DEVELOPMENT OF IRON FORTIFIED CASSAVA *MAHEWU*

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A thesis submitted in fulfillment of the requirements of the degree of Philosophiae Doctor (PhD) at School of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria

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School children can improve dietary iron intake by consuming cassava *mahewu* fortified with iron

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DEDICATION

I have fulfilled my desire with the help of my fabulous family. This doctoral degree is dedicated to them with love and gratitude:

My parents, Salvador and Argentina who are supportive, loving and wise you mean the world to me.

Adelino - brother my achievement would not have been possible without your assistance and love.

Norgia, Aren, Elon and Aurelio – daughter, grandsons and son in law my achievement would not have meaning without you.

DECLARATION

I, Elsa Maria Salvador, hereby declare that the dissertation which I have submitted for the degree Doctor Philosophy at the University of Pretoria is my own work and has not previously been submitted by me to this or any other tertiary institution.

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Elsa Maria Salvador Date

DEVELOPMENT OF IRON FORTIFIED CASSAVA *MAHEWU*

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SUMMARY

In Mozambique 97% of the small scale farmers grow cassava in 43% of the total cultivated land. In 2012 cassava production was estimated at 10.05 million tons. Cassava is the second most important staple food after maize. Consumption of cassava is estimated at 85 kg per person per year. In Mozambique the diet is poor in micronutrients and the prevalence of under-nutrition is estimated at 38%. Anaemia is a serious public health concern, affecting more than 40% of young children, pregnant and nursing mothers. Cassava *mahewu* a fermented non-alcoholic beverage prepared using indigenous traditional technology. Fermentation is known to reduce the toxicity of cyanogenic glycosides in both leaves and cassava roots. It also results in higher levels of vitamins, especially the B group, essential amino acids, improves the digestibility of protein and increases the bioavailability of minerals. The aim of the present study was to investigate whether cassava, in the form of *mahewu*, can be fortified with iron.

Bitter and sweet cassava roots and soil samples, were collected from small scale farmers in four Districts of Mozambique. The four Districts are located in intermediate and high production areas of cassava. The concentrations of iron and other minerals such as aluminum, calcium, copper, manganese, phosphorus, lead and zinc in cassava roots and soil, were determined using an inductively coupled-plasma optical emission spectrometer (ICP-OES), after microwave digestion.

Sweet and bitter varieties of cassava from four Districts in Mozambique were fermented in a food laboratory, under controlled conditions (45°C for 24 hours) and the optimal stage for iron fortification was determined. Samples were collected at hour 0 and hour 24 for microbial analysis, acid concentration and total solids determination. Fortification with ferrous sulfate ($FeSO_4.7H_2O$) and ferrous fumarate ($C_4H_2FeO_4$) were investigated. The total iron content of the fortified cassava *mahewu* was determined using Microwave Digestion Accelerated Reaction System (MARS) and ICP-OES, while the bioaccessibility

of iron in fortified cassava *mahewu* was assessed using the *in vitro* dialysability procedure.

Results showed that the mineral content of soil from the four districts differed significantly ($p < 0.05$). There was also a significant difference ($p < 0.05$) in the mineral concentration of the cassava roots from the four varieties, but no difference between sweet or bitter types. The concentration of minerals was found to be significantly higher in soil, than in roots. This difference was greatest for iron concentration, which was not detectable in the root samples, although soil concentration was up to 24.78 mg/kg. Although the reason for this was not determined, this lack of iron in the roots supported iron fortification of *mahewu*.

