NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

COMPARATIVE NUTRITIONAL ANALYSIS OF *TYLOSEMA ESCULENTUM* (MARAMA BEAN) GERMPLASM COLLECTION IN NAMIBIA

By

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In the Faculty of Health and Applied Sciences

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DECLARATION

I Paidamoyo Mataranyika hereby declare that the work contained in the thesis entitled **Comparative analysis of the nutritional status of *Tylosema esculentum* (Marama bean) germplasm collection in Namibia** is my own original work and that I have not previously in its entirety or in part submitted it at any university or other higher education institution for the award of a degree.

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DEDICATION

I dedicate this work to my grandmother, Esther Susan Zvafadza Mvere Kutya Soko.

ABSTRACT

Purpose of the research:

Malnutrition is a medical condition caused by an unbalanced diet, typically characterised by stunting, wasting and underweight in children. Worldwide, malnutrition causes approximately 45% of all deaths among children under 5 years of age. The largest number of global incidences of malnutrition is observed in developing countries. In Namibia, 24% of children within this age group are stunted while wasting is at 6.2%, (the highest in Southern Africa). The main causes of malnutrition in Namibia are low education of mothers or caregivers of the children and food insecurity usually correlated to the household income. Therefore, treatment efforts usually include nutrition based interventions that involve providing nutritious foods to malnourished children. Protein rich legumes are often used together with cereals to form composite flours. *Tylosema esculentum*, (Burchell) Schreiber, commonly known as Marama bean may be used to treat malnourished children due to its high nutritious value. Indigenous to Namibia, Botswana and South Africa, Marama bean seeds have comparably high protein content ranging between 29% and 39% while lipids are between 32% and 42%. The high nutrition value of Marama bean and its physical attributes allow it to be ground into a flour and used in porridge. Marama bean is an appealing crop to Namibia in particular due to its low cultivation demands as it grows in sandy soils with minimal water requirements and no need for fertilisers.

Objectives:

The aim of this study was to comparatively analyse the nutritional composition of ten Marama bean accessions from the germplasm in Namibia. The objective was to determine the accession most suitable for crop development with the intended use as a biofortifier to provide alternative food sources to malnourished children under the age of 5 years.

Results:

Marama bean seeds from the ten accessions were dried and analysed using several analytical techniques. Ash content was used to determined overall mineral content by mass using dry ashing via a blast furnace. The amount of ash in all samples ranged between 2.1% and 3.5%. Essential minerals analysed using ICP-OES and photospectrometry were calcium, magnesium and phosphorus. The range of concentrations and p values obtained were 750.11 - 2306.22 mg/kg

(p=0.538), 1764.12 - 7415.04 mg/kg (p=0.621) and 4300.81 - 5267.93 mg/kg (p=0.111) respectively. Trace minerals analysed using ICP-OES were iron and zinc. Iron concentration ranged from 53.90 - 322.40 mg/kg and there was no significant difference (p=0.099) between the accessions. Data on zinc concentrations were analysed with non-parametric tests which found the data to be not significantly different ($\chi^2 = 3.073$, $p = 0.961$). The lowest concentration of zinc was 32.2 mg/kg while the highest concentration was 48.8mg/kg. Analysis of the minerals revealed that there was no significant difference in concentration among the ten accessions. Correlation analysis of the minerals within the accessions showed that the distribution of the concentrations of the 5 minerals was similar throughout all accessions. However, pairwise analysis found that the correlations between zinc-magnesium and zinc-phosphorus were significantly different as compared to the rest of the pairs for all accessions. The concentration of crude fatsdetermined using Soxhlet solvent extration ($\chi^2 = 22.934$, $p = 0.006$) and carbohydrates determined by deduction ($\chi^2 = 20.215$, $p = 0.017$) also did not have a significant difference across all accessions. The maximum and minimum amounts observed were 29.93%- 44.06% and 19.41%- 39.04% respectively. Crude protein content was determined using the Dumas combustion method. Protein data had a significant difference ($p<0.001$) with PMBC2 (mean content 34.6%) being the most significant accession.

Conclusion:

PMBC2 was found to be the most suitable accession for crop development and domestication. This study's main contribution with respect to the domestication of Marama bean was the identification of the most superior accession based on nutritional composition.

ABBREVIATIONS, ACRONYMS AND DEFINITIONS

AACC- American Association of Cereal Chemists

AAS- Atomic Absorption Spectroscopy

ANOVA- Analysis of Variance

AOAC- Association of Official Agricultural Chemists

BMI- Body Mass Index

DHS- Demographic and Health Survey

DNA- Deoxyribonucleic acid

EDTA- Ethylenediaminetetraacetic acid

GPS- Global Positioning System

ICP MS- Inductively Coupled Plasma Mass Spectrometry

ICP OES- Inductively Coupled Plasma Optical Emission Spectroscopy

ID- Iron deficiency

MDG- Millenial Development Goal

NMR- Nuclear Magnetic Resonance

NUST- Namibia University of Science and Technology

PEM- Protein-Energy Malnutrition

RDA- Recommended Dietary Allowance

SAM- Severe Acute Malnutrition

SDG- Sustainable Development Goal

UN- United Nations

USDA- United States Department of Agriculture

WHO- World Health Organisation

Bio fortifier- A food supplement used to enhance the content of essential nutrients.

Germplasm- Living genetic resources such as seeds maintained for the purpose of breeding and research uses.

Malnutrition- A state of imbalance with respect to nutritional intake by a person.

Stunting- Reduced growth and development in children due to poor nutrition characterized by low height for age.

Wasting- Loss of weight in children characterized by low weight for height.

Underweight- Composite index for both stunting and wasting reflecting on low weight for age.

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PUBLICATIONS

Some of the work presented in this thesis has been published in part under the title:

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Tylosema esculentum, (Burchell) Schreiber, is an edible leguminous perennial plant indigenous to Botswana, Namibia and South Africa with the ability to survive and thrive in arid conditions. It is known to grow in poor sandy soils with evident heat tolerance (Cullis, Chimwamurombe, Barker, Kunert, & Voster, 2018). It forms a major part of the diets of the natives, being eaten as a snack or in meals. The oil from the seeds is used for frying or in some cases during initiation rituals practiced by some tribes (Martin, Chimwamurombe, & Uzabakiriho, 2012). Marama bean is an underutilised food crop despite its nutritional and economic advantages. Marama bean contains an estimated 29 – 39% protein (Jackson, et al., 2010), significantly higher than soya bean and chickpea (which are estimated to contain approximately between 34.3 - 36.3% and 23% respectively) (Bøhn, et al., 2014; Qayyum, Butt, Anjum, & Nawaz, 2012). Its uses go beyond being eaten as a legume as many products such as cookies have been made from Marama bean (Duodu & Apea-Bah, 2017). The high nutritional value of Marama based on previous studies and traditional knowledge systems, therefore, increases its potential for use within Namibia where many crops cannot thrive in the very arid climate and sandy soils.

Compared to other legumes, Marama bean takes a spot among several highly nutritious but underutilised legumes in Africa which include bambara groundnut (*Vigna subterranea*), cowpea *(Vigna unguiculata*) and lablab *(Lablab purpureus)* (Gulzar & Minnaar, 2016). These legumes have the ability to grow in adverse climates with minimum growth requirements while providing significant levels of essential nutrients such as proteins. Studies have shown that Marama bean seeds have other nutrients such as riboflavin and elements such as calcium, iron, zinc, phosphorus and magnesium (Woollard, Bensch, Indyk, & McMahon, 2016). Apart from being able to thrive in unfavourable conditions, Marama bean possesses health-promoting properties that positively contribute to the prevention of certain diseases. *T. esculentum* contains phenolic compounds with antioxidant properties that contribute to preventing cancer and cardiovascular diseases (Duodu & Apea-Bah, 2017; Kayitesi, de Kock, Minnaar, & Duodu, 2012), while the tubers and leaves from

the Marama bean plant may also be used to treat viral infections linked to diarrhoea (Chingwaru, et al., 2011)

Comparisons of nutrient profiles of Marama bean have been performed within the germplasm collection of Namibia, Botswana and South Africa where Marama bean occurs indigenously. Beans from South Africa contained the highest percentage composition of proteins while samples from Namibia had the highest percentage composition of vitamin E. Protein concentrations ranged between 29 and 39%, while lipids were observed to be between 32 and 42% (Holse, Husted, & Hansen, 2010). Differences observed among the accessions may be attributed to soil composition or microbial associations between the plant and bacteria within the different soil samples (van der Heijden, Bruin, Luckerhoff, van Logtestijn, & Schlaeppi, 2016). This study provided comparative data of the major nutrients of Marama among the three Southern Africa countries however, a gap in knowledge still existed among nutrient profiles of Marama accessions in the respective countries. The data compared only provided information irrespective of accession differences within each population. The study also neglected to explore the full nutritional potential of Marama bean as a biofortifier.

1.2 Health impact of malnutrition

Malnutrition is a medical condition that arises from an unbalanced diet. It is characterised largely by two extremes of nutrition-dependent health complications being undernutrition and overnutrition. Conditions most commonly associated with malnutrition are stunting (low height for age), wasting (low weight for height), underweight (low weight for age), and morbidity (excess weight) (Ziba, Kalimbira, & Kalumikiza, 2018). Therefore, undernutrition is defined as the condition that arises from consuming inadequate amounts of nutrients while malnutrition is developed by continuous undernutrition. The onset of disease or rapid weight loss due to poor nutrition is then referred to as acute malnutrition. However, in cases where it goes untreated, conditions such as marasmus and kwashiorkor may arise resulting in a condition known as severe acute malnutrition (SAM) (Ghosh-Jerath, Singh, Jerath, Gupta, & Racine, 2017). Overnutrition, on the other hand, results in excessive weight gain increasing the chances of children under the age of 5 years becoming obese (Kalu & Etim, 2018).

The largest concern on malnutrition lies in the number of children affected by it every year. It is estimated that one-third of all child and infant deaths in developing countries are due to

malnutrition with African and Asian countries having the highest cases (Kalu & Etim, 2018). Table 1.1 shows statistics of malnutrition in Sub-Saharan countries based on information obtained from the Demographic and Health Surveys (DHS) of the respective countries. Similar statistics were observed in South Africa, (iLembe district, KwaZulu-Natal), where 21.1% of children under the age of 5 years were stunted. The trends of which are a reflection of the state of the whole nation (Dukhi, Sartorius, & Taylor, 2017).

Table 1.1: Statistics of child malnutrition (0- 5 years) in some countries of Sub-Saharan Africa

Country	Year	Stunting (%)	Wasting (%)	Underweight (%)
Burundi	2010	57.7	5.8	28.8
eSwatini	2007	28.9	2.5	5.4
Ghana	2014	18.8	4.7	11.0
Lesotho	2014	33.2	2.8	10.3
Malawi	2010	47.1	4.0	12.8
Mozambique	2011	42.6	5.9	14.9
Namibia	2013	23.8	6.2	13.4
Niger	2012	43.9	18.0	36.4
Nigeria	2013	36.8	8.7	28.8
Tanzania	2016	34.4	4.5	13.7
Zambia	2014	40.1	6.0	1.5
Zimbabwe	2011	32.0	3.0	9.7

Data represented in the table is based on the most recent Demographic and Health Surveys (Akombi, Agho, Merom, Renzaho, & Hall, 2017).

The causes of child malnutrition vary but are mostly influenced by social, economic and cultural shortcomings. The most significant and constant cause within different countries' case studies was the education level of the child's caregivers, observing that less educated mothers would most likely have malnourished children (Akombi, Agho, Merom, Renzaho, & Hall, 2017; Kalu & Etim, 2018). Maternal health also contributes to child health as it was noted that mothers with lower body mass index (BMI) were more disposed to have malnourished children. This together with low income in a family contribute negatively towards the health and wellbeing of children under the age of 5 years (Kalu & Etim, 2018). However, the main signs and symptoms of malnutrition may also be linked to food insecurity and the absence of varieties of food choices (Motbainor, Worku, & Kumie, 2015). These challenges become a national problem though it affects certain communities. Food security in a nation or community is threatened by poor

management of resources and at times agricultural challenges that result in less food available to communities (Vink, 2012).

Malnutrition leads to several primary conditions that may result in long term mental and physiological ailments. Some of the common effects of malnutrition observed in children under the age of 5 years include protein-energy malnutrition (PEM), typically characterised by kwashiorkor or marasmus (Figure 1.2.1), and micronutrient deficiencies which include deficiencies in vitamin A, iodine, iron and zinc (Bain, et al., 2013). Malnourished children between 0-59 months in rural villages in Malawi were observed to contain low serum concentrations of essential amino acids. The study highlighted the link between stunting and essential amino acid content. It was also noted that protein deficiency subsequently results in repressed protein and lipid synthesis, limiting cell growth (Semba, et al., 2016). Most minerals are required in the diet attributable to the complex role they play in the physiological intergrity of the body for general growth and development. Iron deficiency (ID) is of particular importance as it is the most common type of micronutrient deficiency in the world. Given that iron is required for signal transduction and oxygen transport in the bloodstream, ID is associated with poor neurodevelopment, retarded growth and impaired immune response increasing susceptibility to infections (Domello, et al., 2014; Cole & Kramer, 2016).

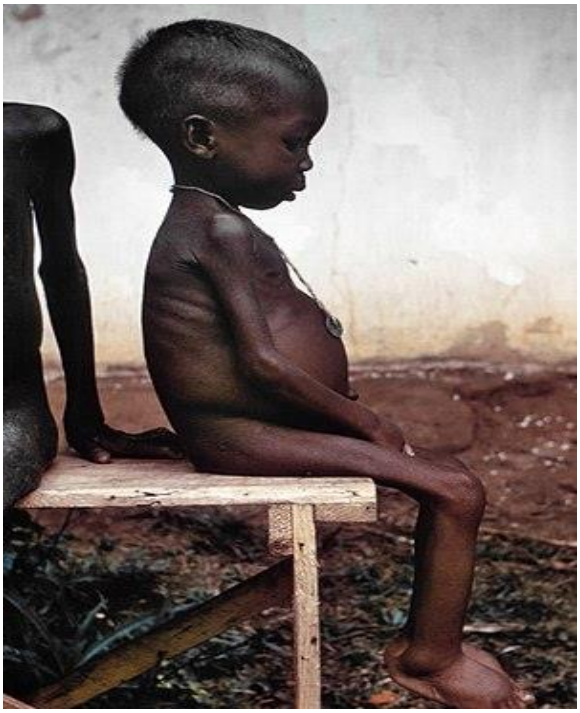

Figure 1.2.1: Severely malnourished child (Photo by Lyle Conrad)

The effects of malnutrition distress the entire human body going as far as altering the natural gut microbiota such as the diminution of *Bifidobacterium longum* in the gut, indicating severe acute malnutrition. As *B. longum* is a pathogen repressor within the gut, depletion leads to an obvious susceptibility to bacterial infections. Resolutions to the bacterial infections would be the administration of antibiotics, probiotics or a drastic change in diet (Million, Diallo, & Raoult, 2016). However, it has been found that permanent disruption of the gut microbiota may occur in severe cases which may lead to death. In some cases, persistent impaired development was observed as a result (Alou, et al., 2017).

Secondary effects of malnutrition include developmental or intellectual delays and susceptibility to infections due to immune dysfunction which may contribute to lower chances of survival and increased risks of morbidity (Mehta, et al., 2013; Goudet, Griffiths, Bogin, & Madise, 2016). Poor school performance and psycho-social problems are the most common challenges to arise in the long run from malnutrition. This may become an economic burden on the state (Akombi, et al., 2017). Furthermore, malnutrition puts a physiological strain on children with cardiovascular and metabolic complications arising at later stages (Ramos, Dumith, & César, 2014). As malnutrition has been of growing concern many studies have gone into finding solutions for not only the effects of malnutrition but the causes as well. The causes and effects of malnutrition have therefore led to a need to find alternative food sources or solutions to the causal factors.

Clinical treatment options include treating symptoms which may require admission (especially in cases of severe acute malnutrition), were provision of nutrition rich food and counseling of caregivers is availed. Children suffering from undernutrition are usually treated or rehabilitated from home without the need for admission (Ghosh-Jerath, et al., 2017). The first treatment choice is usually a nutrition based intervention were the children are given nutrition-rich foods in order to restore balance. This is achieved by either providing food or counseling caregivers of the malnourished children on the best ways to help nurse the children back to health. Goudet, et al., (2016) analysed ways of combating malnutrition especially in economically challenged communities such as slums with the majority being from India, Bangladesh and Kenya. On review of the literature, it was found that nutrition-specific interventions were needed to tackle malnutrition and its effects. These included counseling guardians of the malnourished children, providing direct nutritional intervention such as school feeding programs or Child Health institutional based intervention which comprised of access to health care and sanitation.

Supplementation was the leading type of intervention which included micronutrient fortification of food and nutrient-specific diets. However, feeding programs may be unsuccessful in treating malnutrition in communities due to cultural factors among others (Mas-Harithulfadhli-Agus, Hamid, & Rohana, 2018). The failure of these feeding programs therefore, entails a revision of the strategies and approach to the communities that assistance is rendered.

