for preservation. This showed less knowledge about the nutritional benefits it provides.

The findings of this work indicated that when HPA line is crossed with LPA for generations, genes could be fixed to produce LPA progenies. The progenies showed grain phytate levels of 1.7 -16.2 mg/g compared to the positive control, CM32 (26.2mg/g), and negative control, LP16 (62.8 mg/g), which reduced phytic acid content to 93% compared to LPA parent line (CM32). This present an opportunity for breeders to identify maize varieties with LPA, multiply them for commercial and rural communities benefit. Overall, it appears that LPA maize has potential to be used in place of HPA maize. Low phytic acid maize could assist the community by availing minerals and fight hidden hunger.

7.3 Recommendations

7.3.1 Recommendations for improvement of the study

a) This study was conducted in KwaZulu-Natal, Ntambanana municipality targeting only two villages. This limits the study findings for other rural households. Therefore, it is essential to perform further sensory evaluations using low phytic acid maize to assess cultural acceptability.

A challenge faced by the citizens is that they have inadequate access to information resources and sources to help them make optimal choices about their nutrition and diet. With this, there is a felt need to invest in social programs, which could influence nutrition through improved information availability.

b) Studies assessing the bioavailability of minerals in traditional processing methods.

c) Assess the effect on nutritional composition of more traditionally processed maize-based foods: foods, which as seen in this study staple diets of the concerned communities.

APPENDICES SECTION

APPENDIX A: SURVEY QUESTIONNAIRE IN ENGLISH

SECTIONA: Socio-Demographic Information

1. Gender

1. Male	
2. Female	

2. Age of participant

1. Below 20	2. 20-40	3. 41-50	4. Above 50

3. Marital Status

1. Single	2. Married	3. Divorced	4. Widowed	5. other (specify)

4. Are you the household head?

1. Yes	
2. No	

5. Indicate the number of people in the household per age category

1. 0-12 months	2. 1-5 years	3.6-12 years	4.13-19 years	5.20-35 years	6.36-59 years	7.59 and above

6. Level of Education

1.No formal	2. Primary school	3. Secondary school	4. Tertiary education

7. Employment status

1.Employed full time	2.Employed part time	3.Unemployed

8. Source of income

1.Wages	2.Salary	3.Pension	4.Grant	5.Other (specify)

9. Average money in the household

1.Below 800	2. R801- R1500	3.R1501- R3500	4. Above R3500

Maize consumption

10. Do you plant maize?

Yes ☐ No ☐

11. How often do you consume maize?

1. always	2. very often	3. fairly often	4. sometimes	5.almost never	6. never

12. Have you ever heard about fortification?

Yes ☐ No ☐

13. Do you eat fortified food?

Yes ☐ No ☐

14. Which maize meal do you prefer?

Local maize ☐ Commercial maize ☐

Refer to Q 14, why do you prefer the one you chose above?

……………………………………………………………………………………………………. .

15. Which maize processing methods do you use in your household most frequently (rank them from1- 5

Fermentation	Roasting	soaking	milling	germination

16. How much quantity of commercial maize do you buy in a month? (give it in kgs)

17.

Processing method	**questions**			
	How often do you		Why do you **processing** your **maize**	For how long do you **processing** your **maize**
fermentation	1 - Always			(in days)
	2 - very often			
	3 - fairly often			
	4 - sometimes			
	5- almost never			
	6 - never			
Soaking	1 - Always			(in hours)
	2 - very often			
	3 - fairly often			
	4 - sometimes			
	5 - almost never			
	6 - never			
Milling	1 - Always			
	2 - very often			
	3 - fairly often 4 - sometimes			

	5 - almost never			
	6 - never			
Roasting	1 - Always			
	2 - very often			
	3 - fairly often			
	4 - sometimes			
	5 - almost never			
	6 - never			
Germination	1 - Always			
	2 - very often			
	3 - fairly often			
	4 - sometimes			
	5 - almost never			
	6 - never			
other	1 - Always			
	2 - very often			
	3 - fairly often			
	4 - sometimes			
	5 - almost never			
	6 - never			

18. Which relish do you couple your maize meal with?
……………………………………………………………………………………………… ……

19. Do the teenagers practice or accompany you when you processing your maize?

Yes ☐ NO ☐

20.