A significant difference ($p < 0.05$) in pH was observed between *mahewu* fortified with ferrous sulfate (4.5) and ferrous fumarate (4.3), with the latter being similar to the control. At the beginning of fermentation the acidity was 0.06% and at the end 0.30%. The total solids of fermented *mahewu* were 9.6%. The microorganisms responsible for fermentation were predominantly lactic acid bacteria (LAB) and yeast. The pH and acidity was different to that reported in the fermentation of maize *mahewu*. The fermenting microorganisms and total solids were similar to previous findings for maize *mahewu.*

The total iron content of *mahewu* fortified with ferrous sulfate was significantly higher ($p < 0.05$) than *mahewu* fortified with ferrous fumarate. Both the amount and percentage of bioaccessible iron were higher in *mahewu* fortified with ferrous sulfate compared to *mahewu* fortified with ferrous fumarate. It was found that *mahewu* made using the bitter variety of cassava and fortified with ferrous sulfate provided a more bioaccessible source of iron. The stage of fortification was not found to affect the total iron content nor the iron bioaccessibility.

It was concluded that cassava roots do not take up iron, even when soils are high in this metal. Thus the selection of varieties with better uptake of minerals and fertilization of soils is recommended, but may not increase concentration of essential minerals sufficiently to maintain health in vulnerable communities where cassava is the main staple. This was the first study in which fermentation of traditional cassava *mahewu* was carried out under repeatable, controlled

conditions. It is recommended that fortification occurs at the end of the fermentation when done at household level. However, when flour is being milled in larger villages, it could be fortified prior to sale in informal markets. There is also the possibility of large scale commercial applications.

It is concluded that bitter varieties of cassava will deliver more bioaccessible iron to the consumer. Ferrous sulfate is more suitable as iron fortification source for cassava *mahewu* than ferrous fumarate and also is more stable in *mahewu*. Fortification of cassava *mahewu* could contribute to the iron intake of vulnerable population.

In addition, although fortification at the end of fermentation would probably be ideal for rural communities, as it could be made available in sugar sprinkled into the traditional product, it was shown that fortification of flour used to make *mahewu* would result in significantly higher availability. It is recommended that iron fortification of *mahewu* is implemented both at subsistence and commercial level. This should form part of a communication strategy at community level by the state, in order to improve public health in vulnerable communities in Mozambique.

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CHAPTER 1
GENERAL INTRODUCTION

1.1. Background

Between 2005 and 2007, it was estimated that 38% of the population of Mozambique was undernourished. In 2008, 44% of children less than five years of age were found to be undernourshed.[1] In particular, iron deficiency and its resultant anaemia cause severe public health problems. It is estimated that 45% of the population are iron deficient.[1,2] Population groups most affected by iron deficiency anaemia are pre-school children, expectant woman and women of reproductive age.[1]

It is considered that iron deficiency causes approximately half of the cases of anaemia diagnosed worldwide.[1,3,4] Factors causing iron deficiency anaemia include the high cost of animal products; taboos about dietary intake of animal foods;[5] high levels of hunger resulting in consumption of “empty” carbohydrates; internal parasites like intestinal worms or schistosomiasis; and menstruation.[6,7] Dietary iron deficiency, caused by low intake or bioavailability, can occur in both industrialized and developing countries.[8] Often, consumers in developing countries, suffer from dietary iron deficiency due to consumption of plant derived foods, which contain iron inhibitors such as phytates.[7]

Optimal dietary iron consumption is 18 mg/day.[9] The most common intervention to address iron deficiency anaemia, is iron fortification of food.[10] One of the objectives of iron fortification is to improve micronutrient status in vulnerable populations.[10,11] The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations, suggest iron fortification of staple foods, to prevent dietary iron deficiency and thus reduce anaemia in consumers.[12]

Cassava is a carbohydrate staple cultivated mainly in African and Latin America countries.[13] Either a tropical climate with precipitation >1000 mm,[14] or a sub-

tropical climate with rainfall <600 mm[15] are suitable for cassava cultivation. The optimal temperature for cassava cultivation lies between 23°C and 35°C.[16] In Mozambique the climate is tropical and humid, with annual rainfall of 1200 mm and an ambient temperature ranging between 23°C and 26°C, which explains why cassava is widely grown and consumed.[17] More than 70% of global cassava cultivation occurs in Africa,[13] with Nigeria ranking as the highest producer and Mozambique in 5th position.[18] The edible part of cassava includes roots and leaves, which respectively comprise 50% and 6% of a matured cassava shrub.[19] Cassava root is the most popular staple in Africa with consumption estimated at about 80 kg per person, per year.[20] In Mozambique more than 90% of total cassava production is for human consumption.[21]