1.3 Prevalence of malnutrition in Namibia

The prevalence of malnutrition in Namibia raises a lot of concerns because there is an evident correlation between malnutrition and low-income families. In Namibia, 24% of children under the age of 5 are stunted while wasting is at 6.2%, (the highest in Southern Africa) (Mtambo, Katoma, & Kazembe, 2016). As shown in Table 1.1, the highest occurrences of stunting were found to be in the Ohangwena and Hardap regions (largely rural areas). The lowest cases of stunting were observed in the Erongo and Kunene regions. In 2013, malnutrition trends showed a decline in the number of children exhibiting stunting, wasting and being underweight since 2007 (Ministry of Health and Social Services, 2014a; Akombi, Agho, Merom, Renzaho, & Hall, 2017).

The main causes of malnutrition in Namibia are attributed to the education level of mothers and household income. Statistics show that mothers who had education beyond high school have a 9% chance of having stunted children under the age of 5 years while mothers with little to no education have a 31% chance of having stunted children (Ministry of Health and Social Services, 2014b). While low household income highlighted the unavailability of a regular supply of nutritious food. It was also noted that children with underlying conditions such as Human Immunodeficiency Virus (HIV) were less likely to recover fully from the effects of malnutrition with some cases resulting in mortalities (Mdala & Mash, 2015). It should be noted that the nutritional status of the mother also affects the child immensely increasing the risk of disease. It is upon this backdrop that feeding programs have been established within Namibia as an attempt to tackle malnutrition (Mtambo, Katoma, & Kazembe, 2016).

Table 1.2: Malnutrition statistics for Namibia

Region in Namibia	Stunting (%)	Wasting (%)	Underweight (%)
ǁKaras	27.0	5.6	12.1
Erongo	15.2	8.1	9.9
Hardap	29.1	8.2	17.8
Kavango	23.9	8.5	15.0
Khomas	12.8	3.5	9.1
Kunene	19.4	6.1	11.9
Ohangwena	36.5	5.4	16.3
Omaheke	26.9	10.4	18.1
Omusati	24.2	6.0	14.6
Oshana	19.8	4.5	8.2
Oshikoto	26.3	8.5	20.7
Otjozondjupa	20.1	4.3	6.5
Zambezi	18.6	5.7	10.5

Data obtained from the Demographic Health Survey (Ministry of Health and Social Services, 2014b)

1.4 Significance of the study

Malnutrition challenges in children under the age of 5 years has been of increasing concern leading to it being included in the Millennium Developmental Goals (MDGs) by both the United Nations (UN) and the World Health Organisation (WHO) a target that ended in 2015 (World Health Organisation, 2018). As the target came to a close Sustainable Developmental Goals (SDGs) took over with a target of 2030. The SDGs include a goal to end hunger and improve nutrition for all, which is SDG 2. It states that malnutrition causes approximately 45% of deaths among children under the age of 5 years worldwide, a value too high to be ignored (United Nations, 2018). Malnourished children within this age group in Namibia have been noted to be more inclined to stunting. As a result, child stunting potentially leads to developmental health complications such as retarded growth and impaired cognitive (Bain, et al., 2013). Protein deficiency in this age group is a well-established fact by WHO (World Health Organisation, 2002).

Attempts by the government of Namibia to alleviate malnutrition have been aligned with providing food rations and developing strategies and policies to enable efficient delivery of food rations. However, most implementations are in the form of monitoring children's health at clinics and the provision of micronutrients such as vitamins and zinc. The Ministry of Health and Social Services of Namibia, therefore, has a unit designated to the provision of therapeutic and

supplementary food (Ministry of Health and Social Services, 2014a). The collective aim of these efforts is to provide affordable and suitable healthy alternatives to children suffering from malnutrition and the communities at large.

Therefore, research has gone into developing other ways to curb malnutrition such as incorporating plant proteins into cereals (Temba, Njobeh, Adebo, Olugbile, & Kayitesi, 2016). In other countries such as Uganda, efforts to curb malnutrition go beyond providing rations but include providing seed material to poor communities and interactive workshops with farmers to encourage the farming of sustainable crops (Mujjukizi, Adhiambo, & Ruolahti-Virtanen, 2014). Another possible solution to malnutrition would be incoperation of new crops that favour nutrition. This is acheivable through domestication of nutritionally valuable crops. The process of domestication includes the selection of crops with favourable traits such as nutritional quality, tolerance to pests and diseases and growth patterns (Østerberg, et al., 2017). Therefore, this research will reference indigenous knowledge systems of the San and Otjiherero people of Namibia who have been known to use *T. esculentum*, known to them as ozombanui. Hence, it is essential to comparatively evaluate Marama bean accessions in Namibia for their complete nutritional profiles in order to determine the best accession for use in domestication and future production.

CHAPTER 2

LITERATURE REVIEW

2.1. Crop Plants and Food Security

Food security is described as a situation whereby affordable and nutritious food is readily and sufficiently accessible to people (Kline, et al., 2017). The accessibility of affordable, nutritious food is largely affected by the availability of it in communities which is influenced by several factors. Government funding is one such factor as it was noted that funds allocated by a government towards research and improving the agriculture sector of a nation have a constructive bearing on food security. For instance, food security in Ethiopia, Kenya, Mozambique and Sierra Leone improved by approximately 6% for every 10% allocated to agriculture from the total budget of those countries (Fontan Sers & Mughal, 2019). Though valid, economic rations are not the largest determinants of food security as natural influences are now known to play a part in the availability of food.

Natural events like pest infestations, drought and flooding also contribute to food insecurity. Though to a lesser extent, pest infestations can affect food security in the seasons they manifest. These infestations have a tendency to affect the nutritional quality of the crops harvested (Bruce, 2010). Droughts and floods, on the other hand, may be results of more long term changes in the weather, climate change. The effects of climate are well documented and known to adversely affect food security in sub-Saharan Africa (Connolly-Boutin & Smit, 2016). Drought, in particular, caused approximately 630 000 Namibians to experience food insecurity resulting in nutrition-related health challenges increasing within the population. Crops such as moringa (*Moringa oleifera*) and other drought-tolerant species or varieties, therefore, have been studied over the years for their ability to thrive in arid conditions (Huber, Huber, Ananias, & Knott, 2017).

Namibia is predominantly an arid country. In addition to this, the effects of climate change continue to affect it. Efforts to help the agricultural sector in Namibia are costly with the budget allocated for the 2018/2019 period being approximately N$1 billion. The Ministry of Agriculture, Water and Forestry aims to decrease food insecurity in the nation by 13% and increase food production by 25% using the annual budget allocations (Republic of Namibia, 2018). Despite the

large financial investment into agriculture, several crops fail to thrive without extensive assistance like greenhouse structures, fertilisers and irrigation. The common bean (*Phaseolus vulgaris*) for example requires fertiliser and inoculation of the soil with Rhizobium bacteria. The crop also requires a constant supply of water in clay loam soils (Jacobi, 2008).

2.2. Biofortification

Biofortification is a technique used to improve the micronutrient profiles of crops. The most common method employed is breeding, in which plants are selected and grown taking into consideration the preferred genetic and phenotypic characteristics or chemical compositions (Mamedov, et al., 2017). The principle of biofortification lies behind technological advancements in biology and agriculture that have allowed for plant modification such as selective breeding. There is a need to provide food crops with sufficient amounts of nutrients that are otherwise prevalent deficiencies within populations. In recent times, this has driven the exploration of this scientific aspect of biofortification. In this study, biofortification will be taken to mean the nutritional enhancement of food by the addition of nutrient-rich foods (Talsma, Melse-Boonstra, & Brouwer, 2017).

With regards to biofortification, targeted foods for nutrient enhancement are usually cereals and legumes which make up many of the staple foods in Africa. The combination of cereals and legumes (usually high in micronutrients and proteins) is the most likely biofortification to be done (Temba, et al., 2016). Cereals such as sorghum and maize are typically selected for biofortification while varying types of legumes may be used to form a composite mixture as was done with Marama bean flour and soybean flour (Kayitesi, et al, 2012; Awolu, Omoba, Olawoye, & Dairo, 2017). The beneficial properties of legumes which include high nutritional compositions and other health-promoting compounds such as phenolics make them favourable candidates of biofortification. Legumes are also more readily available and less costly compared to equivalent protein animal sources (Soria-Hernández, Serna-Saldívar, & Chuck-Hernández, 2015). The fortified cereal flours may be used to make porridge or other products capable of treating malnourished children. In other instances, fortified foods are packaged into ready-to-eat meals that are delivered to regions with the highest occurrences of malnutrition or experiencing food insecurity (Osborn & Morley, 2016).

Nutrient composition determination found that ground bean had the highest protein content of 25.39% along with the highest levels of Vitamin A, Vitamin B, iron and iodine. Cowpea flour was found to contain the highest levels of calcium and zinc, while the protein content was 24.34% (Chikwendu, 2015). The study by Chikwendu (2015), compared legumes and a commonly utilized cereal with respect to nutritional composition, however, only one cultivar for each of the legume species was used and the samples were obtained from local markets limiting the accuracy of the research as the origins of the samples were not documented. The study mentioned above does highlight the possibility of using legumes, in this case in the form of flour, to fortify other foods in rural and/or urban regions.

2.3. Use of bio-fortifiers

The concept of food alternatives or complementary feeding is of great significance as it plays a major role in the prevention of malnutrition. Complementary feeding is defined as supplementary feeding given to an infant or toddler in the event that breastfeeding is no longer sufficient (Dewey, 2013). Complementary feeding is described as a system that supports breastfeeding by offering either an alternative or additional nutritional sustenance. It includes infant age timelines and suggested foods along with methods of preparation (Romero-Velarde, et al., 2016). In an effort to introduce complementary feeding to both urban areas and rural areas, the government of Botswana endorsed the use of a weaning food locally known as Tsabana. The main ingredients, soya bean and sorghum, offer protein and energy provision to infants recently weaned (Kopong, 2013).

The use of fortified foods is not exclusive to children who are under the age of 5 years as elderly people are at risk of developing malnutrition. This is influenced by the presence of other diseases, food security and economic factors (Morilla-Herrera, et al., 2016). As a result, malnutrition in older individuals is typically characterised by weight loss and general ill-health which may include frailty. Therefore, fortified foods are best used to combat malnutrition among those over the age of 65 years, an at-risk age group. Supplementation of diets with fortified foods has proven successful in most cases especially with nutrient-specific food that aims to target nutrients that are most likely deficient (Artaza-Artabe, Sáez-López, Sánchez-Hernández, Fernández-Gutierrez, & Malafarina, 2016). Approaches to deal with the issue of malnutrition among the elderly vary with supplementation of deficient nutrients as in the case of vitamins or provision of meals being some of the most common. It should be noted that the provision of fortified foods has been very

successful in most cases (Hoffman, 2017). However, the success of food supplementation among the elderly is hindered by pre-existing disorders or subsequent complications.

Biofortification of foods is not only an excellent way to combat malnutrition but it is important to note that it is also a cost-effective method of tackling malnutrition (Bouis & Saltzman, 2017). Legumes are widely used as food supplements as they are nutritionally sufficient and economically practicable to acquire in most countries. The health benefits of pulses include the ability to reduce the risk of coronary heart disease and they possess antioxidants that act as anti-cancer agents. Biofortification of legumes whether during growth, (as seeds) or during the processing of the crops has proven to be effective and recommended (Jangir, Kumar, Lakhran, & Meena, 2017). The use of biofortifiers is not limited to combating malnutrition alone as food security challenges can be solved by increasing agricultural production or variety. Food security, a contributing economic factor with respect to malnutrition, may be solved by biofortification (Bouis & Saltzman, 2017). Therefore, biofortification using legumes such as Marama bean becomes a multifaceted beneficial approach as Marama bean is a drought tolerant plant. Its use, therefore, as a component of supplementary foods is vital, not only for malnourished children but also for countries fraught with food security (Cullis, et al., 2018).

2.4. Nutritional evaluation

In order to determine suitable crops to use for biofortification, experimental analyses are performed on potential biofortifiers. These experimental analyses are done to determine the nutritional compositions of the crops with the aim of finding the most suitable biofortifiers (Temba, et al., 2016). Nutrients of interest are typically those most commonly appearing as deficient in malnourished individuals such as proteins and minerals (iron and calcium). The objectives of these studies, therefore, are to determine the most nutritionally superior crops to fortify food. Methods employed include extraction, chemical tests, chromatographic, spectrophotometric analyses and various Association of Official Agricultural Chemists (AOAC) approved methods (Kalidass & Mohan, 2012; Soria-Hernández, Serna-Saldívar, & Chuck-Hernández, 2015). The nutritional composition of Marama bean has been of great interest as studies were done to determine the nutritional profile of the legume. Studies also sought to determine chemical profiles and composition differences of Marama bean including antioxidants and phytochemicals of Marama bean samples from different countries. Differences which, when analysed, were found to be mostly induced by seasonal differences (Müseler & Schonfeldt, 2006;

Holse, Husted, & Hansen, 2010). Though the Marama bean species (*T. esculentum*) from Botswana, Namibia and South Africa were analysed and nutritionally profiled (Holse, Husted, & Hansen, 2010), no study has comparatively analysed the nutritional composition of Marama bean among accessions in Namibia. To bring more light to nutritional evaluation as a concept, the methods typically used to analyse the nutritional profiles of legumes are described below.

2.4.1. Ash Content

The ash content of a sample shows the amount of mineral content within a sample. It follows the principle that removing all water and organic matter such as carbon-based compounds like sugars, carbohydrates, proteins and lipids present within the sample leaving minerals (Holse, Husted, & Hansen, 2010). Dry ashing and wet ashing are the 2 main methods employed to remove organic material in order to determine the weight equivalent concentration of minerals. They vary largely in the treatment applied to the sample before incineration and the equipment used.

Dry ashing

Dry ashing entails the use of dried samples usually ground to a powder and burned at high temperatures. The samples are placed in crucibles of appropriate size and material and incinerated between 500°C and 900°C for 2-24hrs in a muffle furnace. The advantages of dry ashing over wet ashing include lower risk factors and that it is much simpler however, there are risks of volatilization and possibility of temperature resistance of some pyrolytic organic compounds. It is also costly to run as it requires a lot of electricity and requires a lot of time to run the ashing process (Enders & Lehmann, 2012).

Wet ashing

Wet ashing involves treatment of samples with acid and oxidising agents before ashing in high-pressure systems depending on the sample type and acid used. The temperature and duration of the ashing depend largely on the type of sample and ashing method (Harris & Marshall, 2017; Kalagbor & Opusunju, 2015). The most common acids used are hydrochloric acid, nitric acid and perchloric acid, sulphuric acid and in some cases hydrogen peroxide (H_2O_2) is used. The acids are used in varying combinations to maximise oxidation of the samples. Wet ashing is largely advantageous because it requires less processing time however, the corrosive reagents used are dangerous and may react violently posing a large threat (Enders & Lehmann, 2012).

Microwave ashing

Advances in technology have developed ways to determine ash content using microwave technology. This technique follows the use of a microwave muffle furnace which ashes samples (wet) in significantly less time (Harris & Marshall, 2017). This method is followed by determination of the minerals via any standard mineral analysis method. The microwave technique is time effective as it reduces analysis time from hours to minutes (Yang, Li, Guoxjxj, & Yan, 2013).

2.4.2. Protein analysis

Proteins are essential nutrients especially during the early stages of life as they are required during cell division which occurs rapidly during infanthood through early childhood. For this reason, children under the age of 5year require sufficient amounts of proteins (Kalu & Etim, 2018). In addition, proteins play a crucial role as an energy source while also being part of the crucial components in the body. Proteins perform important enzymatic activities while playing roles in the transportation of other proteins and biochemical compounds across cells walls (Mæhre, Dalheim, Edvinsen, Elvevoll, & Jensen, 2018). In the absence of an adequate amount of proteins (which act as buffers), blood pH becomes compromised. Therefore, protein deficiency is one of the leading causes of acidosis in malnourished children (Maguy, Pierre, Lou, & Michel, 2018). As a consequence, the importance of proteins in the diets of children and determination in foods cannot be sidelined.

Evaluation of protein content is a quantitative process that can be done with different methods. The basic methods aim to determine the nitrogen content taken to be directly proportional to that of the particular protein content within the samples. Instruments may also be used to directly detect the protein quantity. These techniques namely the Kjeldahl Method and the use of instruments can be used to determine protein quantity (Chang & Zhang, 2017).

Kjeldahl Method of Nitrogen Determination

Determination of the nitrogen content is one such technique. This method is known as the Kjeldahl method, which essentially breaks down all organic compounds within the food sample and uses the nitrogen content as the target for analysis. This is a chemical process that relies on the digestion of the sample with the aid of a catalyst followed by titration (Sáez-Plaza,

Michałowski, Navas, Asuero, & Wybraniec, 2013). The basic principle behind the Kjeldahl method is that a strong acid (usually sulphuric acid) is used to break down all organic compounds, in the presence of a salt and a catalyst retaining nitrogen. However, the largest drawback of the Kjeldahl method is the environmental risks posed by the reagents used in the determination of nitrogen (Silva, Detmann, Franco, Palma, & Rocha, 2016). The risks cannot be ignored therefore, alternatives need to be employed in the determination of nitrogen.