Question?	1 Strongly disagree	2 disagree	3 Neither agree nor disagree	4 agree	5 Strongly agree
Do you think local maize is nutritious					
Do you think commercial maizeis nutritious					

21. Have you ever heard of genetically modified maize (GMO)? Yes ☐ No ☐

22. Do you consume GMO foods?

Yes ☐ No ☐

SECTION B: FDSC status of the HH – food access & diet diversity

Household Food security status food access

Question number	Questions	Coding	If yes Question continue	Answer
1.	In the past four weeks did you worry that your household would not have enough food?	0 = No 1 = Yes	Q1a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	
2.	In the past four weeks were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?	0 = No 1 = Yes	Q2a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	
3.	In the past four weeks did you or any household member have to eat a limited variety of foods due to a lack of resources?	0 = No 1 = Yes	Q3a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	
4.	In the past four weeks did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?	0 = No 1 = Yes	Q4a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	
5.	In the past four weeks did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?	0 = No 1 = Yes	Q5a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	
6.	In the past four weeks did you or any household member have to eat fewer meals in a day because there was not enough food?	1 = Yes 0 = No	Q6a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	

7.	In the past four weeks was there ever no food to eat of any kind in your household because of lack of resources to get food?	0 = No 1 = Yes	Q7a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	
8.	In the past four weeks did you or any household member go to sleep at night hungry because there was not enough food?	0 = No 1 = Yes	Q8a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	
9.	In the past four weeks did you or any household member go a whole day and night without eating anything because there was not enough food?	0 = No 1 = Yes	Q9a. **How often did this happen?** 1 = Rarely (1–2 times) 2 = Sometimes (3–10 times) 3 = Often (more than 10 times)	

Household dietary diversity in 24 hours

Question number	Food group	Examples	YES=1 NO=0
1	CEREALS	corn/maize, rice, or any other grains or foods made from these (e.g. bread, noodles, porridge or other grain products)	
2	WHITE ROOTS AND TUBERS	white potatoes, white cassava, or other foods made from roots	
3	VITAMIN A RICH VEGETABLES AND TUBERS	pumpkin, carrot, squash, or sweet potato that are orange inside	
4	DARK GREEN LEAFY VEGETABLES	dark green leafy vegetables, including wild forms + locally available vitamin A rich leaf such as amaranth, cassava leaves, spinach	
5	OTHER VEGETABLES	other vegetables (e.g. tomato, onion, eggplant) + other locally available vegetables	
6	VITAMIN A RICH FRUITS	ripe mango, green apple, papaya and 100% fruit juice made from these + other locally available vitamin A rich	
7	OTHER FRUITS	other fruits, including wild fruits and 100% fruit juice made from these	
8	ORGAN MEAT	liver, kidney, heart or other organ meats or blood-based foods	
9	FLESH MEATS	beef, pork, lamb, goat, rabbit, game, chicken, duck, other birds, insects	
10	EGGS	eggs from chicken, duck, guinea fowl or any other egg	
11	FISH AND SEAFOOD	fresh or dried fish or shellfish	
12	LEGUMES, NUTS AND SEEDS	dried beans, lentils, nuts, seeds or foods made from these (e.g., peanut butter)	

APPENDIX B: SENSORY EVALUATION QUESTIONNAIRE

IMIBUZO YOKWAMUKELEKA KWEZIDLO EZINCANYANA EZENZIWE NGOMBILA.

Inombolo onikezwe yona: --------------------------------

Inombolo yesampulo: -------------------------------------

Ubulili: --

Iminyaka: |__||__|

IMIYALELO

- Yakaza umlomo ngamanzi ngaphambi kokuba uqale.
- Hlola ukudla okuphambi kwakho. Shono ukuthi ucabangani ngendlela okunambitheka ngayo, iphunga, umbala, indlela okuzwakala ngayo emlonyeni kanye nendlela okuthanda ngayo nje. Khombisa lokhu ngokubeka uphawu [X] eduze kobuso obuqondene nomuzwa wakho
- Yakaza umlomo ngamanzi emva kokudla isidlo ngasinye nangasinye. Ungayakaza futhi nanoma ngasiphi isikhathi ngenkathi uhlola lokudla.
- Uma unomubuzo ungabuza.

APPENDIX C: NOTIFICATION OF ETHICS APPROVAL: The Humanities and Social Science Ethics Committee, University of KwaZulu Natal

MAKA UBUSO OBUBODWA NGO **X** *OKUVUMELANA NESINQUMO SAKHO NGEZIHLOKWANA EZIBHALIWE*

UKUNAMBITHEKA

Kubi impela	Kubi	Mhlawumbe kumnandi noma mhlawumbe kubi	Kumnandi	Kumnandi impela

UMBALA

Kubi impela	Kubi	Mhlawumbe kumnandi noma mhlawumbe kubi	Kumnandi	Kumnandi impela

IPHUNGA

Kubi impela	Kubi	Mhlawumbe kumnandi noma mhlawumbe kubi	Kumnandi	Kumnandi impela

UKUZWA NGESANDLA

Kubi impela	Kubi	Mhlawumbe kumnandi noma mhlawumbe kubi	Kumnandi	Kumnandi impela

ISINQUMO JIKELELE

Kubi impela	Kubi	Mhlawumbe kumnandi noma mhlawumbe kubi	Kumnandi	Kumnandi impela

SIYABONGA!!!!