The starch content of cassava roots is high, comprising more than 80% of the dry matter (DM), while approximately 4% is fibre.[22] Protein levels are low, ranging between 1% and 3% DM.[23] The concentration of vitamins and minerals in cassava roots are also low,[22] with the exception of vitamin C (15 to 45 mg/100g per edible portion), which is destroyed by cooking. [24,25] As they contain cyanogenic glycosides, cassava roots are generally cooked or fermented to reduce toxicity, so the Vitamin C is not available to most consumers. In Mozambique, *mahewu* is a popular traditional, non-alcoholic, fermented beverage prepared from cooked cassava roots.[26] It has been reported that fermentation increases the nutrient density of carbohydrate staple foods.[27]

1.2. Problem statement

Micronutrient deficiencies are an important cause of ill health in communities living in Africa, including Mozambique.[10,28] Dietary deficiencies of minerals, including iron, have been identified as widespread public health problems.[10,29] In undernourished populations the per capita intake of dietary iron appears to be decreasing annually according to the WHO.[30] Iron deficiency, can have negative economic, social and health consequences. Health consequences include chronic anemia; an increased risk of maternal and childhood morbidity and mortality; deficient physical and cognitive development in children; and reduced work productivity in adults.[31,32]

Cassava is the second highest staple food in Mozambique with 90% of cassava production, used for human consumption. *Mahewu* is a popular fermented drink that is made at home from either fresh, cooked cassava root or cassava flour. However, it is unknown whether the level of bioavailable iron in this staple food contributes sufficient dietary iron to meet the needs of vulnerable populations in Mozambique. Cassava products consumed in Mozambique are not currently fortified with iron as the food fortification programme is still being developed. If cassava *mahewu* could be fortified with iron that was bio-available, this would improve nutrient density as well as providing a dietary source of iron to prevent iron deficiency anaemia.

1.3. Aim and objectives

1.3.1. Aim

The aim of this study was to investigate if cassava *mahewu,* could be fermented under controlled conditions and indicate the optimal stage at which it could be fortified to achieve bioavailable iron.

1.3.2. Objectives

1. To investigate whether the iron concentration in cassava roots was sufficient for the nutritional needs of consumers or needed to be increased in order to prevent iron deficiency anaemia.
2. To determine whether the iron concentration in Mozambique soils, influences the concentration of iron in cassava roots grown in that soil.
3. To compare the mineral concentration of sweet and bitter cassava varieties grown in different parts of Mozambique
4. To ferment cassava *mahewu* under controlled conditions and standardize a method for cassava *mahewu* preparation.
5. To identify the optimal stage for cassava *mahewu* fortification with iron.

6. To evaluate the bioaccessibility of iron in fortified cassava *mahewu* using an *in vitro* dialysability method.

7. To compare the bioaccessability of ferrous fumarate and ferrous sulphate used to fortify cassava *mahewu* made of sweet or bitter root types, at the beginning or end of fermentation.

1.4. Rationale

The importance of this study was to gain insight into the concentration and bioaccessibility of iron in cassava *mahewu* before and after fortification, in order to reduce iron deficiency anemia in vulnerable populations. In addition, the functional microorganisms in *mahewu* would result in a bio-active food that includes digestible protein and vitamin B, thus reducing nutritional anaemia not necessarily linked to iron deficiencies.

1.5. Study design

The process flow for this study is depicted schematically in Figure 1.1 below.