Using the Kjeldahl method, it was found that the highest protein content of the 3 species (and 9 accessions), was 19.40% a significantly lower value compared to Marama bean, extracted from *R. rufescens* (Kalidass & Mohan, 2012). Another study on cowpea and other crop plants namely sorghum (*Sorghum sudanese*), lablab (*Lablab purpureus*), and mucuna (*Mucuna pruriens*) estimated the content of protein to be 22.00%, that is 2.34% less than that estimated in the previous study. Protein analysis was done following the Kjeldahl method according to AOAC as mentioned in studies referenced above (Gwanzura, Ng'ambi, & Norris, 2012). These differences may indicate nutrient variations which may arise between cultivars as the former cowpea samples were collected in Nigeria while the latter was from South Africa.

Legumes like many cereals can be ground into fine flour which may be added to typical cereals to fortify them due to the high protein content, as seen with the nutritional composition of *Kerstingella geocarpa* flour (ground bean), *Vigna unguiculata* flour (cowpea), and *Triticum* spp. flour (commercial wheat). In order to determine the nutritional composition of the fortified flours, the chemical composition of the samples was determined. The seeds from the legumes (ground bean and cowpea) were fermented, dried and milled into a flour in order to determine the chemical composition. Protein content evaluation was done using the micro Kjeldahl method followed by distillation (Chikwendu, 2015). The distillate was used in a titration process were the titre value was subsequently used to determine the protein content using the equation below:

$$Protein\ (\%) = \frac{T \times 14.01 \times 0.01 \times 6.25 \times 20 \times 100}{1000 \times 0.2} \quad (1)$$

Where;

T: Titre value

14.01: Atomic mass of nitrogen (gmN)

0.01: Normality of the acid

6.25: Protein conversion factor

20: Dilution factor

100: Percentage

2: Weight of sample

Dumas combustion method

In order to determine nitrogen, a weighed sample is combusted at high temperatures in the presence of oxygen, resulting in the release of carbon, water and nitrogen. Removal of carbon and water leaves nitrogen in special columns. The actual nitrogen content is then determined by using conversion factors (Mihaljev, Jakšić, Prica, Ćupić, & Živkov-Baloš, 2015). The safety and environmental risks associated with the traditional methods of nitrogen analysis are incentive to use safer and more efficient methods. Modern advancements with technology have contributed to the development of equipment that can determine protein quantity (Mæhre, et al., 2018).

The main difference between the Dumas combustion method and the Kjeldahl is the type of nitrogen compounds determined. The former method determines all nitrogen compounds including inorganic nitrogen-based compounds while the latter only determines the organic nitrogen compounds even then some types of organic nitrogen cannot be determined (Müller, 2017). Essentially the Dumas method is able to detect higher levels of nitrogen in the same samples in significantly less time (Beljkaš, et al., 2010). As such fast and easy to use machines were developed with the Dumas method as the principle behind their function.

Protein analysis was performed on different soybean, *Glycine max*, types with the aim to determine trait loci responsible for lipid and protein synthesis within the soybean genome using germplasm accessions as reference. In order to determine protein content, harvested seeds were dried and ground into a flour and tested for the amount of nitrogen using the LECO CHN 2000 analyser. The obtained value was multiplied by 6.25 in order to determine the protein content of the seeds (Hwang, et al., 2014). A study on underutilized legumes, similar to Marama bean, was done using three accession samples of three *Rhynchosia* species. Description of the results was done in reference to amino acids. Extracted proteins were hydrolysed with 6N HCl at 110°C for 24hrs *in vacuo* before being dissolved in citrate buffer (pH2.2). The analysis was continued with an LKB-Biochrome Automated Amino acid Analyser (Kalidass & Mohan, 2012).

2.4.3. Mineral analysis

Essential elements required in the diet are referred to as minerals or trace elements depending on the requisite quantities with each mineral serving a crucial function in the metabolism. Trace elements or micro-elements are minerals required in low quantities compared to other minerals. Iron and zinc are some of the essential trace elements. They both function as important constituents of enzymes allowing for the versatility of enzymes with respect to specific processes (Mohd-Taufek, et al., 2016). Essential minerals refer to minerals that are most abundant in the body and therefore required as such. This is typical of minerals such as calcium, phosphorus and magnesium (Geissler & Powers, 2017). Calcium is the main component of bones in the body hence, calcium is required for the maintenance of bone integrity. It is also vital for muscular function, cell signaling and metabolic processes. Phosphorus, on the other hand, is an important constituent of genetic material. Phosphorus is also an integral component of metabolic energy (Kraft, 2015; Adams, Tlotliso Sello, Qin, Che, & Han, 2018). On the other hand, magnesium is especially functional in enzymatic processes while also contributing to metabolic energy (de Baaij, Hoenderop, & Bindels, 2015). Iron is important as it is a component of blood in the haemoglobin complex with anaemia being the most common symptom of iron deficiency (Eussen, Alles, Uijterschout, & van der Horst-Graat, 2015). Iron is crucial as an oxygen transporter in the haemoglobin structure, therefore, it is central to metabolic processes and DNA synthesis (Adams, et al., 2018). Though a trace mineral, zinc contributes to the process of DNA replication, cell differentiation and composition of certain enzymes. It should be noted that a diet rich in antioxidant compounds found in whole grains and nuts (phytates) can lead to zinc deficiency as they prevent absorption of zinc (Adams, et al, 2018). Consequently, deficiencies of these minerals compromise the health of the individuals by reducing bioactivity and negatively affecting integral structures (Kalu & Etim, 2018).

Mineral determination in food samples is a broad spectrum of analytical techniques. The basic principle of mineral analysis of food samples is first destroying any organic matter within the sample followed by increasing the purity of the mineral to be determined and finally resolution by spectroscopy or chromatography and other similar analytical methods (Food Safety and Standards Authority of India, 2015). Sample preparation is done by removal of organic material in a sample by ashing. The remaining residue is ideally mineral content from which analysis may be done (Poitevin, 2016). Sample preparation may also be done by extraction and concentration of

metals using acids via microwave digestion in what is known as microwave-assisted digestion. Microwave digestion entails the use of acids in an enclosed system preventing contamination of samples and evaporation of volatile acids (Saydut, 2010). The subsequent procedures depend highly on the technique used to determine the mineral content which may be inductively coupled plasma-optical emission spectrometry (ICP-OES), atomic absorption spectroscopy (AAS) or flame photometry among others (Olaoye, Ubbor, & Uduma, 2016; Saydut, 2010).

Atomic Absorption Spectroscopy

AAS is a method used to determine mineral content in samples. Samples are treated in order to discharge the separable atoms using a combination of flammable gases. The principle of AAS is based on the absorption of UV light by the atoms at different wavelengths changing the state of the atoms which is translated into concentration of the specific element in the sample (Paul, et al., 2014). Variations of this technique exist, for example, flames atomic absorption spectroscopy and graphite furnace atomic absorption spectrometry, both also being used to ascertain mineral content. The former was used to establish the concentration of cadmium (II) and lead (II) in food and water samples. Tests during the study found that sample preparation, pH and ions present in the samples influenced the outcome of the results to various degrees, therefore knowledge of the minerals to be determined and multiple tests are essential to accurately determine the concentration (Dasbası, Saçmacı, Ülgen, & Kartal, 2015).

Nutritional evaluation of underutilized legumes, similar to Marama bean, was done in a study using three accession samples of three *Rhynchosia* species; *R. filipes, R. rufescens and R. suaveolens.* The samples were collected and analysed for calcium, potassium, magnesium, manganese, copper, sodium, zinc and iron using the atomic absorption spectrophotometer. Phosphorus was analysed spectrophotometrically after being incubated with triple acid solution. The minerals were found to be of sufficient amounts to satisfy the recommended dietary allowances (RDA). However, within this study, it was not mentioned which of the three species had the highest contents of any of the fatty acids as was the case for the minerals as well (Kalidass & Mohan, 2012).

Inductively Coupled Plasma Spectrometry

Inductively coupled plasma (ICP) spectrometry is another technique used to determine the concentration of elements including organic compounds. This technique also has variations in the

form of inductively coupled plasma optical emission spectrometry or inductively coupled plasma mass spectrometry (ICP-MS) (Bressy, Brito, Barbosa, Teixeira, & Korn, 2013). The success of ICP OES and ICP-MS to determine minerals relies largely on minimization of sample contamination and ion loss usually achieved by microwave digestion. The samples are prepared in individual vessels minimizing contamination (Muller, et al., 2016).

The principle behind ICP-MS is that ions have to be extracted and separated based on mass-to-charge ratio. A detector within the equipment then identifies an ion signal relative to the concentration within the sample. Therefore, determination is by mass using mass spectrometry. Standards may be used to determine the concentration using them as reference (Batsala, Chandu, Sakala, Nama, & Domatoti, 2012). This technique has been used in mineral determination of Marama bean. In order to define the precision of the results, standards were used along with the samples and blanks. Mineral levels similar to those of peanuts were observed for calcium, magnesium, phosphorus and potassium while significant levels of microelements (copper, iron and zinc) similar to soybeans were observed (Holse, Husted, & Hansen, 2010). ICP-MS is thus more sensitive with regards to analyzing samples while ICP-OES requires precise sample preparation to limit false positive or incorrect readings.

2.4.4. Fat analysis

Fats naturally occur as triglycerides made up of fatty acids. Fatty acids may occur as trans-fatty acids as isomers of unsaturated fatty acids. Fats are known to contribute to the process of digestion and uptake of fat-soluble vitamins (Kayitesi, et al, 2012; Osborn & Morley, 2016). Fats in foods also contribute to the energy level of food. Maintenance of healthy hair and skin is largely influenced by oils obtained in the diet. Fatty acids in the form of lipids play a crucial role in the formation of cell membranes providing stability to cells. It is important to note though that the healthiest forms of fats have been observed and identified from seeds such as sunflower and rapeseed with certain legumes (e.g. soya bean) as a close competitor of healthy fatty acids (Mathew, et al., 2014; Niveditha, Sridhar, & Chatra, 2012). Nevertheless, the quantities and types of fats consumed play a crucial role in the health of the individual. High quantities of saturated fats have been linked to cardiovascular diseases with high fatalities. Therefore, amounts of fats in food need to be monitored and diets adjusted accordingly (Osborn & Morley, 2016). The basic

functions and importance of fatty acids in the diet necessitate the need to determine and control the amount of fats in foods.

The determination of fatty acid content is based on extraction using solvents. Varying supplementary treatments in the form of solvents may be applied so as to to determine the specific lipids present in a sample (Bahrami, et al., 2014). Therefore, various solvents are used for extraction and analysis depending on sample type and target lipids. The most common type of method is the Soxhlet extraction method however, AOAC methods may be used usually tailored to the availability of reagents and preferred procedure. Nonetheless, a need for less time consuming and environmentally friendly techniques saw the development or equipment that allows for rapid determination of fats in food samples.

Soxhlet's method

The Soxhlet fat extraction method is a traditional method approved by the American Association of Cereal Chemists (AACC) (1983). It requires the use of non-polar solvents such as petroleum ether to extract lipids. Extraction requires the use of thimbles and a Soxhlet extractor (Bahrami, et al., 2014; Yadav, 2017). The main disadvantages of the Soxhlet method are that it is a very time-consuming technique that requires precision. The solvents used in the Soxhlet's pose toxic threats and therefore are not environmentally friendly (Devaraj, et al., 2018). Fatty acid composition via Soxhlet extraction in species of underutilised cooked and fermented beans found between 1-2% of total lipid. Further analysis by gas chromatography determined the different fatty acids and their compositions (Niveditha, Sridhar, & Chatra, 2012). Fat content analysis of the samples using a modified method such as Tecator Soxtech method requires calculations to determine the actual fat content. The weight of the container and sample are used to determine percentage fat content using the equation below (Chikwendu, 2015):

$$Fat\ (\%) = \frac{X_2 - X_1}{W} \times \frac{100}{1} \quad (2)$$

Where;

X2: Final weight of cup

X1: Initial weight of cup

W: Weight of sample

X2-X1: Weight of fat

Other techniques have been developed and comparisons have been done to establish the most effective and favourable method.

Bligh and Dyer method

The Bligh and Dyer technique is a solvent extraction method that relies on the polarity of chloroform and methanol with water aiding in the final extraction of lipids from samples (Bligh & Dyer, 1959). The principle behind this method is motivated by the knowledge that lipids are either polar or nonpolar therefore both polar and nonpolar solvents are used in any single extraction. (Breil, Vian, Zemb, Kunz, & Chemat, 2017). The main advantage offered by the Bligh and Dyer method is the ability to extract both polar and nonpolar lipids unlike methods such as the Soxhlet which can only extract nonpolar solvents under reflux. However, in order to maximise the amount of extracts obtained using this method it should be carried out as a two-step procedure which is time-consuming (Bahrami, et al., 2014), but effective. A similar lipid extraction procedure may also be used as described by Folch, Leese and Sloane Stanley (1957).

Comparisons of the Bligh and Dyer and Soxhlet methods aimed to determine the most effective method. Due to the different polarities of solvents and eventually target lipids, a difference is expected in amount of lipids extracted. The Bligh and Dyer fat extraction method was noted to generally extract more lipids compared to the Soxhlet, nevertheless, it was observed that the latter extracted more unsaturated fatty acids than saturated fatty acids (Niveditha, Sridhar, & Chatra, 2012; Munir, Imtiaz, Sharif, Haq, & Naz, 2016).

Further analysis of lipids entails the use of advanced techniques to determine the specific lipids and the concentrations in the samples. Lipid analysis was done by Maran pulsed NMR (Resonance Instruments, Whitney, Oxfordshire, UK), before a procedure known as field induction decay-spin echo procedure (Hwang, et al., 2014). This study used 298 accessions found within USDA Soybean Germplasm Collection for experimental analysis however, it did not provide comparative information with respect to the protein content of the different accessions. Gas chromatography may also be employed to analyse fatty acids as was done utilizing a flame ionization detector in gas chromatography (ASHMACO, Japan; Model No: ABD20A). Some of the essential fatty acids extracted were linoleic acid and linolenic acid, while palmitic and stearic acid were some of the unsaturated fatty acids mentioned (Kalidass & Mohan, 2012).

2.4.5. Carbohydrate determination

Carbohydrates in legumes are of great interest and importance due to their positive controlling influence on bowel habit, intestinal microbiota, glucose homeostasis and cholesterol blood level known as cholesterolemia. This is attributed to high levels of resistant starch that allows for a low glycemic index (GI) in the legumes (Clemente & Olias, 2017). The value of carbohydrates goes beyond maintenance as carbohydrate specific diets have been employed as dietary intermediations in the treatment of certain diseases that affect not only children but adults as well. Celiac disease and Crohn's disease are some of the most common diseases managed by adhering to carbohydrate specific diets which regulate the intake of complex carbohydrates. Though the dietary management of the above-mentioned diseases is largely paired with medication, research has noted that the diet plays a major role in management post diagnosis and initial treatment (Obih, et al., 2016). This reveals that though carbohydrate deficiency is not largely documented, the benefits of this specific group of nutrients are undeniable.

This method of carbohydrate determination employed is commonly used in nutritional determination of different food samples however, slight variations of the equations exist. In this research, the total content of ash, crude protein and crude fate were used as the determinants of carbohydrate content. In other cases, the sum total of crude fibre, moisture, ash, crude protein and crude fat content is used to determine carbohydrate content (Awolu, et al., 2017). The largest concern with both methods of carbohydrate determination is the equations failure to put into account the presence of compounds that are not carbohydrates within the samples which may include tannins, waxes and organic acids. This method highly depends on the analysis and accuracy of the results of the other nutrients, which may be problematic in the event that undetected errors would have occurred (Holse, Husted, & Hansen, 2010). In order to avoid discrepancies in carbohydrate content determination, AOAC methods that analyse carbohydrates inclusive of the dietary fibre may be employed for analysis. The main advantage this approach has is that the data obtained will be more comprehensive and accurate (Kalidass & Mohan, 2012).

2.5. Problem Statement

Malnutrition is a condition that arises from one not eating the correct proportions of nutrients leading to several health conditions. Some of the common effects of malnutrition in children that

are under the age of 5 years include protein-energy malnutrition (PEM), typically characterised by kwashiorkor or marasmus, and micronutrient deficiencies. The most common deficiencies being in iron, iodine, zinc and vitamin A. Secondary effects of malnutrition include developmental or intellectual delays and susceptibility to infections due to immune dysfunction (Mehta, et al., 2013). Iron deficiency (ID) is of particular importance as it is the most frequent type of micronutrient deficiency among humans. It is associated with poor neurodevelopment, retarded growth and impaired immune response (Domello, et al., 2014). The causes and effects of malnutrition have led to a need to find alternative food sources or solutions to the other contributing factors as the number of cases of malnutrition in Namibia continue to rise. Provision of alternative food sources, especially a climate sustainable local food crop, becomes of paramount importance. Especially since the semi-arid and arid climate of Namibia does not support many crops. Therefore, given that the Marama bean accessions within the germplasm collection of Namibia have not been nutritionally evaluated individually, it is important to ascertain which one of them has the highest nutritional value that can be used as fortifiers to feed malnourished children that are under the age of 5 years and possibly the entire population.

2.6. Objectives

2.6.1. To determine the micronutrient and macronutrient composition of the different Marama bean accessions with specific attention to the following;

i. Protein content
ii. Fat content
iii. Micronutrients (calcium, iron, magnesium, phosphorus, and zinc) content
iv. Carbohydrate content

2.6.2. To determine the Marama bean accession with the most significant nutritional composition among the collected samples.