APPENDIX C: CONSENT FORM IN ZULU

27 January 2016

Ms Thulisi Myeni 214533041
School of Agriculture, Earth and Environmental Science
Pietermaritzburg Campus

Dear Ms Myeni

Protocol reference number: HSS/0782/015M
Project title: Effect of indigenous processing methods and breeding on phytic acid content, nutritional quality and consumer acceptance of white maize

Full Approval – Expedited Application

In response to your application received 23 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)
Humanitities & Social Scinces Research Ethics Committee

/pm

Cc Supervisor: Dr Unathi Kolanisi
Cc Academic Leader Research: Professor Onisimo 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 / snymanm@ukzn.ac.za / mohunp@ukzn.ac.za
Website: www.ukzn.ac.za

1910 - 2010
100 YEARS OF ACADEMIC EXCELLENCE

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

8 APPENDIX D: CONSENT FORM IN ENGLISH

My name is Thulisiwe Myeni and I am a full-time student at the University of KwaZulu-Natal registered for Masters in Agriculture (food security). I would like you to participate in a study of evaluating the effect of indigenous processing methods and breeding on phytic acid content, nutritional quality and consumer acceptance of white maize. Therefore, you will be required to taste maize food product and further rate each sample using a simple picture scale indicating the views on the taste, texture and overall acceptability.

It is essential to know that:

- Participation in this study is voluntary, participant are free to leave the study anytime they wish
- There will be no form of payment for participating in the study
- All information will be kept confidentiality and will only be used for the purpose of this study
- All information will be destroyed when it is no longer needed

For further information with the study you may contact Dr Kolanisi who is the supervisor of the study at 033 260 6342 or kolanisi@ukzn.ac.za

Declaration:

I ……………………………… (Full name and surname) hereby confirm my understanding of the questionnaire and I understand that there will be no risks from the study and I may withdraw if I desire since the study is voluntary.

……………………………………….

……………………………………………

Signature Date

HSSREC Research Office UKZN Govan Mbeki Building
Westville Campus 031 260 4557
E-mail: mohunp@ukzn.ac.za

APPENDIX E: CONSENT FORM IN ZULU (IFOMU LEMVUME)

Igama lami ngingu Thulisiwe Myeni, ngingumfundi eNyuvesi yaKwazulu-Natal, ngenza i-Masters kwi Agriculture/kwezolimo (Food Security). Ngingathanda ukuthi ube yingxenye yalolu cwaningo mayelana nokwamkeleka kokudla okwenziwe ngombila. Lokhu kusho ukuthi uzodingeka ukuthi unambithe ukudla okwenziwe ngombila bese ukhombisa imibono yakho mayelane noku nambitheka, ukuzwakala emlomeni, iphunga, umbala kanye nesimo sonke jikelele.

Kubalulekile ukuthi wazi okulandelayo:

- Abantu abayingxenye yalolucwaningo ngokuvolontiya, abantu abayingxenye yalolucwaningo bavumelekile ukuthi bashiye phakathi kwalo uma bafisa akukho lutho olubi oluyokwenziwa kubona.
- Ayikho imali eyotholwa abantu abayingxenye yalolucwaningo.
- Imininingwane ezotholakala ngeke isetshenziselwe okunye okuseceleni, izosebenziswa kulolucwaningo kuphela. Imininingwane yabantu abazobe beyingxenye yalolucwaningo izogodlwa.
- Yonke imininingwane yalolucwaningo izolahlwa uma ingasadingeki.
- Uma udinga eminye imininingwane ngalolucwaningo ungathintana no Dkt. Kolanisi ongumphathi walolucwaningo. Utholakala kule nombolo-033 260 6342 noma kolanisi@ukzn.ac.za.

Izwi lobufakazi:

Mina______________________________________ (Amagama aphelele nesibongo) ngiyaqiniseka ukuthi ngichazelekile kahle ngalembuzo engizobuzwa yona futhi ngiyasiqonda isizathu salolucwaningo nokuthi yonke imininingwane etholakele izohlolwa. Ngiyavuma ukuba ingxenye yalolucwaningo, ngiyaqonda ukuthi kuyavolontiywa ukuba ingxenye yalolucwaningo nanokuthi ngingashiya phakathi uma ngifisa.

____

Sayina Usuku

HSSREC Research Office UKZN

Govan Mbeki Building

Westville Campus

031 260 4557

E-mail mohunp@ukzn.ac.za