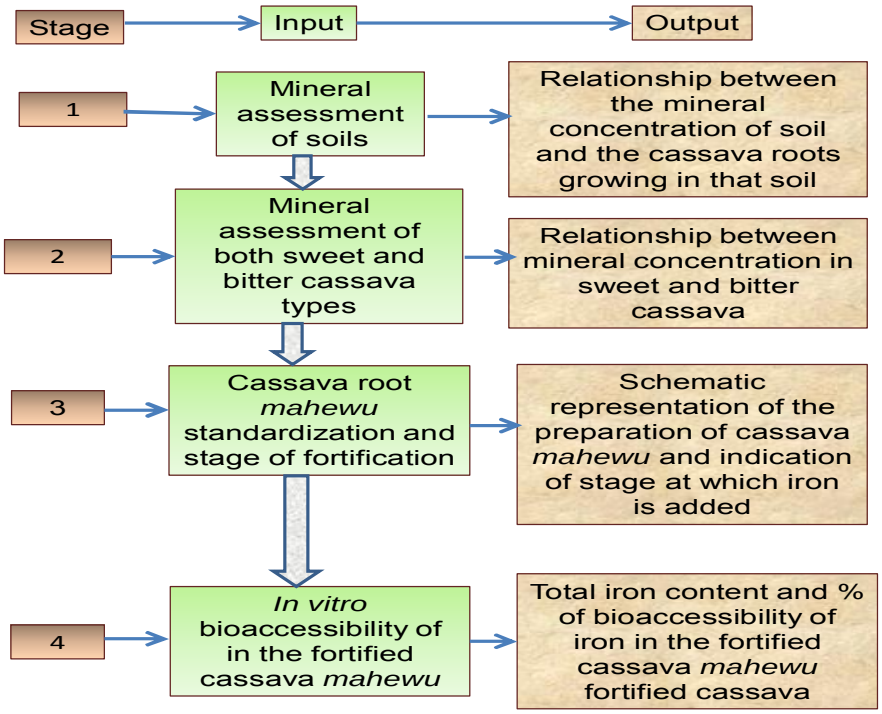

Figure 1.1: Schematic summary of the project

This was a quantitative experimental study design that compared the mineral concentration of samples of soils and cassava root, as well as the differences between iron bioaccessibility in sweet and bitter varieties fortified with two different iron salts during standardized fermentation to produce *mahewu*.

1.6. Thesis structure and outline

Chapter 1 summarises the background and motivation for this investigation and lists the aims and objectives.

Chapter 2 is a literature review of cassava, micronutrient deficiencies and anaemia as well as food fortification particularly food fortification with iron. The objective of the review was to understand the importance of cassava in the diet of African populations, in particular the rural vulnerable populations in Mozambique. This has been published as an overview of cassava in Mozambique.

Chapter 3 indicates how extraction using microwave digestion and analysis with inductively coupled plasma optical emission spectroscopy could be used to compare the mineral concentration in cassava roots to that of the soil, and investigate whether this was influenced by the type of cassava. Samples of soil and cassava roots (both bitter and sweet types), were collected from four Districts of Mozambique considered as intermediate and high producers of cassava. This paper has been submitted for publication. It has also been submitted as an abstract to the International Spectroscopy Conference 2015.

Chapter 4 describes the development of a standardized method, applicable at commercial or village level for fermentation of cassava roots to make cassava *mahewu* based on indigenous knowledge in Mozambique. The objective was not only to standardize the method but to also assess the optimal stage for iron fortification. This paper has also been submitted for publication.

Chapter 5 compares the bioaccessibility of two types of iron compounds (ferrous sulfate and ferrous fumarate), used to fortify mahewu made from sweet or bitter types of cassava roots, using *in vitro* dyalisability. It also compares

whether bioavailability is increased or decreased by the stage at which the iron compound is added. This paper has been submitted for publication.

Chapter 6 focuses on the overall discussion, conclusions, recommendations, perspectives and limitations of the study.