2.7. Research questions

2.7.1. Considering the general nutritional profile of Marama what are the comparable nutritional values among the different accessions of Marama bean in Namibia?

2.7.2. Given the determined nutritional statuses of the Marama bean samples, which accession has the highest nutritional values?

2.8. Significance

The current state of malnutrition across the world has resulted in the need to find solutions with respect to all aspects of the problem. The most prominent solutions, therefore, focus on nutrition specific solutions. As a result, there is a need to find affordable and nutritious supplementary food to provide to malnourished children that are under the age of 5 years in Namibia. Marama bean is a nutritious plant indigenous to Namibia with minimal economic requirements to cultivate. Consequently, there is a need to explore the nutritional potential as a supplement and to also define the best accession for domestication and further cultivation. The potentials of Marama bean will not end with it being a nutritious supplement but will include its use as a fortifier of other foods such as cereals. Findings will be made available for use to motivate the commercial production of Marama with the hope to reduce malnutrition in Namibia. It is hoped that recommendations from this study will further motivate the domestication of this valuable indigenous legume in Namibia as support to ongoing research on Marama bean domestication.

CHAPTER 3

MATERIALS AND METHODS

3.1. Introduction

Marama bean (*Tylosema esculentum*) seeds from the north-eastern parts of Namibia were collected for nutritional analysis. The study took a quantitative approach in analysing the samples with one sample set of 10 accessions. The 10 accessions were chosen based on their diversity and favourable phenotypic traits, particularly early flowering, high number of seeds per pod and high number of seeds per plant. For each sample, the seeds were dehulled and ground to a flour to allow for ease of analysis. The macronutrients quantified were proteins, fats and carbohydrates, while micronutrients analysed were minerals (calcium, iron, magnesium, phosphorus and zinc using spectrophotometry and spectrometry). The data obtained were analysed using SPSS (Version 22) using analysis of variance statistical tests. Figure 3.1 shows an overview of the process.

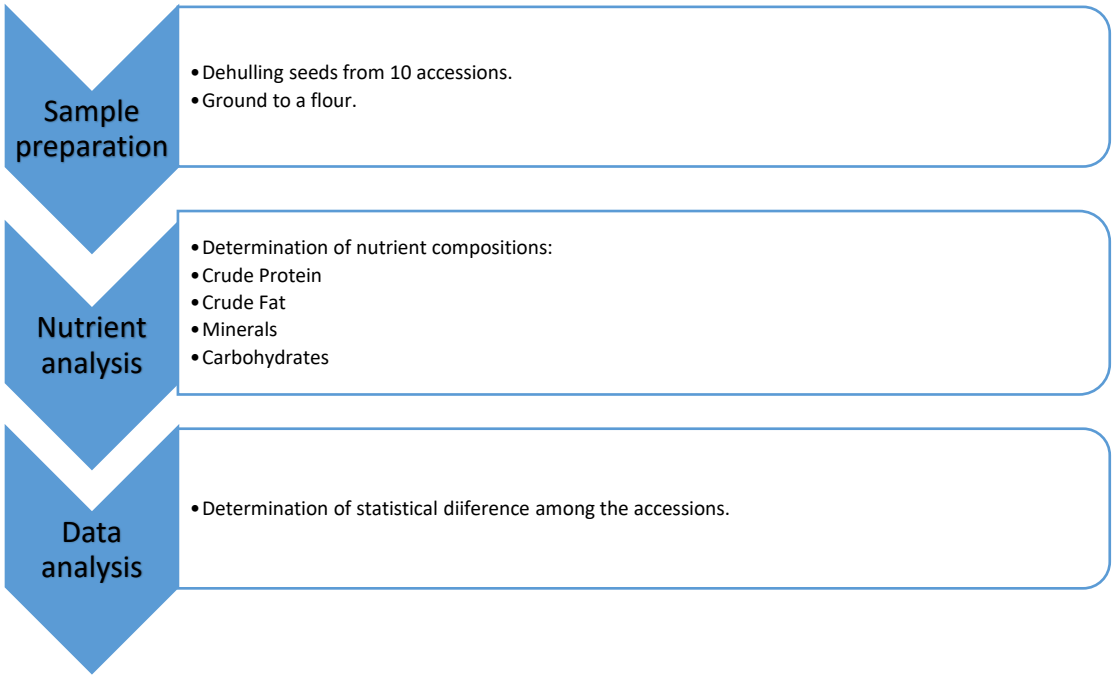

Figure 3.1 Presentation of the experimental implementation applied during research.

3.2. Sampling area

Marama bean grows indigenously in the poor sandy soils of South Africa, Botswana and Namibia, Figure 3.2a below shows the generalised locations within these three countries where it grows. This study will focus on the populations that grow in Namibia with specific attention to the Otjozondjupa region in northeastern Namibia (Figure 3.2b). The Otjozondjupa region experiences two types of climates namely hot desert and hot semi-arid climates with Köppen-Geiger classifications of BWh and BSh respectively (Climate-Data.org, 2018). The soils of the Otjozondjupa region are sandy and well-drained with a significantly low nutrient status that inhibits agricultural proficiency (Strohbach & Kutuahuripa, 2014).

Figure 3.2a Region of *Tylosema esculentum* growth in Southern Africa

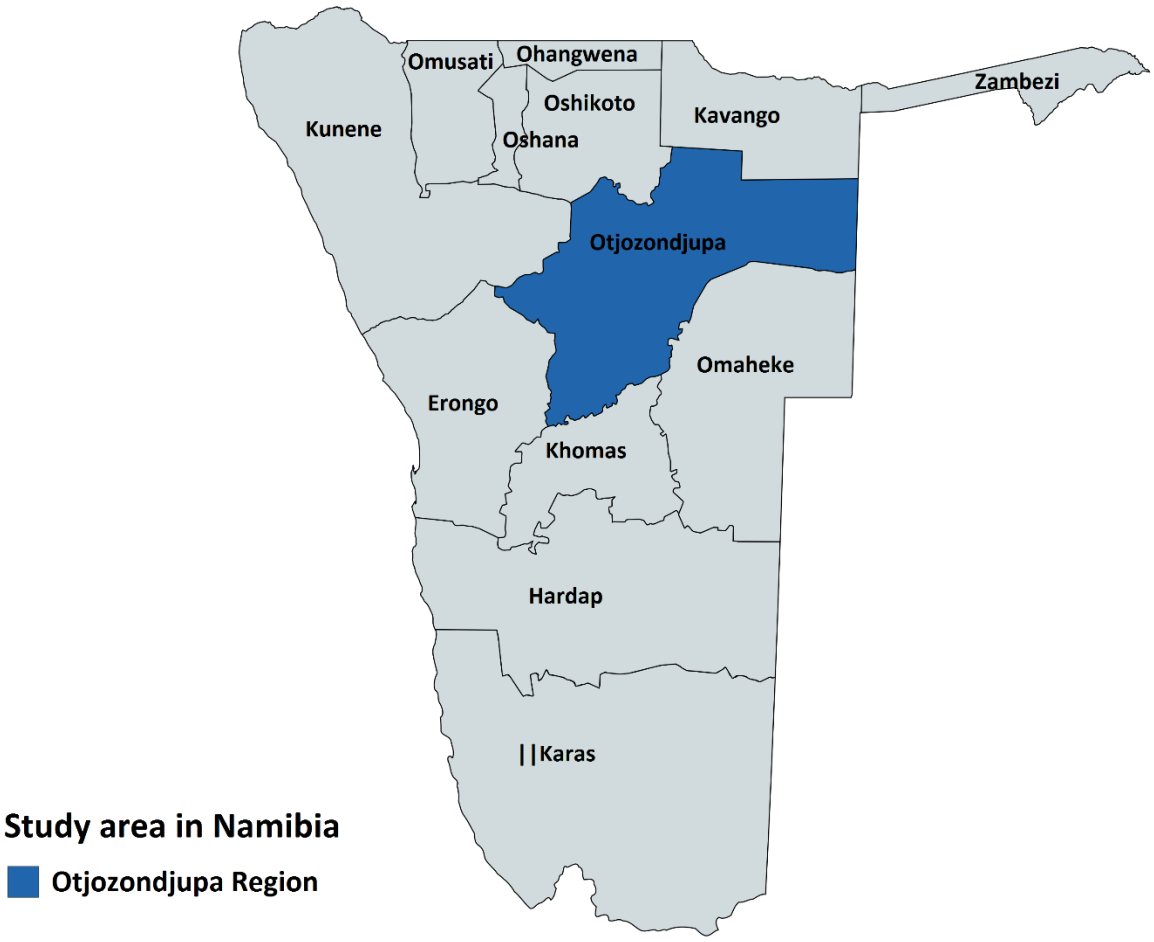

Figure 3.2b Otjozondjupa Region, Namibia

3.3. Sampling method

Marama bean accessions were collected during the 2016 harvest season from the Otjozondjupa region from the sites listed in Table 3.3.1. For each accession, the seeds, tuber, leaves and stem were collected. This study focused on the nutritional composition of the seeds (Figure 3.3.1). From a total of 521 accessions, 10 accessions were sampled from the wild and based on phenotypic characteristics as representatives of the Marama bean population in Namibia. Plants selected had variable number of seeds per pod (1-3 seeds) and seeds emerging during different times of flowering. Table 3.3.1 shows the accessions collected with the corresponding sites, Global Positioning System (GPS) locations and region in Namibia.

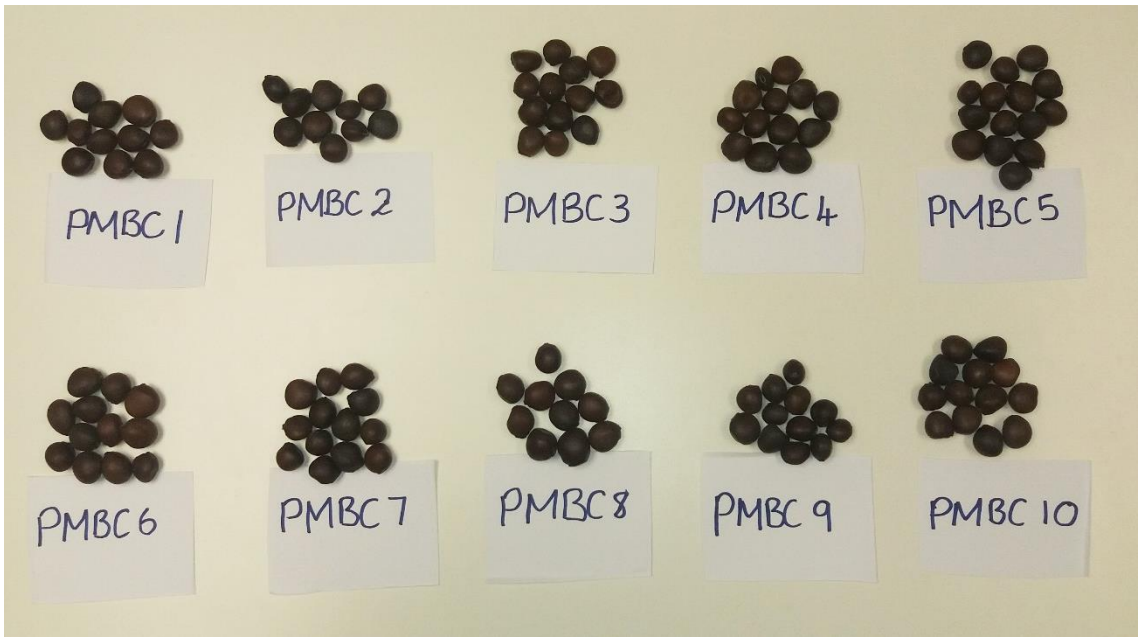

Figure 3.3.1: Marama bean seeds with corresponding accession labels

Table 3.3.1: Table of collected samples and their corresponding sites

Accession name	GPS Location	Site Collected	Region in Namibia
PMBC1	S 21°20'96.1" E 17°46'39.3"	Imkerhof	Otjozondjupa
PMBC2	S 21°15'02.8" E 17°43'34.1"	Imkerhof-Osire	Otjozondjupa
PMBC3	S 21°08'61.8" E 17°37'10.7"	Osire	Otjozondjupa
PMBC4	S 21°08'61.8" E 17°37'10.7"	Ombujondjou plot	Otjozondjupa
PMBC5	S 20°31'60.3" E 17°97'89.0"	Ombujondjou roadside	Otjozondjupa
PMBC6	S 20°41'50.5" E 17°85'73.2"	Okongoho	Otjozondjupa
PMBC7	S 20°41'75.4" E 17°87'20.0"	Okararakanua	Otjozondjupa
PMBC8	S 20°59'19.0" E 17°90'94.9"	Okararakanua	Otjozondjupa
PMBC9	S 20°59'50.6" E 18°08'63.9"	Okarombaranga	Otjozondjupa
PMBC10	S 20°66'79.3" E 18°14'68.3"	Okondjatu	Otjozondjupa

3.4. Sample preparation

A modified method for sample preparation was used following one described by Kayitesi, et al., (2012). Air dried Marama been seeds were de-hulled using a hammer. They were stored in Ziploc bags at -20°C to reduce the possibility of spoilage, moisture absorbance and to prevent oxidation. For analyses, the samples were ground into a flour (Figure 3.4.1) using a laboratory mill. Analyses of minerals, crude fats and proteins were done at the Namibia University of Science and Technology (NUST) in the Department of Natural and Applied Sciences and the Ministry of Agriculture, Water and Forestry. Ash content, on the other hand was determined in the Department of Mining and Process Engineering laboratory, NUST.

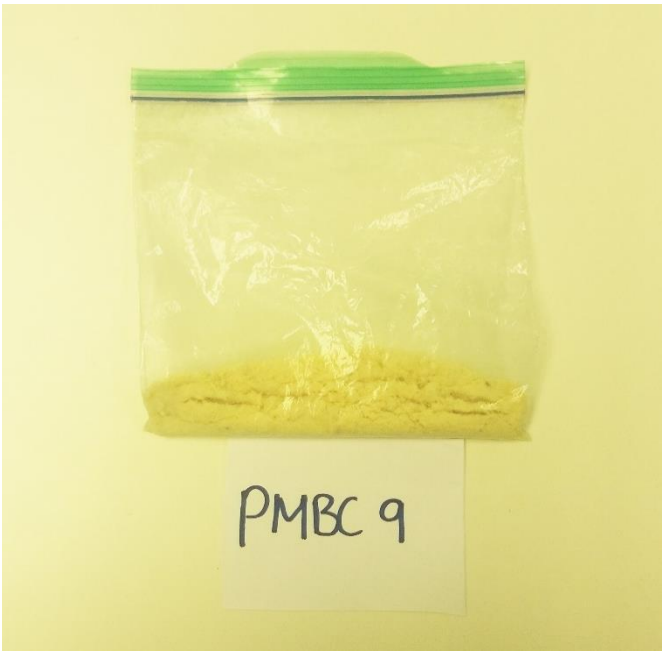

Figure 3.4.1: Marama bean flour in a sealed Ziploc bag

3.5. Ash determination

Determination of ash content was done using Marama bean cotyledons which were weighed at approximately 3.0 g each and placed in porcelain crucibles and incinerated at 500°C for 24hrs and then 650°C for 4hrs until the weight was stable (Enders & Lehmann, 2012). Ashing was considered complete when residue appeared grey (Figure 3.5.1). The crucibles and sample were weighed before and after ashing and samples were treated in triplicates. Percentage of ash was calculated following the equation:

$$\% \, Ash \, (dry) = \frac{M_{c+a} - M_c}{M_{c+s} - M_c} \times 100 \quad (3)$$

Were:

M_{c+a}= Mass of crucible + ash residue

M_{c+s}= Mass of crucible + sample

M_c= Mass of empty crucible

Figure 3.5.1 Marama bean samples after ashing using a blast furnace oven

3.6. Crude Protein Analysis

Crude protein determination was done using the **LECO TruSpec® Micro** N-Nitrogen/Protein Analyzer (CHN628) following the Dumas combustion method (Mihaljev, et al., 2015). Prior to analysis, blank runs were done in order to determine whether any impurities existed in the system. Thereafter, a standard sample of EDTA (Ethylenediaminetetraacetic acid) was run as a control sample. Approximately 140 mg of each sample including the control was weighed into tin foil cups. Samples were folded and loaded into the auto-sampler. A standard factor of 6.25 was applied to all samples in protein content calculations. Results were determined as percentage crude protein.

3.7. Mineral analysis

ICP-OES Analysis

Approximately 300 mg of each sample was digested for calcium, iron, magnesium and zinc analysis using the **PerkinElmer Titan MPS**™ Microwave system using a 20 ml mixture of aqua regia (HCl and HNO_3, 3:1). A blank sample was prepared by adding 20ml aqua regia into an empty vessel and treated like other samples. The target temperature for the running program was set at 200°C for a period of 5 minutes while the second target temperature was set at 175°C for 2 minutes. The holding temperature was set at 50°C for 3 minutes. After which the digested samples were transferred to 15ml falcon tubes and stored at 4°C until analysis.

In order to determine the concentration of minerals in the samples, a **PerkinElmer® Optima™** 8000 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) instrument was used following the instructors manual. The digested samples were prepared for analysis by adding 5ml of sample and 5ml distilled water into 15ml test tubes. Standard solutions for calcium, iron, magnesium and zinc were prepared following calculations described by the instructors manual. The standard solutions and blank were used to calibrate the ICP-OES program before analysis.