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CHAPTER 2

OVERVIEW OF PRODUCTION, CONSUMPTION AND NUTRITIONAL VALUE OF CASSAVA IN MOZAMBIQUE*

2.1. Abstract

Both soils and climate in Mozambique suit cassava cultivation and nine million tons fresh weight is produced annually, with a consumption of 85 kg per person per year. The roots are a staple carbohydrate and cooked leaves are served as a vegetable. Cassava is essential to food security, as it is a subsistence crop. Roots and leaves contain vitamin C and some minerals but are deficient in proteins and amino-acids. Although cassava is cultivated by about 63% of the population, cyanogenic glycosides and other anti-nutritional factors, threaten food safety. Cassava root is known to be low in essential micronutrients and this may contribute to the high level of dietary anaemia in vulnerable populations in Mozambique. There are more than 100 varieties, but the more drought and insect resistant bitter types predominate. Traditional products made from cassava that rely on sun-drying, cooking or fermentation to reduce toxicity include "*rale*", "*xinguinha*", "*karakata*" "*mahewu*" and "*oteka*". Cassava flour has replaced up to 20% of wheat flour in bread, for economic reasons. An overview of the distribution, consumption patterns and nutritional value of cassava in Mozambique could contribute to knowledge, as much of the existing data has not been published. Food safety and nutritional value could be improved by commercializing the production of traditional products and fortifying the easily grown staple carbohydrate. This could improve the health of vulnerable rural populations.

Key words: Cassava consumption, cassava production, cyanogenic glycosides, mineral fortification, Mozambique, traditional foods.

* This Chapter has been published (Appendix 7)

2.2. Introduction

The cassava plant (*Manihote esculenta* Crantz) originated in Brazil and has been introduced globally in order to provide a staple carbohydrate, particularly in developing countries where the climate is not suitable for large scale production of wheat and maize.[1-3] In Mozambique, about nine million tons fresh weight of cassava is produced annually.[4] The roots are a staple carbohydrate and cooked leaves are served as a vegetable. The estimated consumption of cassava roots amounts to about 85 kg per person per year.[5,6] It is essential for food security and is grown as a subsistence crop by about 63% of small scale farmers as well as on a large scale for commercial purposes.[7] Although the roots and leaves contain vitamin C and minerals they are deficient in proteins and amino-acids.[8-11]

Cyanogenic glycosides and other anti-nutritional factors in cassava, threaten food safety in a large proportion of rural communities in Mozambique, where drought and insect resistant bitter types predominate.[12] Diseases linked to consumption of cassava include both acute and chronic cyanide poisoning, as well as Konzo, which is associated with under-nutrition, sulphur and iodine deficiency.[13-17] Anaemia is a problem in vulnerable populations such as young children, nursing and pregnant women, the aged or and those suffering from malaria or HIV infections. Nearly half these cases of anaemia have been linked to chronic dietary iron deficiency.[18-20]

Traditional products made from cassava rely on sun-drying, cooking or fermentation to reduce toxicity. These include "*rale*", "*xinguinhaò ñkarakataò* "*mahewu*" and "*oteka*". Approximately 20% of the wheat flour used to make bread in Mozambique, has been replaced with cassava flour.[21-26]

2.3. Cassava (*Manihote esculenta* Crantz)

The word ''cassava'' comes from *casaba*, the name given by the Arawak Indians to the root. It is known as ''*yucaõ* in Spanish, ''*manioc*õ in French, ''*mandioca*õ in Portuguese; ''*cassave*õ in Dutch and "*maniok*" in German.[27]

Cassava (*Manihot esculenta* Crantz) is native to Brazil,[2] and during the 16th and 17th centuries it was dispersed widely by the Portuguese to tropical and subtropical areas of Africa, Asia and the Caribbean (Fig 2.1).[28]