Photospectrophotometric Analysis

The analysis of phosphorus was done via a colorimetric technique using **Hach® Lange** DR6000 Benchtop **S**pectrophotometer following a method described by Agri Laboratory Association of Southern Africa (6.2.5) (2007)**.** Reagents and standards were prepared as below;

Ammonium molybdate-ammonium metavanadate reagent - 25 g of ammonium molybdate and 1.25 g of ammonium metavanadate were added to 300 ml of distilled water before being dissolved by warming the solution.

Phosphorus stock solution- 0.879 g of dried potassium dihydrogen orthophosphate was dissolved in 200 ml water before addition of 1 ml hydrochloric acid and a drop of toluene.

Sample solution and analysis

Into 50ml volumetric flasks, 5 ml of sample were added followed by 5 ml of 5M hydrochloric acid and 5ml ammonium molybdate-ammonium metavanadate reagent (resulting in a 10x dilution). The sample was diluted to 50 ml and set aside for 30 minutes. A standard curve was established

using standard solutions. Absorbance was measured at 400 nm in 10mm cuvettes for the blank and all samples. A 10x dilution factor was applied to all samples.

Phosphorus concentration was determined by applying the difference between the sample reading and blank into the following equation:

$$P(mgkg^{-1}) = \frac{C_g \times V \times 50}{5 \times m} \quad (4)$$

Where:

C_g= Difference between sample and blank readings

V= Total volume of the sample digest solution (50ml)

m= mass of sample in g

5 = Sample aliquot (5ml)

50= Dilution of sample aliquot (10x dilution)

3.8. Crude Fat analysis

Crude fat was determined using the Soxhlet Method (Agri Laboratory Association of Southern Africa, 2007). Approximately 0.2 g of boiling stones were weighed into Soxhlet flasks and dried in an oven for 15 minutes at 105°C. The flasks were placed in a desiccator until they reached room temperature. Using tongs, the flasks were labelled and weighed. The mass was recorded. Into each flask, 60 ml of petroleum ether was added for the extract of crude fat. Thereafter, approximately 3g of each Marama bean flour sample was weighed into extraction thimbles. The samples were placed into a Soxhlet extractor (Velp® Scientifica SER 148 Solvent Extractor) and immersed into the Soxhlet flasks with the program set as below. After extraction, the flasks were removed and dried in an oven at 105°C for 15 minutes until all the petroleum ether was removed and cooled in a desiccator until they were at room temperature. Using tongs, the flasks were removed and weighed, the mass was recorded. By subtracting the mass of the empty Soxhlets flasks from the mass of the flasks and the extracted crude fat, crude fat mass was determined. Therefore, fat content was calculated as a percentage of the total sample mass.

Soxhlet extractor program:

1. Immersion- 60 minutes
2. Washing- 60 minutes
3. Recovery- 60 minutes

3.9. Carbohydrate determination

The total carbohydrate content was determined by estimating its content following the description by Holse, Husted, and Hansen (2010). The carbohydrate content was estimated by calculating the percentage difference of ash, crude protein and crude fat from the sample weight using the equation:

$$Carbohydrate\ content = 100\% - (\%\ Ash + \%\ Crude\ Protein + \%Crude\ Fat) \quad (5)$$

3.10. Data analysis

The data collected were analysed using SPSS (Version 22). The Shapiro-Wilk test was used to determine normality while Kruskal Wallis was employed as the non-parametric test of choice to determine significance. Parametric tests were done using one-way analysis of variance (ANOVA) and probability was accepted at $p<0.05$. Analysis of nutrients was done in duplicates and triplicates. and in the event of calcium, magnesium and zinc, testing was done in replicates of 5. Means were calculated for all samples and presented graphically.

CHAPTER 4

RESULTS

In order to determine normality of data obtained, the Shapiro-Wilk test was performed on all data sets. The one-way analysis of variance (ANOVA) test was used to ascertain whether significant differences existed between sample means in cases in which the data were normally distributed while Kruskal Wallis was used for non-parametric analysis of data not normally distributed. While the significance level was $p \leq 0.05$ and all analysis was done using IBM SPSS Statistics (Version 22). Table 4.5 shows concentrations of all nutrients from the 10 accession samples.

4.1. Ash Content of Marama bean seeds

The percentage of ash content was between 2.13% being the lowest and 3.46% being the highest. The data were not normally distributed (Shapiro Wilk p = 0.002). The subsequent Kruskal Wallis test revealed no significant differences in ash among Marama beans ($\chi^2 = 9.267$; p = 0.413). The graph (Figure 4.1) shows the medians did not differ significantly among selected accessions.

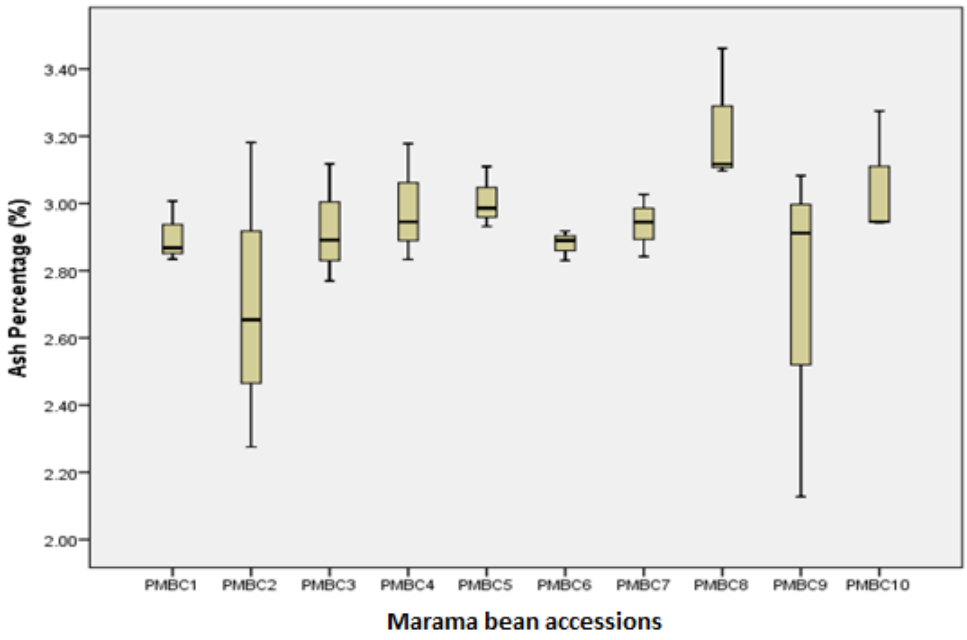

Figure 4.1 Box plot graph for ash medians

4.2. Crude Protein Content of Marama bean seeds

The data were subjected to the Shapiro-Wilk test for normality, which showed the data to be normally distributed (p=0.631). The descriptive statistics of the protein data indicates the minimum (30.1%) and maximum (34.8%) protein content observed from all accessions analysed with an average amount of 32.7%. Statistically significant differences existed among the samples with respect to protein content ($p < 0.001$). The calculated means for each accession group are presented in Figure 4.2.

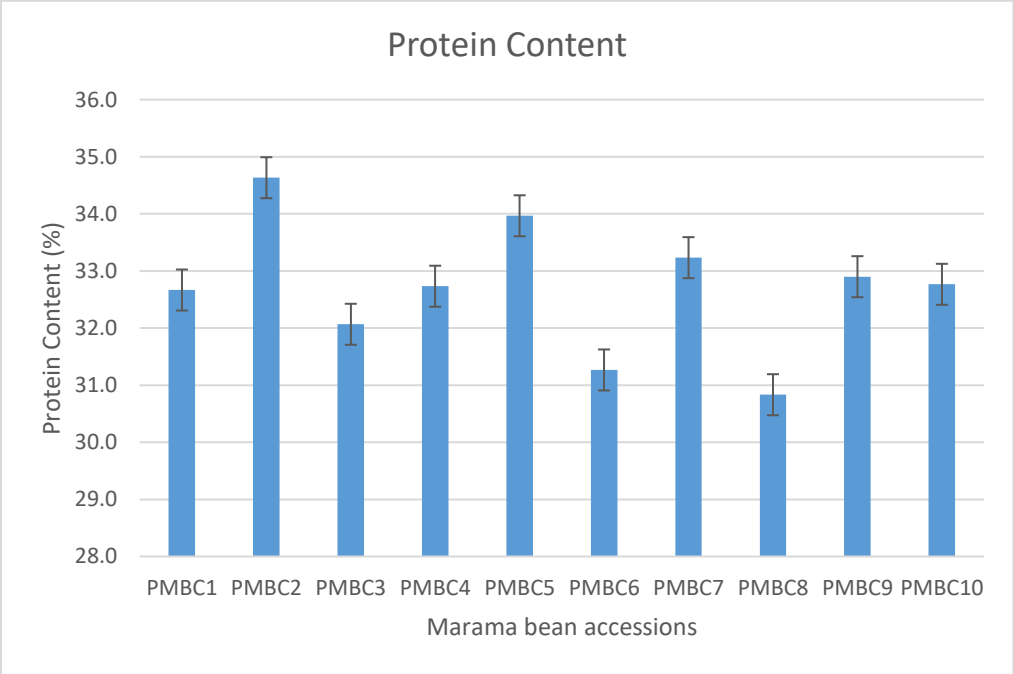

Figure 4.2 Mean Protein content within each accession

A Tukey post hoc test revealed that of the 45 pairwise comparisons performed, 27 comparisons were statistically significant indicated by an asterisk (*) in Table 4.1. Of the observations deemed statistically significant, 17 comparisons had a significantly higher mean difference while 10 comparisons had significantly lower mean differences. The highest mean difference was observed between PMBC2 and PMBC8 ($p < 0.001$) with the former being higher. The lowest mean difference was observed between PMBC8 and PMBC9 ($p < 0.001$) with the former being significantly lower. P values of the Tukey post hoc analysis are shown in Appendix B.

Table 4.1: Multiple comparisons of mean differences of Marama bean accessions

Sample	PMBC1	PMBC2	PMBC3	PMBC4	PMBC5	PMBC6	PMBC7	PMBC8	PMBC9	PMBC10
PMBC1	1	-1.967*	.600	-.067	-1.300*	1.400*	-.567	1.833*	-.233	-.100
PMBC2		1	2.567*	1.900*	.667	3.367*	1.400*	3.800*	1.733*	1.867*
PMBC3			1	-.667	-1.900*	.800	-1.167*	1.233*	-.833	-.700
PMBC4				1	-1.233*	1.467*	-.500	1.900*	-.167	-.033
PMBC5					1	2.700*	.733	3.133*	1.067*	1.200*
PMBC6						1	-1.967*	.433	-1.633*	-1.500*
PMBC7							1	2.400*	.333	.467
PMBC8								1	-2.067*	-1.933*
PMBC9									1	.133
PMBC10										1

* The mean difference is significant at $p \leq 0.05$ level.

4.3. Mineral Concentration of Marama bean seeds

Essential elements

The concentrations of the minerals were measured in mg/kg. The descriptive statistics are shown in Table 4.2. The difference between the highest and lowest mean concentrations (observed from marama bean accessions PMBC7 and PMBC9) was 47.3%, while the mean concentrations of phosphorus had a 19.6% difference between the highest and lowest concentrations. PMBC2 had the highest mean concentration while PMBC10 had the lowest. However, magnesium had the largest percent difference of means of 79.9% (PMBC3 and PMBC5).

Table 4.2 Descriptive statistics of calcium, magnesium and phosphorus.

	N	Mean	Std. Deviation	Minimum	Maximum
Calcium concentration (mg/kg)	50	1405.52	361.85	750.11	2306.22
Magnesium concentration (mg/kg)	50	2925.55	816.30	1764.12	7415.04
Phosphorus concentration (mg/kg)	20	4565.03	296.12	4300.81	5267.93

Data obtained for calcium, magnesium and phosphorus concentrations were not normality distributed (Shapiro Wilk, $p < 0.05$). Therefore, non-parametric treatments were applied to all data sets. The Kruskal Wallis Test was performed in order to determine significant differences. The p-values for calcium, magnesium and phosphorus were 0.538, 0.621 and 0.111, respectively There was no significant difference ($p > 0.05$) among the concentrations of the individual elements among the 10 accessions. The medians of the three data sets are shown in Figures 4.3, 4.4 and 4.5.

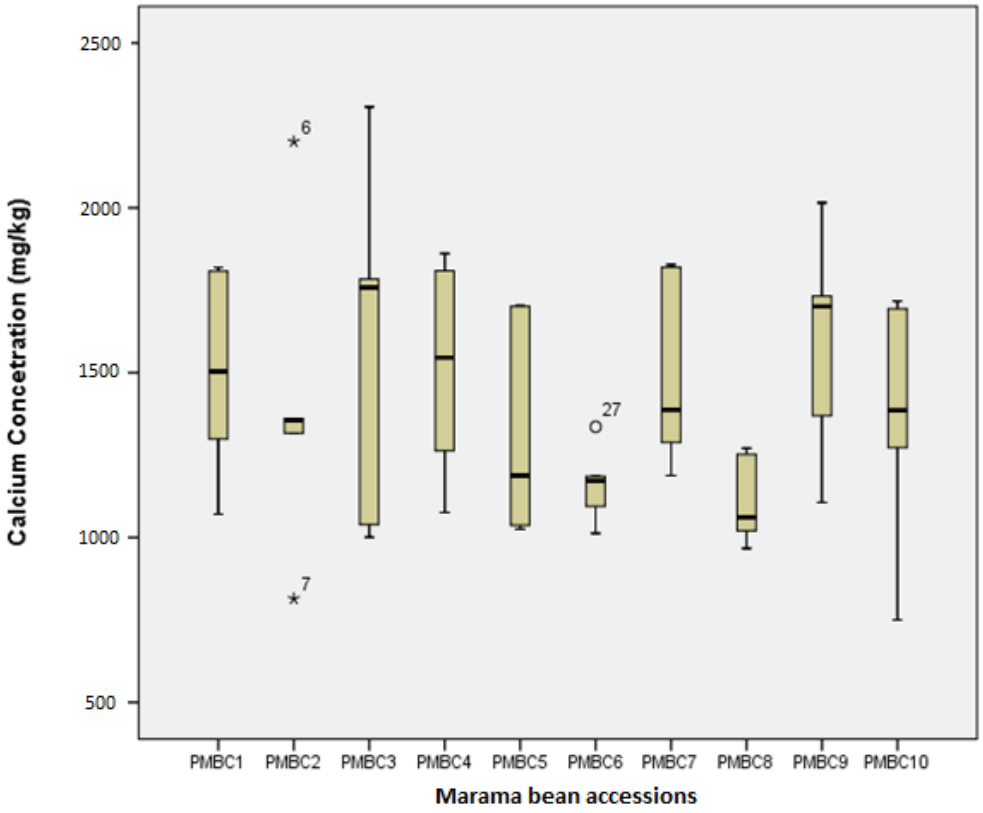

Figure 4.3 Boxplot for Calcium concentration

The Kruskal-Wallis H test for calcium concentration showed that there was no statistically significant difference among the medians of accessions of Marama bean analysed, $\chi^2 = 7.959$, $p = 0.538$, df = 9.

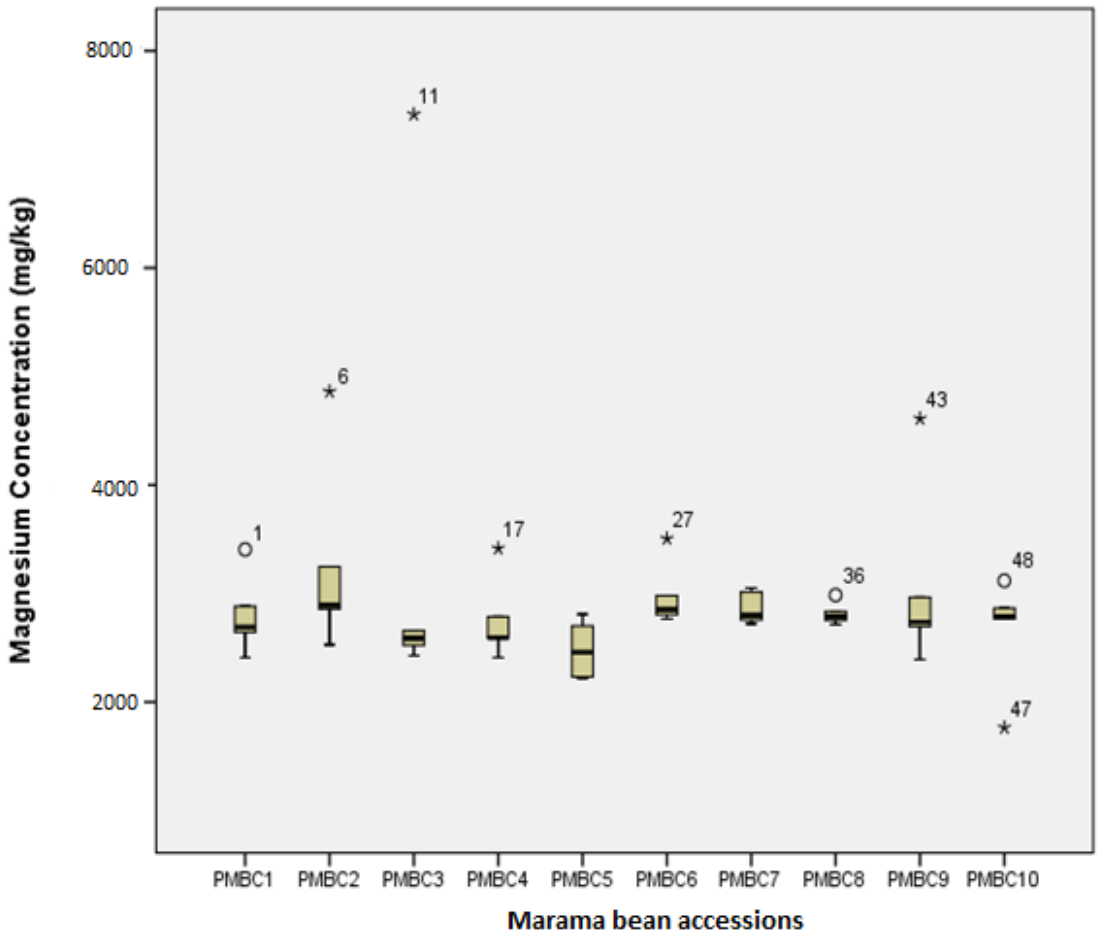

Figure 4.4 Boxplot of Magnesium concentration

Kruskal-Wallis H tests of magnesium concentration showed that there was no statistically significant difference among the accessions, $\chi^2 = 7.159$, $p = 0.621$, df = 9. The medians reflected in the boxplot show similarity with respect to the concentration of magnesium in the Marama bean samples.