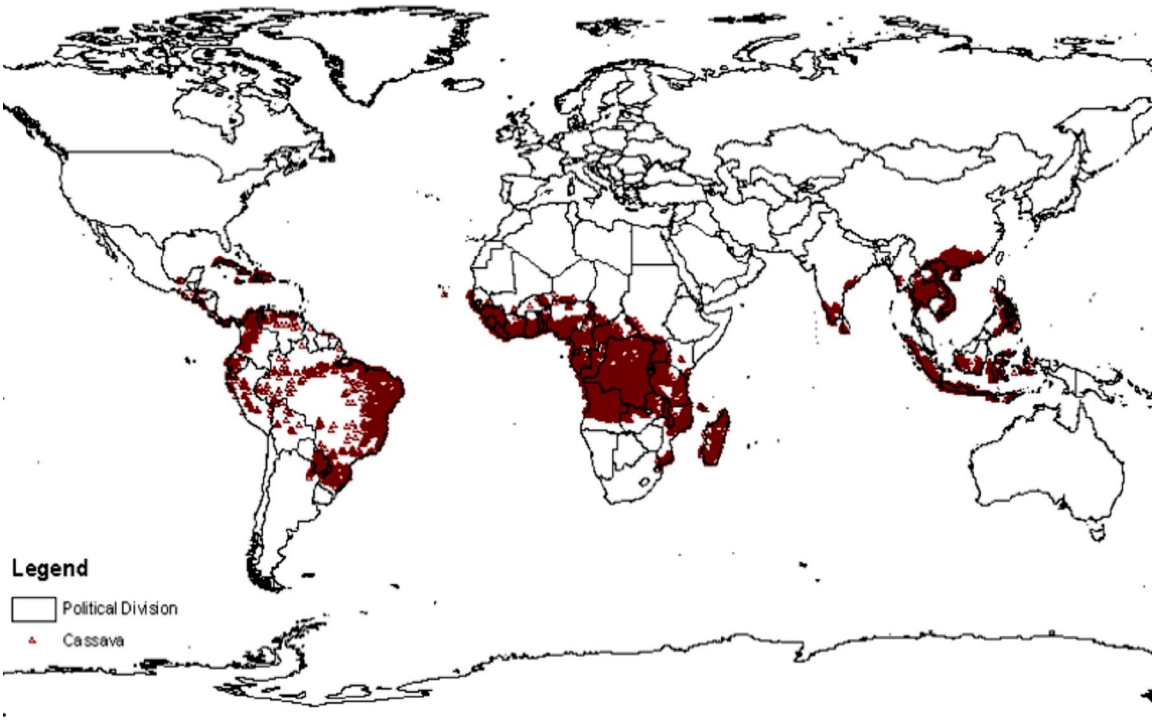

Figure 2.1: Global cassava distribution.[28]

More than 50% of global cassava production takes place in Africa, 34% in Asia and 15% in Latin America.[29] Between 500 million and 1 billion people consume cassava. In the tropics, it is the foodstuff most frequently consumed after rice and maize.[30]

Cassava has become a staple food in many countries, due to its tolerance to drought, poor soil conditions and difficult crop environments.[31] The main cassava producing countries in Africa include Nigeria, Democratic Republic of Congo, Ghana, Angola, Mozambique, Tanzania, Uganda, Malawi, Cameroon and Benin (Figure 2.2).[32]