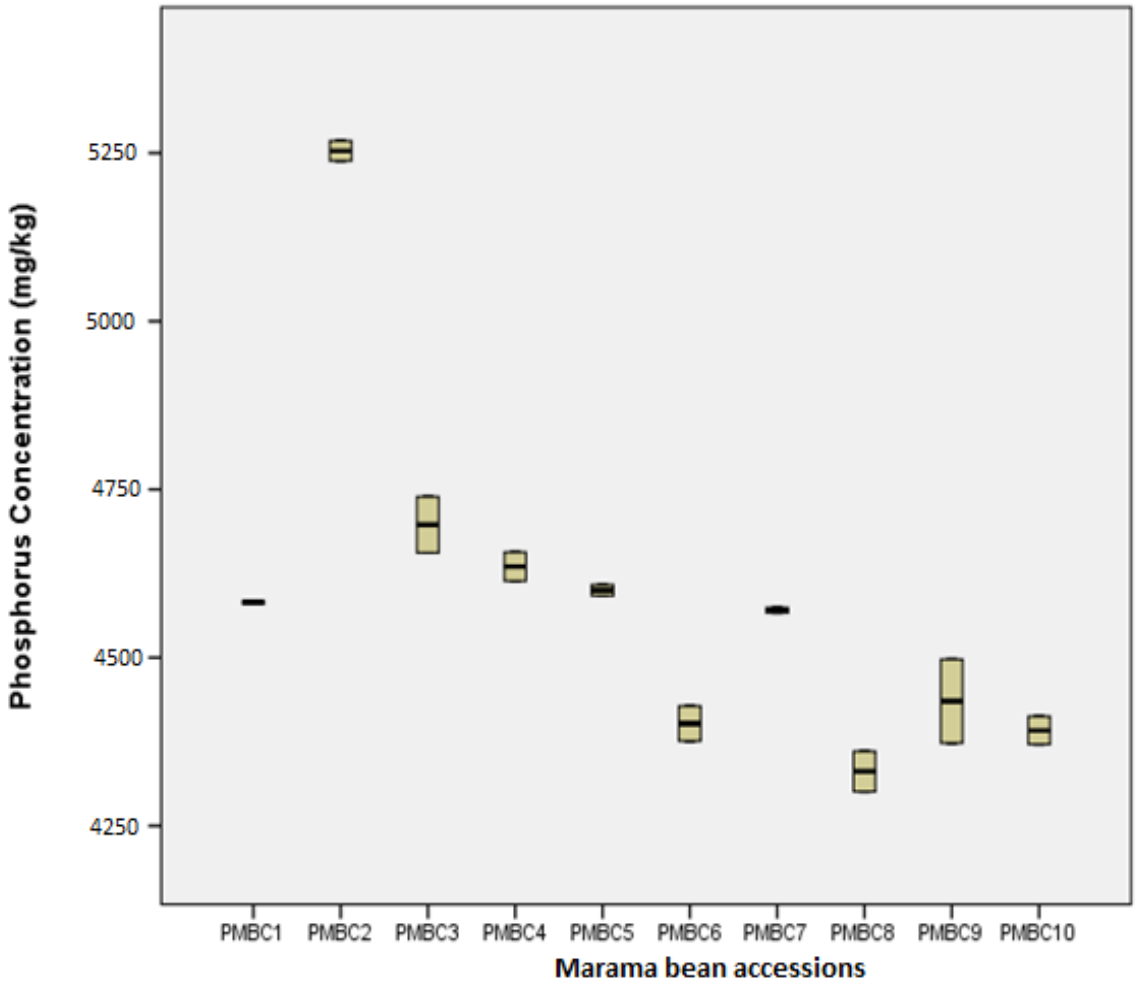

Figure 4.5 Boxplot of Phosphorus concentration

Non-parametric analysis (Kruskal-Wallis H) of phosphorus concentration in Marama bean seeds indicated that there was no statistically significant difference in nutrient concentration among the accessions analysed, $\chi^2 = 14.343$, $p = 0.111$, df = 9.

Trace elements

The descriptive statistics for data obtained on concentrations of trace elements are presented in Table 4.3. The table indicates the ranges of data obtained as maximum and minimum values. Concentrations of iron normally distributed (p = 0.598) and subsequently subjected to one-way ANOVA. There was no significant difference among the samples for the concentration of iron (p = 0.099). Figure 4.6 shows the means for the concentration of iron. Marama bean from the accession labelled PMBC4 had the highest mean concentration of iron at 322.4 mg/kg while the lowest recorded concentration was 53.9 mg/kg from PMBC7.

Table 4.3: Descriptive statistics for iron and zinc

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
Iron concentration (mg/kg)	30	107.712	110.904	34.716	618.174
Zinc concentration (mg/kg)	50	36.251	10.215	20.313	78.246

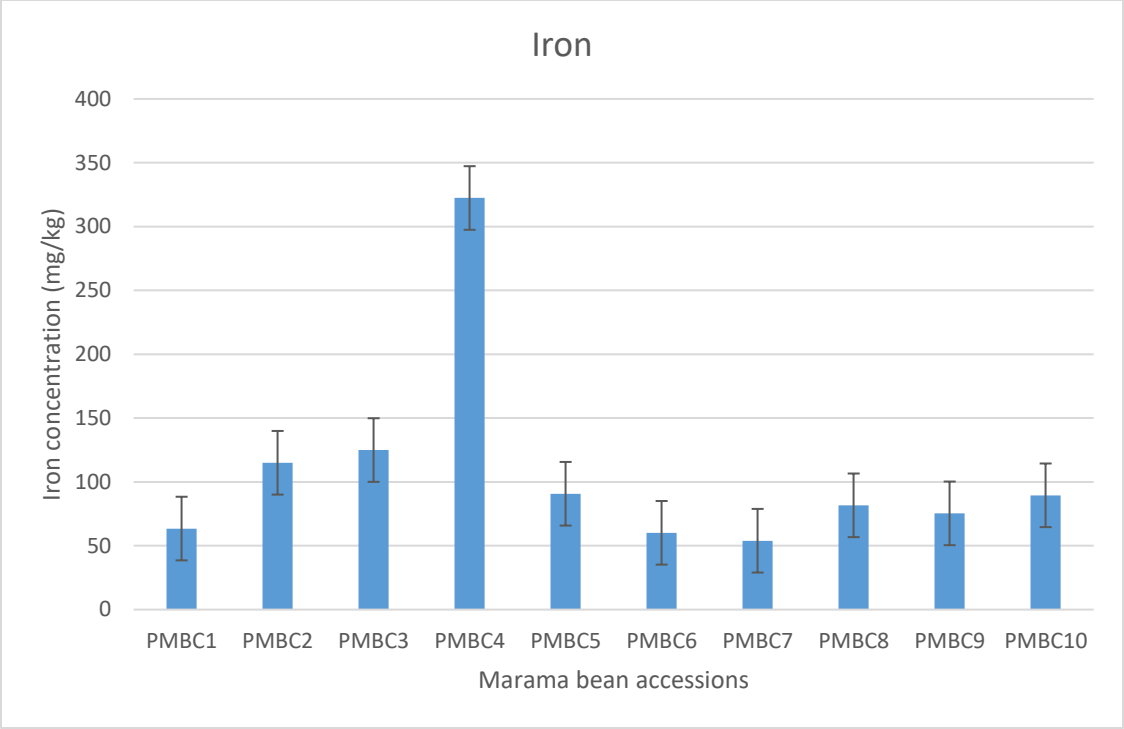

Figure 4.6 Distribution of iron content in Marama bean accessions

The Shapiro-Wilk test for normality for zinc concentrations was not normally distributed and therefore had to be subjected to the Kruskal Wallis test. The data obtained for zinc concentrations had no significant statistical difference, $\chi^2 = 3.073$, $p = 0.961$, df = 9. Figure 4.7 as a box plot graph shows there is no normality based on the medians from each sample set. A similarity in median heights is evident in the graph with the box plots exhibiting corresponding overrall heights except for PMBC4 and PMBC10 which were seen to be too high and too low, respectively with regards to their neighboring box plots. As shown in Table 4.3, the lowest concentration of zinc was in PMBC5 (32.2 mg/kg) while the highest concentration was in PMBC3 (48.8 mg/kg).

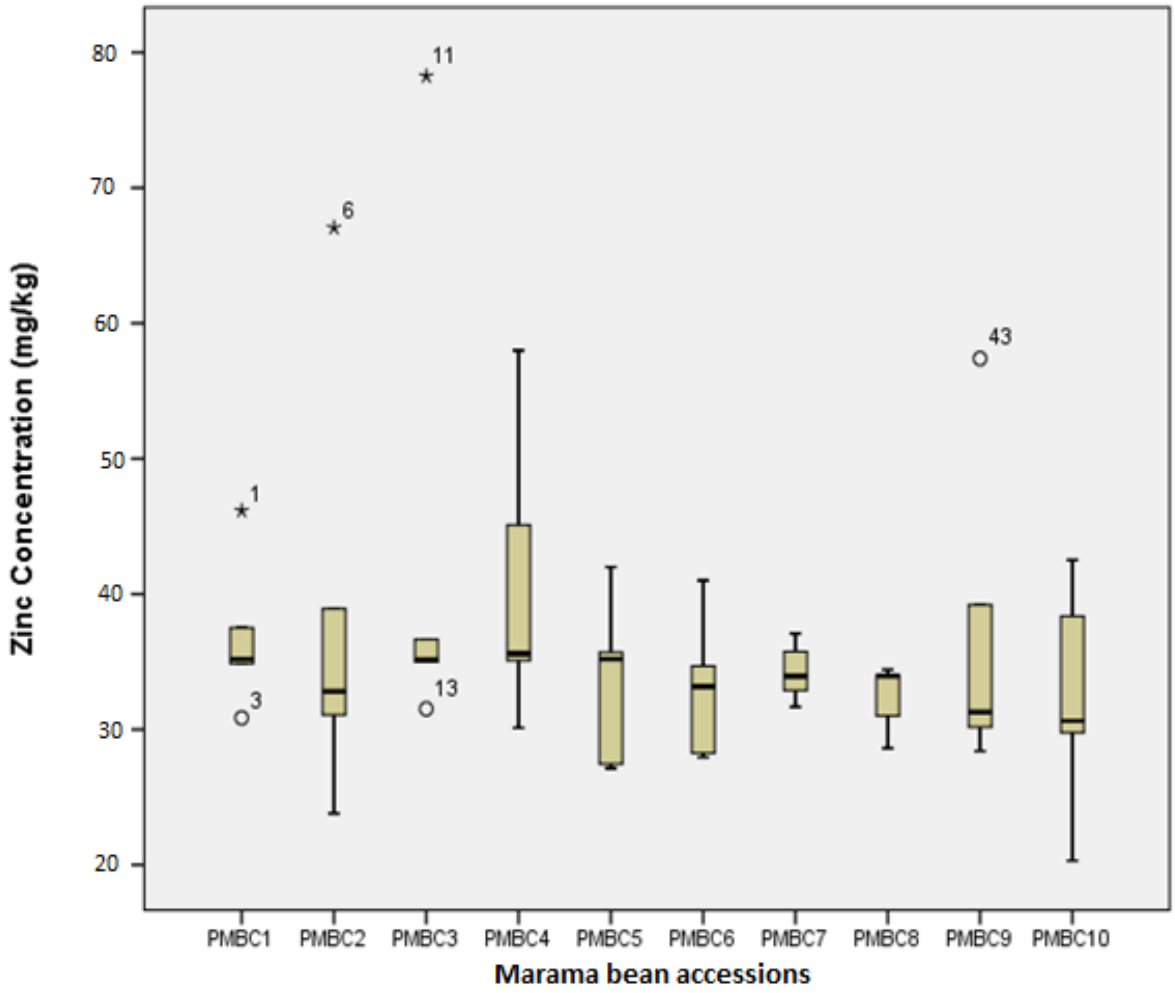

Figure 4.7 Graph of box plots for calculated zinc means

A composite analysis was performed on all minerals in order to determine whether distribution differences were present within accessions. This analysis was done by executing a multiple comparison analysis of the 5 mineral data sets. The analysis was performed to determine subgroup differences with respect to the 10 accessions. The distribution of the concentrations of the 5 minerals was similar throughout all accessions shown by the low significance therefore, the null hypothesis was rejected. However, pairwise analysis found that the concentrations between zinc-magnesium and zinc-phosphorus were significantly different as compared to the rest of the pairs for all accessions (Table 4.4).

Table 4.4: p values for multiple comparison analysis on all accessions based on minerals analysed

Marama bean Accession	All Minerals	Zinc-Magnesium	Zinc-Phosphorus
PMBC1	0.001	0.006	0.010
PMBC2	0.001	0.005	0.009
PMBC3	0.002	0.005	0.023
PMBC4	0.001	0.005	0.009
PMBC5	0.002	0.009	0.013
PMBC6	0.001	0.005	0.009
PMBC7	0.001	0.005	0.009
PMBC8	0.001	0.005	0.009
PMBC9	0.002	0.004	0.020
PMBC10	0.001	0.005	0.009

4.4. Crude Fat Content of Marama bean seeds

Data obtained from fat analysis was presented as a percentage of samples' weight. The mean crude fat content for all samples was 39.3%, while the lowest fat content was 29.9% and the highest being 44.1%. The Shapiro-Wilk test for normality showed that the data were not normally distributed and therefore the non-parametric test, Kruskal Wallis had to be applied to the data. The Kruskal Wallis H test result was observed to be $\chi^2 = 22.934$, $p = 0.006$, df = 9. This indicates that the data collected on the amount of crude fat in Marama bean samples were significantly different. Figure 4.8 shows the medians from crude fat determination for each accession. PMBC7 and PMBC8 had similar percent content of crude fat (41.6% and 41.7% respectively) the lowest mean was in PMBC6 (37.0%).

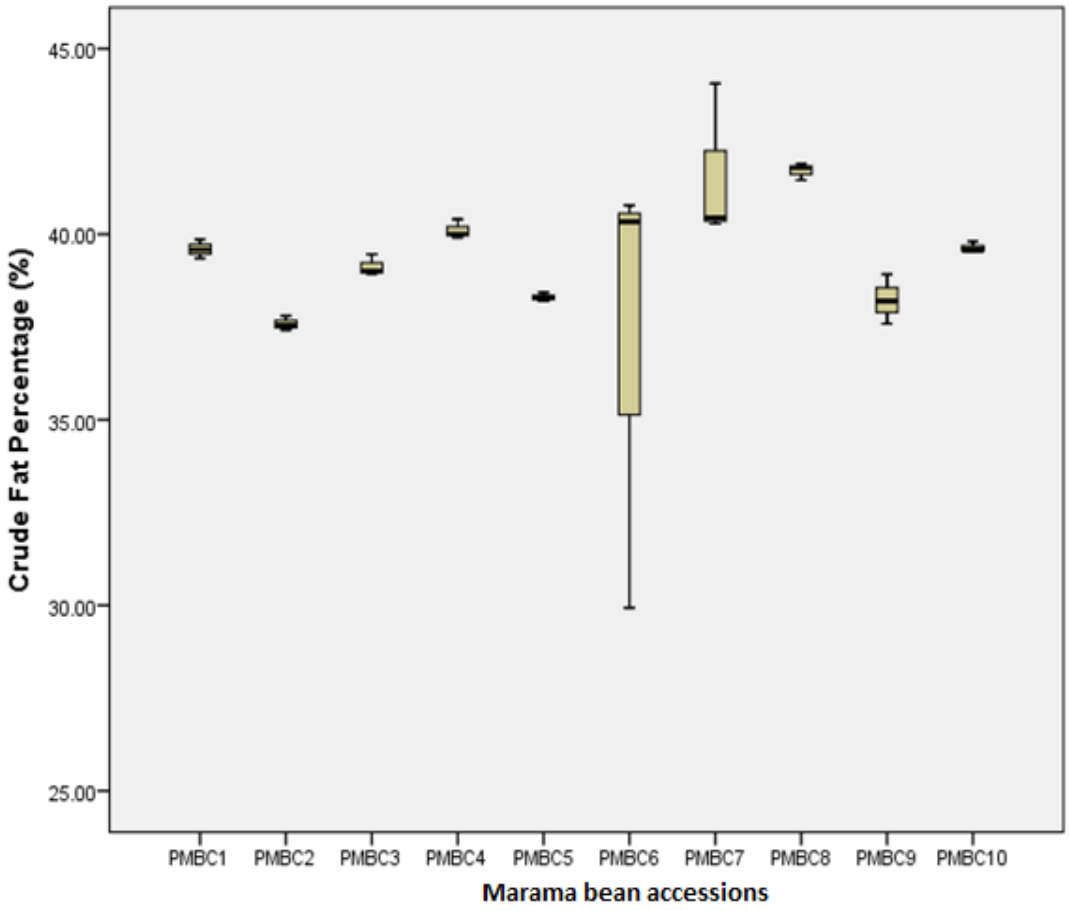

Figure 4.8 Boxplot for content of crude fat in Marama bean accessions

4.5. Carbohydrate Content of Marama bean seeds

Carbohydrate content ranged from 19.4 to 39.0%. The Shapiro Wilk test for normality showed the data to be not normally distributed ($p < 0.001$). Therefore, a non-parametric test (Kruskal Wallis) was applied to the data. There was no significant difference ($\chi^2 = 20.215$, df = 9, $p = 0.017$) among the samples of Marama bean accessions. The mean carbohydrate content for all Marama bean accession was 25.1%. Figure 4.9 indicates medians of carbohydrate content.

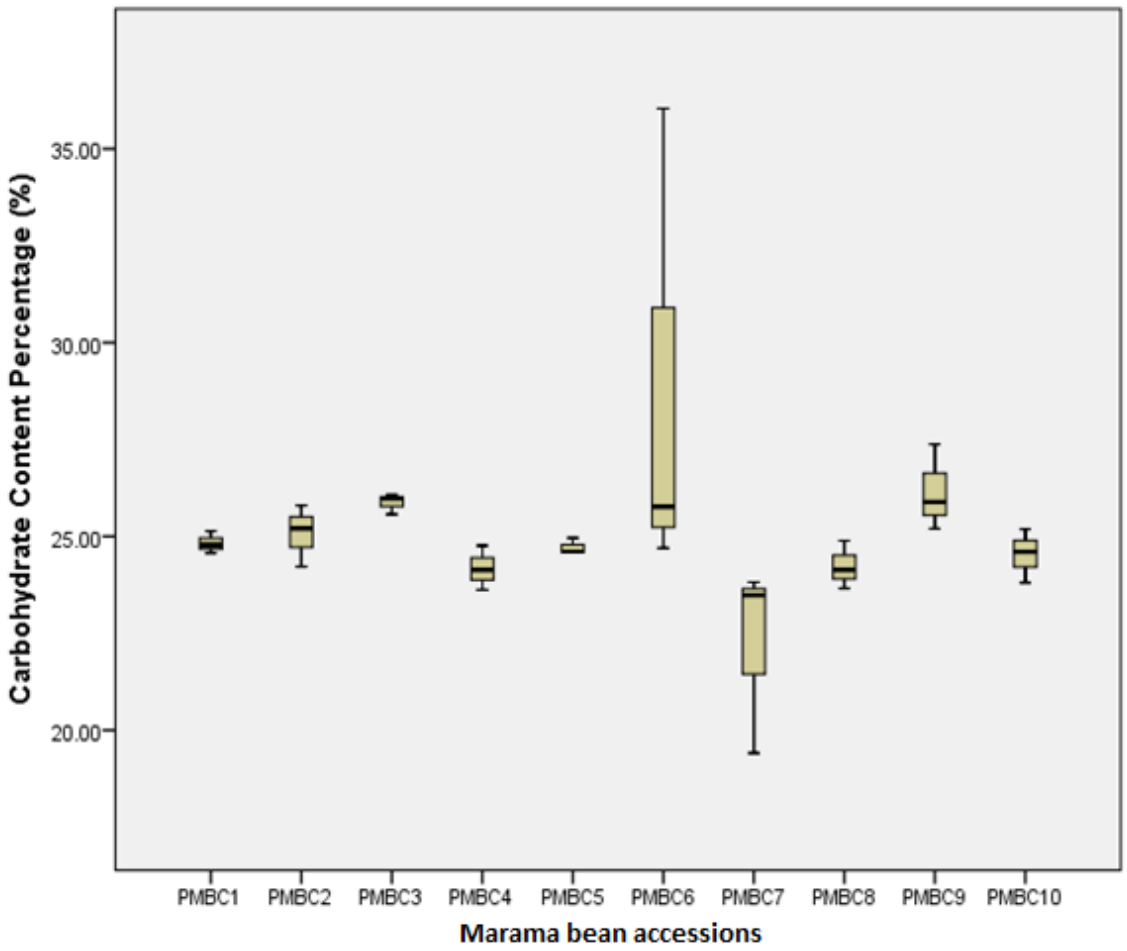

Figure 4.9 Median distribution of carbohydrate content within the different accessions

A Kruskal Wallis pairwise multiple comparison analysis was performed on protein, crude fat and carbohydrate content to determine whether there were any differences present within the accessions based on distribution. The distribution of the content of carbohydrates, crude fat and proteins were found to not have a statistically significant difference among the 10 accessions since $p < 0.05$. The null hypothesis as a result was neglected. Nevertheless, pairwise analysis determined that a difference was present between carbohydrate and crude fat content throughout all the accessions ($p = 0.021$). Figure 4.10 shows the graphical representation of the pairwise comparison and the data of the pairwise comparisons of carbohydrates, porteins and fats for PMBC2.

Each node shows the sample average rank of Nutrient.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Carbohydrate-Protein	3.000	2.227	1.347	.178	.534
Carbohydrate-Crude Fat	6.000	2.227	2.695	.007	.021
Protein-Crude Fat	3.000	2.227	1.347	.178	.534

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Figure 4.10 Pairwise comparison of Crude fats, Crude Proteins and Carbohydrates from PMBC2.

Table 4.5: Concentrations of nutrients analysed are presented as means.

Accession	Ash (%)	Calcium (mg/kg)	Magnesium (mg/kg)	Phosphorus (mg/kg)	Iron (mg/kg)	Zinc (mg/kg)	Carbohydrate (%)	Crude Fat (%)	Protein (%)
PMBC1	2.90	1500.157	2806.454	4582.200	63.46230	36.9170	24.83	39.60	32.67
PMBC2	2.70	1409.653	3277.048	5253.165	114.98440	38.7325	25.08	37.59	34.63
PMBC3	2.93	1577.684	3522.287	4697.535	124.95460	43.3043	25.87	39.13	32.07
PMBC4	2.99	1511.192	2758.562	4635.390	322.38910	40.7828	24.17	40.11	32.73
PMBC5	3.01	1331.102	2484.267	4600.025	90.73140	33.4912	27.72	38.30	33.97
PMBC6	2.88	1160.177	2982.325	4401.865	60.12180	33.0113	28.84	37.02	31.27
PMBC7	2.94	1502.261	2868.190	4570.240	53.93130	34.2670	22.24	41.59	33.23
PMBC8	3.23	1114.406	2815.086	4330.810	81.65770	32.3918	24.23	41.71	30.83
PMBC9	2.71	1584.766	3080.350	4435.370	75.37450	37.2934	26.16	38.24	32.90
PMBC10	3.06	1363.752	2660.823	4391.800	89.51300	32.3162	24.53	39.64	32.77

CHAPTER 5

DISCUSSION

This study aimed to determine the most nutritionally superior Marama bean accession from selected samples. This was done by determining the nutritional profiles of 10 accessions. The samples were analysed for both macronutrietnts and micronutrients. Statistical analysis revealed the most nutritionally superior Marama bean accession. The data presented in this study was collected from populations collected from the Otjozondjupa region, Namibia.

5.1. Ash Content of Marama bean seeds

Analysis of the different important nutrients of Marama bean populations has been carried out from bean populations from 3 countries namely Botswana, Namibia and South Africa. The purpose was to determine the nutritional composition of Marama bean from different populations (Holse, Husted, & Hansen, 2010).The ash content of any crop is used to provide a percentage content of minerals in samples of interest (Jhaumeer Laulloo, Bhowon, Soyfoo, & Chua, 2018). It reflects the total amount of minerals in a sample, however, it does not show the concentrations and is not selective of the minerals that are left, therefore, even toxic heavy metals (if present) are included in the ash mass (Zhou, et al., 2016). The results for ash content were between 2.7% and 3.2%, values consistent with previous studies on Marama bean samples from Botswana, Namibia and South Africa where ash contents of 2.5% to 3.7% were reported (Holse, Husted, & Hansen, 2010; Müseler & Schonfeldt, 2006). In a study by Holse, Husted, & Hansen, the ash content of treated Marama bean flours indicates similar values, although, an increase in ash content was noted in partially or fully defatted flours. These treated flour samples had ash values between 2.7% and 2.9% for full fat flours to 4.2% and 4.7% for partially defatted flours with the higher values in each case being for unheated samples (Kayitesi, et al., 2012).

5.2. Crude protein of Marama bean seeds

The protein content of Marama bean previously analysed was found to be between 30.1% - 34.8%. These values correspond with a previous study that found the crude protein content to be between 29% - 38% (Amonsou, Taylor, Beukes, & Minnaar, 2012). An analysis of 3 Marama bean samples harvested between 2001 and 2004 found the crude protein content ranging between

33.97% and 36.94% with an average value of 35.24%. The study found that the climate during the time samples were collected had a lesser impact on nutritional composition compared to the seasonal influence on plant growth (Müseler & Schonfeldt, 2006). The protein content parallels closely to that of soya bean which has a protein content of approximately 37.69% with a range between 36.9 - 40.1% (Etiosa, Chika, & Benedicta, 2017). Comparison of Marama bean with soya bean shows that they fall within range of each other with Marama bean falling short of soya bean by 2%. Due to their high protein contents, both Marama bean and soya bean are suitable candidates for nutrient supplements and food alternatives (Amonsou, et al., 2012). When compared with other legumes, it is observed that Marama bean is superior to other commonly consumed legumes. Cowpea (*Vigna unguiculata* L. Walp) has a crude protein content range between 23.16 - 28.13% while kidney beans or common beans (*Phaseolus vulgaris*) have an average crude protein content of 20.09% (± 0.52) (Gerrano, et al., 2019; Qayyum, et al., 2012). Therefore, the protein content of Marama bean accessions within this study compares favourably with other legumes with a crude protein content range of 30.1 - 34.8%. Statistical analysis revealed that PMBC2 had the highest mean crude protein content of the Marama bean accessions.

5.3. Mineral content of Marama bean seeds

Marama bean, as an underutilised legume with great potential, is highly comparable to other legumes such as soybean and chickpea while peanuts are a common entrant as a nutritional source. Soybean is a nutrient rich legume with significant values of the major minerals. The content of zinc and iron (essential trace elements) from one study were approximately 27.0 and 164.0 mg/kg, respectively. Calcium, magnesium and phosphorus were found to be 3003.6, 2582.4 and 6952.0 mg/kg, respectively. The highly competitive values obtained were from soybean samples from West Africa (Benin City, Nigeria) (Etiosa, Chika, & Benedicta, 2017). Cowpeas have mineral values much less than that of soybean (Gerrano, et al., 2019).

However, their mineral content is worth mentioning as cowpeas also provide significant amounts of nutrients and minerals. The content of calcium compared to that of soybean is significantly low with ranges between 0.07 - 0.16 mg/kg. The concentrations of magnesium and phosphorus were higher with ranges between 1856.0 – 2274.0 mg/kg and 4625.0 – 5924.0 mg/kg respectively. Iron and zinc content ranged from 60.64 - 105.97 mg/kg and 32.63 - 51.08 mg/kg (Gerrano, et al.,

2019). It can be seen that in both legumes, soybean and cowpea, the most abundant macro element is phosphorus with a sharp contrast being observed in the amount of calcium which may be attributed to the type of crop and soil conditions (Marles, 2017).

The mineral content of Marama bean is highly comparable to that of soybean. Calcium, in particular, was observed to range between 750.11 - 2306.22 mg/kg. Though lower compared to the mean calcium content of soybean it was considerably higher than that observed in cowpea varieties. Unlike in soybean and cowpea tests, the highest concentration of any macro element observed in Marama bean was magnesium were the minimum concentration observed was 1764.12 mg/kg and the maximum was 7415.04 mg/kg. Phosphorus concentration ranged from 4300.81 - 5267.93 mg/kg, the lowest values observed of this mineral among the three legumes mentioned in this section. The highest was observed in soybean.

Compared to soybean and cowpea, mean iron concentrations in Marama bean were lower to that of soybean. It should be noted that there was a 1666% difference between the lowest and highest concentrations of iron in Marama bean a reflection of the vast differences that may be present within acessions. However, the mean zinc concentrations were higher in Marama bean compared to both soybean and cowpea. Müseler and Schonfeldt (2006) when analysing Marama bean seeds from Namibia and Botswana found that the mean concentrations for zinc and calcium were 62 and 2410.0 mg/kg, respectively, values close to double of mean concentrations determined in this study. However, the mean concentrations of iron, phoshorus and magnesium (39.5, 4540.0 and 2745.0 mg/kg, respectively) in the same study were lower than those determined in this study with the greatest difference being observed in the concentrations of iron.

The values of mineral content in Marama bean indicate a strong similarity compared to data collected on Marama bean varieties from Botswana (BO0603), Namibia (NA0701) and South Africa (SA0703) shown in Table 5.1. The table also includes the values observed for accession PMBC2 which was observed to contain the highest protein and phosphorus contents. Both iron and zinc concentrations from PMBC samples (Namibia germplasm collection) were the highest in comparison to the other samples analysed from Botswana, Namibia and South Africa in a past study, an indication of the superiority of Marama bean accessions in that respect (Holse, Husted, & Hansen, 2010).

Table 5.1: Mineral content (mg/kg) of Marama bean varieties and Marama bean accessions

Mineral	NA0701*	BO0603*	SA0703*	PMBC	PMBC2**
P	4050–4576	3307–3383	5488–5594	4301-5268	5238-5268
Mg	3580–3593	2330–2647	3712–3783	1764-7415	2528-4860
Ca	937–1462	2038–2176	1313–1361	750-2306	814-2201
Zn	31–39	33–33	38–39	20-78	24-67
Fe	12–14	13–14	35–40	35-618	75-151

*Ranges of mineral content of Marama bean samples analysed by Holse, Husted and Hansen (2010).

**Ranges of mineral content of accession PMBC2.

The distribution of concentration was observed to be most different between zinc and phosphorus for all accessions. The distribution was analysed to determine where the most difference, if any, was among the 10 accessions. It was revealed that among the 10 Marama bean accessions, the distribution was highly similar therefore no significant difference was observed.

5.4. Crude fat content of Marama bean seeds

Comparing Marama bean with other similar legumes, soybeans are the most nutritionally competitive legumes however, the fat content of Marama bean is typically twice that of soybeans. Soybeans are known to contain between 17.0 - 20.0% fats while Marama beans previously analysed were found to contain between 32 - 42% fats (Holse, Husted, & Hansen, 2010), values that correspond with the crude fat content determined within this study (29.9 - 44.1%). However, edible species seeds of *Rynchosia* have a far less crude fat content ranging between 3.3 - 4.4% compared to Marama bean accessions (Kalidass & Mohan, 2012). Compared to a previous analysis of Marama bean seeds from Namibia and Botswana, (39.9 and 40.2%, respectively) (Müseler & Schonfeldt, 2006), Marama bean seeds analysed in this study had similar content of 39.3%. The difference in crude fat content may allude to the possible use of these legumes' plant oils in maintaining optimal cardiovascular health. Plant-based oils which are known to be low in saturated fats, thereby reducing and reversing the effects of coronary diseases, a trait most crucial to the health of all individuals (Sanchez, et al., 2019).

5.5. Carbohydrate content of Marama bean seeds

The carbohydrate content determined was presented as an estimate from the proximate content of Marama bean accessions. Moisture content was not considered in Marama bean accession

samples used in this research as the samples were dried before analysis, therefore, there were minuscule amounts of moisture. Carbohydrate content determined ranged from 19.4 to 36.0%, with the highest calculated percentage being taken as an anomaly as it deviated from the previously determined maximum amount of carbohydrates in Marama beans of 24.0%. Prior studies have estimated the carbohydrate content of Marama bean samples from Botswana, Namibia and South Africa to be between 19.0 - 24.0% (Holse, Husted, & Hansen, 2010). This estimation found dietary fibre to be the most abundant within the carbohydrates. Another study found the mean carbohydrate content to be approximately 14.1% (Müseler & Schonfeldt, 2006). A calculated estimation much lower compared to the values obtained in this study.

The comparable benefits of Marama bean go beyond the nutritional status of the crop. Its versatility in arid climates particularly like that in most parts of Namibia, make it highly appealing for crop development and eventual cultivation. The common bean, for example, requires fertiliser and inoculation of the soil with Rhizobium bacteria for optimum yield. Furthermore, the crop also requires a constant supply of water in clay loam soils (Jacobi, 2008). Cowpea on the other has less requirements as it grows well in sandy topsoils. It is drought tolerant and is known to thrive well in arid and semi-arid climates. However, to obtain optimum yield fertiliser additives are still required in the form of basal fertilisers added to the soil as part of the preparation before planting (Gerrano, et al., 2019; Jacobi, 2008).

CHAPTER 6

RECOMMENDATIONS AND CONCLUSIONS

6.1. Recommendations

This study focused on Marama bean growing naturally in the Otjozondjupa region of Namibia. It would be worth investigating the nutritional compositions of Marama bean accessions from other regions in Namibia and other countries as well as it would provider a wider range of possibly better varieties. From the findings obtained from this study, it is also suggested that a full vitamin analysis be done in order to profile the Marama bean accessions with respect to both water soluble and fat soluble vitamins particularly vitamin B complexes, vitamin A and vitamin E. It is also recommended that testing be done on composite flours of Marama bean together with other commonly consumed cereals like sorghum and maize to determine the suitability of the compositions to use to treat malnourished children under the age of 5 years. Therefore, it is recommended that trials on the composite flours in different meals be initiated using PMBC2 based on its protein content.

6.2. Conclusion

This research aimed to determine the nutritional profiles of Marama bean accessions in Namibia. Current knowledge of Marama bean in Namibia and Southern Africa includes the chemical composition, phenotypic characteristics and other health-promoting properties of Marama bean. It is also known that differences in the nutritional profiles of Marama bean are largely attributed to the seasonal differences rather than the location in which the plants grow. This study was able to provide nutritional profiles of Marama bean accessions specific to Namibia, a present gap in knowledge. The ash content was used to determine the total amount of minerals by mass. It was found that phosphorus was the most abundant mineral while zinc had the lowest total concentration across all accessions. Analysis of the minerals (calcium, iron, magnesium, phosphorus and zinc) did not find a significant difference among the accessions.

However, differences were observed within the accessions between zinc-magnesium and zinc-phosphorus among the different accessions. Analysis of crude fats and carbohydrates also found that no accession had significantly higher amounts of the nutrients. Protein content, on the other hand, was observed to be significantly different among the 10 accession samples. The greatest difference was observed between PMBC2 and PMBC8 (3.8, Table 4.2) with the latter having a higher concentration of proteins. Therefore, this suggests that with respect to protein content accession PMBC2 is most suitable for use in Marama bean domestication and use as a biofortifier.

In conclusion, Marama bean accessions analysed in this research did not exhibit a significant difference with respect to the concentration of carbohydrates, crude fats and minerals (calcium, iron, magnesium, phosphorus and zinc), therefore, all 10 accessions will be suitable for cultivation with the aim of treating malnutrition in children under the age of 5 years. Protein content, however, was highest in PMBC2. As a result, accession PMBC2 collected from Imkerhof-Osire (S 21°15’02.8” E 17°43’34.1”), as a variety, is recommended for commercial cultivation.

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APPENDICES

Appendix A: HAS Feedback and Ethical Clearance

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FACULTY OF HEALTH AND APPLIED SCIENCES

DECISION/FEEDBACK ON RESEARCH PROPOSAL ETHICAL CLEARANCE

Dear Prof/Dr/Mr/Ms/Other(s): Ms Paidamoyo Natasha Mataranyika

Student No (if applicable):

Research Topic:	COMPARATIVE ANALYSIS OF THE NUTRITIONAL STATUS OF TYLOSEMA ESCULENTUM (MARAMA BEAN) GERMPLASM COLLECTION IN NAMIBIA
Supervisor (if applicable):	Prof Percy M. Chimwamurombe
Co-supervisor(s): if applicable	Mrs Buhlebenkosi Mpofu
Qualification registered for (if applicable):	Master in Health Sciences

Re: Ethical screening application No: REC: HHS 08/2018

The Research Ethics Screening Committee has reviewed your application for the above-mentioned research project. Based on the recommendation of the expert reviewer, the research as set out in the application is hereby:

(Indicate with an X)

Approved: i.e. may proceed with the project	X	
Approved provisionally: i.e. may proceed but subject to compliance with recommendation(s) listed below		
Not approved: Not to proceed with the project until compliance with recommendation(s) listed below and resubmit ethics application for consideration		
IS MINISTRY OF HEALTH & SOCIAL SERVICES (MoHSS) APPROVAL REQUIRED?	YES:	NO: X

It is important to note that as a researcher, you are expected to maintain ethical integrity of your research, strictly adhere to the ethical policy of NUST, and remain within the scope of your research proposal and supporting evidence as submitted to the REC. Should any aspect of your research change from the information as presented, which could have an impact or effect on any research participants/subjects/environment, you are to report this immediately to your supervisor or REC as applicable in writing. Failure to do so may result in withdrawal of approval. Kindly consult your supervisor or HoD if you need further clarification. We wish you success in your research endeavour and are of the belief that it will have positive impact on your career as well as the development of NUST and the society in general.

Ethical issues that require compliance/ must be addressed		
No.	**Ethical issues**	**Comment/recommendation**
1.		
2.		

NB: May attach additional page as required

Full Name (reviewer): Larai Aku-Akai Signature: ..... Date: 19/07/18

Full Name: Munyaradzi Mukesi ... Signature: ..... Date: 20/07/2018
Chair: Ethics Screening Committee

Appendix B: Statistical analysis tables

Post Hoc multiple comparisons of Protein Content among the accessions

Tukey HSD: (Dependent Variable: Protein Content)						
(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PMBC1	PMBC2	-1.96667*	.26583	.000	-2.9080	-1.0253
	PMBC3	.60000	.26583	.454	-.3413	1.5413
	PMBC4	-.06667	.26583	1.000	-1.0080	.8747
	PMBC5	-1.30000*	.26583	.003	-2.2413	-.3587
	PMBC6	1.40000*	.26583	.001	.4587	2.3413
	PMBC7	-.56667	.26583	.528	-1.5080	.3747
	PMBC8	1.83333*	.26583	.000	.8920	2.7747
	PMBC9	-.23333	.26583	.996	-1.1747	.7080
	PMBC10	-.10000	.26583	1.000	-1.0413	.8413
PMBC2	PMBC1	1.96667*	.26583	.000	1.0253	2.9080
	PMBC3	2.56667*	.26583	.000	1.6253	3.5080
	PMBC4	1.90000*	.26583	.000	.9587	2.8413
	PMBC5	.66667	.26583	.321	-.2747	1.6080
	PMBC6	3.36667*	.26583	.000	2.4253	4.3080
	PMBC7	1.40000*	.26583	.001	.4587	2.3413
	PMBC8	3.80000*	.26583	.000	2.8587	4.7413
	PMBC9	1.73333*	.26583	.000	.7920	2.6747
	PMBC10	1.86667*	.26583	.000	.9253	2.8080
PMBC3	PMBC1	-.60000	.26583	.454	-1.5413	.3413
	PMBC2	-2.56667*	.26583	.000	-3.5080	-1.6253
	PMBC4	-.66667	.26583	.321	-1.6080	.2747
	PMBC5	-1.90000*	.26583	.000	-2.8413	-.9587
	PMBC6	.80000	.26583	.140	-.1413	1.7413
	PMBC7	-1.16667*	.26583	.008	-2.1080	-.2253
	PMBC8	1.23333*	.26583	.005	.2920	2.1747
	PMBC9	-.83333	.26583	.111	-1.7747	.1080
	PMBC10	-.70000	.26583	.265	-1.6413	.2413
PMBC4	PMBC1	.06667	.26583	1.000	-.8747	1.0080
	PMBC2	-1.90000*	.26583	.000	-2.8413	-.9587
	PMBC3	.66667	.26583	.321	-.2747	1.6080
	PMBC5	-1.23333*	.26583	.005	-2.1747	-.2920
	PMBC6	1.46667*	.26583	.001	.5253	2.4080
	PMBC7	-.50000	.26583	.681	-1.4413	.4413

	PMBC8	1.90000*	.26583	.000	.9587	2.8413
	PMBC9	-.16667	.26583	1.000	-1.1080	.7747
	PMBC10	-.03333	.26583	1.000	-.9747	.9080
PMBC5	PMBC1	1.30000*	.26583	.003	.3587	2.2413
	PMBC2	-.66667	.26583	.321	-1.6080	.2747
	PMBC3	1.90000*	.26583	.000	.9587	2.8413
	PMBC4	1.23333*	.26583	.005	.2920	2.1747
	PMBC6	2.70000*	.26583	.000	1.7587	3.6413
	PMBC7	.73333	.26583	.216	-.2080	1.6747
	PMBC8	3.13333*	.26583	.000	2.1920	4.0747
	PMBC9	1.06667*	.26583	.019	.1253	2.0080
	PMBC10	1.20000*	.26583	.006	.2587	2.1413
PMBC6	PMBC1	-1.40000*	.26583	.001	-2.3413	-.4587
	PMBC2	-3.36667*	.26583	.000	-4.3080	-2.4253
	PMBC3	-.80000	.26583	.140	-1.7413	.1413
	PMBC4	-1.46667*	.26583	.001	-2.4080	-.5253
	PMBC5	-2.70000*	.26583	.000	-3.6413	-1.7587
	PMBC7	-1.96667*	.26583	.000	-2.9080	-1.0253
	PMBC8	.43333	.26583	.819	-.5080	1.3747
	PMBC9	-1.63333*	.26583	.000	-2.5747	-.6920
	PMBC10	-1.50000*	.26583	.001	-2.4413	-.5587
PMBC7	PMBC1	.56667	.26583	.528	-.3747	1.5080
	PMBC2	-1.40000*	.26583	.001	-2.3413	-.4587
	PMBC3	1.16667*	.26583	.008	.2253	2.1080
	PMBC4	.50000	.26583	.681	-.4413	1.4413
	PMBC5	-.73333	.26583	.216	-1.6747	.2080
	PMBC6	1.96667*	.26583	.000	1.0253	2.9080
	PMBC8	2.40000*	.26583	.000	1.4587	3.3413
	PMBC9	.33333	.26583	.953	-.6080	1.2747
	PMBC10	.46667	.26583	.753	-.4747	1.4080
PMBC8	PMBC1	-1.83333*	.26583	.000	-2.7747	-.8920
	PMBC2	-3.80000*	.26583	.000	-4.7413	-2.8587
	PMBC3	-1.23333*	.26583	.005	-2.1747	-.2920
	PMBC4	-1.90000*	.26583	.000	-2.8413	-.9587
	PMBC5	-3.13333*	.26583	.000	-4.0747	-2.1920
	PMBC6	-.43333	.26583	.819	-1.3747	.5080
	PMBC7	-2.40000*	.26583	.000	-3.3413	-1.4587
	PMBC9	-2.06667*	.26583	.000	-3.0080	-1.1253
	PMBC10	-1.93333*	.26583	.000	-2.8747	-.9920

PMBC9	PMBC1	.23333	.26583	.996	-.7080	1.1747
	PMBC2	-1.73333*	.26583	.000	-2.6747	-.7920
	PMBC3	.83333	.26583	.111	-.1080	1.7747
	PMBC4	.16667	.26583	1.000	-.7747	1.1080
	PMBC5	-1.06667*	.26583	.019	-2.0080	-.1253
	PMBC6	1.63333*	.26583	.000	.6920	2.5747
	PMBC7	-.33333	.26583	.953	-1.2747	.6080
	PMBC8	2.06667*	.26583	.000	1.1253	3.0080
	PMBC10	.13333	.26583	1.000	-.8080	1.0747
PMBC10	PMBC1	.10000	.26583	1.000	-.8413	1.0413
	PMBC2	-1.86667*	.26583	.000	-2.8080	-.9253
	PMBC3	.70000	.26583	.265	-.2413	1.6413
	PMBC4	.03333	.26583	1.000	-.9080	.9747
	PMBC5	-1.20000*	.26583	.006	-2.1413	-.2587
	PMBC6	1.50000*	.26583	.001	.5587	2.4413
	PMBC7	-.46667	.26583	.753	-1.4080	.4747
	PMBC8	1.93333*	.26583	.000	.9920	2.8747
	PMBC9	-.13333	.26583	1.000	-1.0747	.8080

*. The mean difference is significant at the 0.05 level

Appendix C: Concentrations of nutrients analysed

Sample	Ash (%)	Calcium (mg/kg)	Magnesium (mg/kg)	Phosphorus (mg/kg)	Iron (mg/kg)	Zinc (mg/kg)	Carbohydrate (%)	Crude Fat (%)	Protein (%)
PMBC1	2.87	1503.866	3405.528	4580.480	90.306	46.152	24.57	39.86	32.70
PMBC1	3.01	1298.535	2883.221	4583.920	62.083	37.509	25.14	39.35	32.50
PMBC1	2.83	1070.856	2410.523	-	37.999	30.854	24.78	39.59	32.80
PMBC1	-	1819.630	2643.020	-	-	35.190	-	-	-
PMBC1	-	1807.900	2689.980	-	-	34.880	-	-	-
PMBC2	2.28	2200.968	4860.110	5238.400	150.980	67.054	25.80	37.42	34.50
PMBC2	2.65	814.501	2528.307	5267.930	118.640	23.797	25.21	37.54	34.60
PMBC2	3.18	1316.585	3247.004	-	75.334	38.912	24.22	37.80	34.80
PMBC2	-	1360.990	2856.820	-	-	32.820	-	-	-
PMBC2	-	1355.220	2893.000	-	-	31.080	-	-	-
PMBC3	2.77	2306.222	7415.043	4655.960	251.250	78.246	25.57	39.46	32.20
PMBC3	3.12	1039.001	2588.071	4739.110	67.452	36.647	25.98	39.00	31.90
PMBC3	2.89	1000.568	2428.520	-	56.161	31.518	26.07	38.94	32.10
PMBC3	-	1758.260	2523.390	-	-	35.130	-	-	-
PMBC3	-	1784.370	2656.410	-	-	34.980	-	-	-
PMBC4	2.83	1263.368	2787.311	4613.930	247.493	45.120	24.76	39.91	32.50
PMBC4	3.18	1545.620	3415.185	4656.850	618.174	57.990	23.62	40.40	32.80
PMBC4	2.95	1076.069	2409.407	-	101.500	30.124	24.14	40.01	32.90
PMBC4	-	1809.320	2589.350	-	-	35.600	-	-	-
PMBC4	-	1861.580	2591.560	-	-	35.090	-	-	-
PMBC5	2.99	1187.839	2459.578	4608.060	181.171	41.997	24.61	38.20	34.20
PMBC5	2.93	1037.333	2220.133	4591.990	56.307	27.141	24.59	38.28	34.20
PMBC5	3.11	1025.628	2231.216	-	34.716	27.438	24.96	38.43	33.50
PMBC5	-	1703.700	2701.520	-	-	35.190	-	-	-
PMBC5	-	1701.010	2808.890	-	-	35.690	-	-	-

Concentrations of nutrients analysed (continued)

PMBC6	2.92	1094.300	2979.119	4375.900	57.000	34.688	24.70	40.78	31.60
PMBC6	2.83	1335.948	3505.163	4427.830	66.126	41.009	36.04	29.93	31.20
PMBC6	2.89	1013.237	2807.363	-	57.240	33.170	25.77	40.34	31.00
PMBC6	-	1185.120	2766.900	-	-	27.940	-	-	-
PMBC6	-	1172.280	2853.080	-	-	28.250	-	-	-
PMBC7	3.03	1188.135	2720.970	4566.430	42.752	31.674	19.41	44.06	33.50
PMBC7	2.94	1288.481	3012.969	4574.050	53.621	35.746	23.48	40.28	33.30
PMBC7	2.84	1387.159	3051.758	-	65.421	37.095	23.82	40.44	32.90
PMBC7	-	1827.490	2756.490	-	-	32.890	-	-	-
PMBC7	-	1820.040	2798.760	-	-	33.930	-	-	-
PMBC8	3.12	1061.378	2985.532	4300.810	112.983	34.422	24.89	41.89	30.10
PMBC8	3.10	1020.318	2833.174	4360.810	65.717	33.949	24.14	41.46	31.30
PMBC8	3.46	967.142	2787.033	-	66.274	33.988	23.66	41.78	31.10
PMBC8	-	1252.160	2715.170	-	-	28.610	-	-	-
PMBC8	-	1271.030	2754.520	-	-	30.990	-	-	-
PMBC9	2.91	1106.277	2391.143	4497.600	48.976	28.406	25.89	38.20	33.00
PMBC9	2.13	1369.251	2964.160	4373.140	74.356	39.212	27.38	37.59	32.90
PMBC9	3.08	2015.383	4611.358	-	102.791	57.399	25.20	38.92	32.80
PMBC9	-	1732.080	2737.960	-	-	31.280	-	-	-
PMBC9	-	1700.840	2697.130	-	-	30.170	-	-	-
PMBC10	2.95	1272.426	2865.124	4371.000	98.515	38.374	24.60	39.55	32.90
PMBC10	2.94	750.1082	1764.121	4412.600	46.200	20.313	25.19	39.57	32.30
PMBC10	3.28	1385.828	3118.590	-	123.825	42.515	23.81	39.81	33.10
PMBC10	-	1693.750	2789.220	-	-	29.750	-	-	-
PMBC10	-	1716.650	2767.410	-	-	30.630	-	-	-