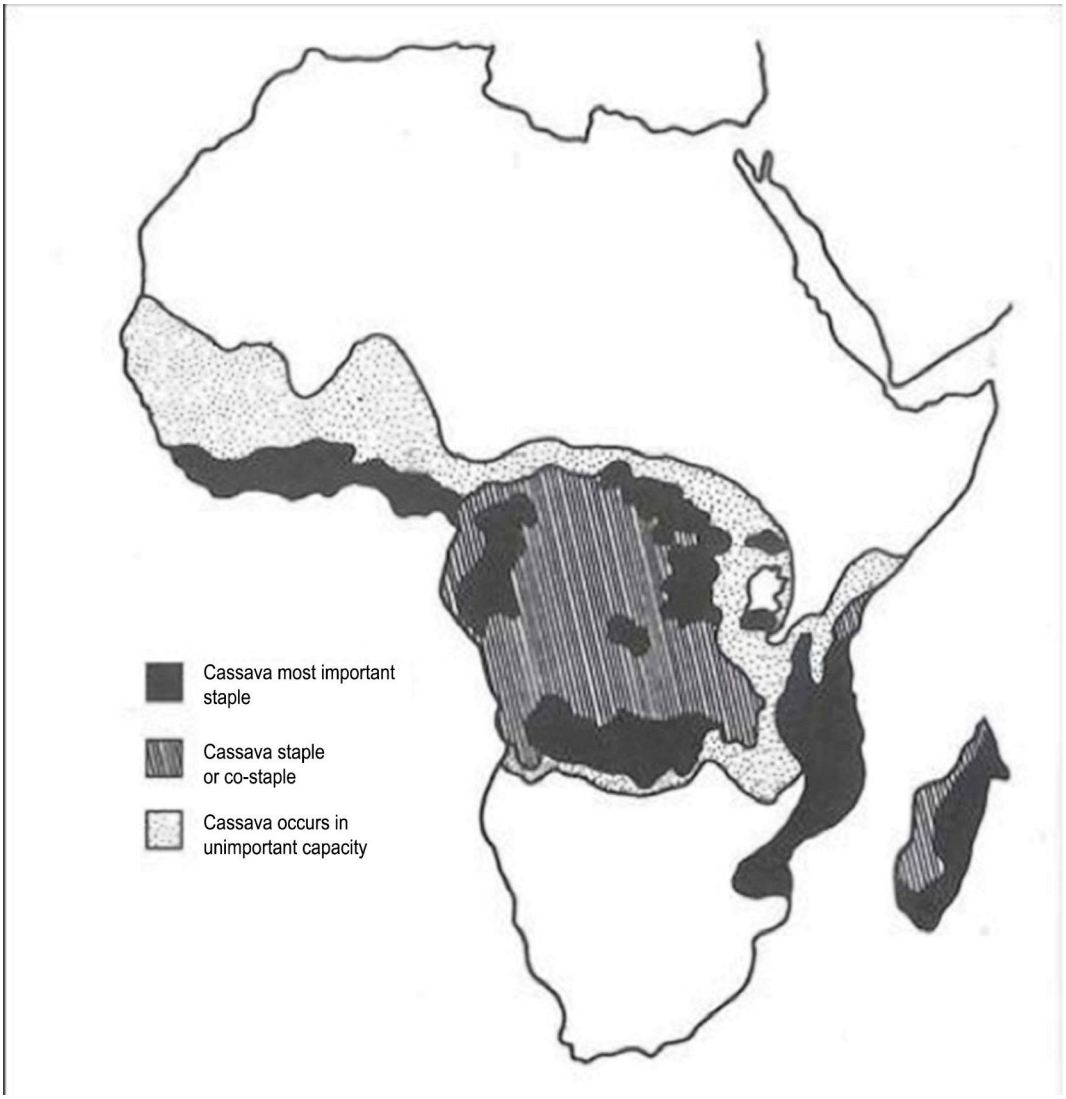

Figure 2.2: Cassava production areas in Africa.[10]

2.3.1. Cassava in Mozambique

In Mozambique the total cultivated land is estimated at 5 632 781 ha and 96.4% of this land is used by small-scale farmers.[33] Approximately 43% of the total cultivated area is used for cassava production by subsistence and small scale family farmers.[33] The country is divided into 10 agro-ecological zones, which are based on altitude, climate (precipitation and temperature) and soil type.[34] There are four agro ecological zones where cassava is mainly cultivated: R1, R2, R7, and R8 (Fig 2.3).

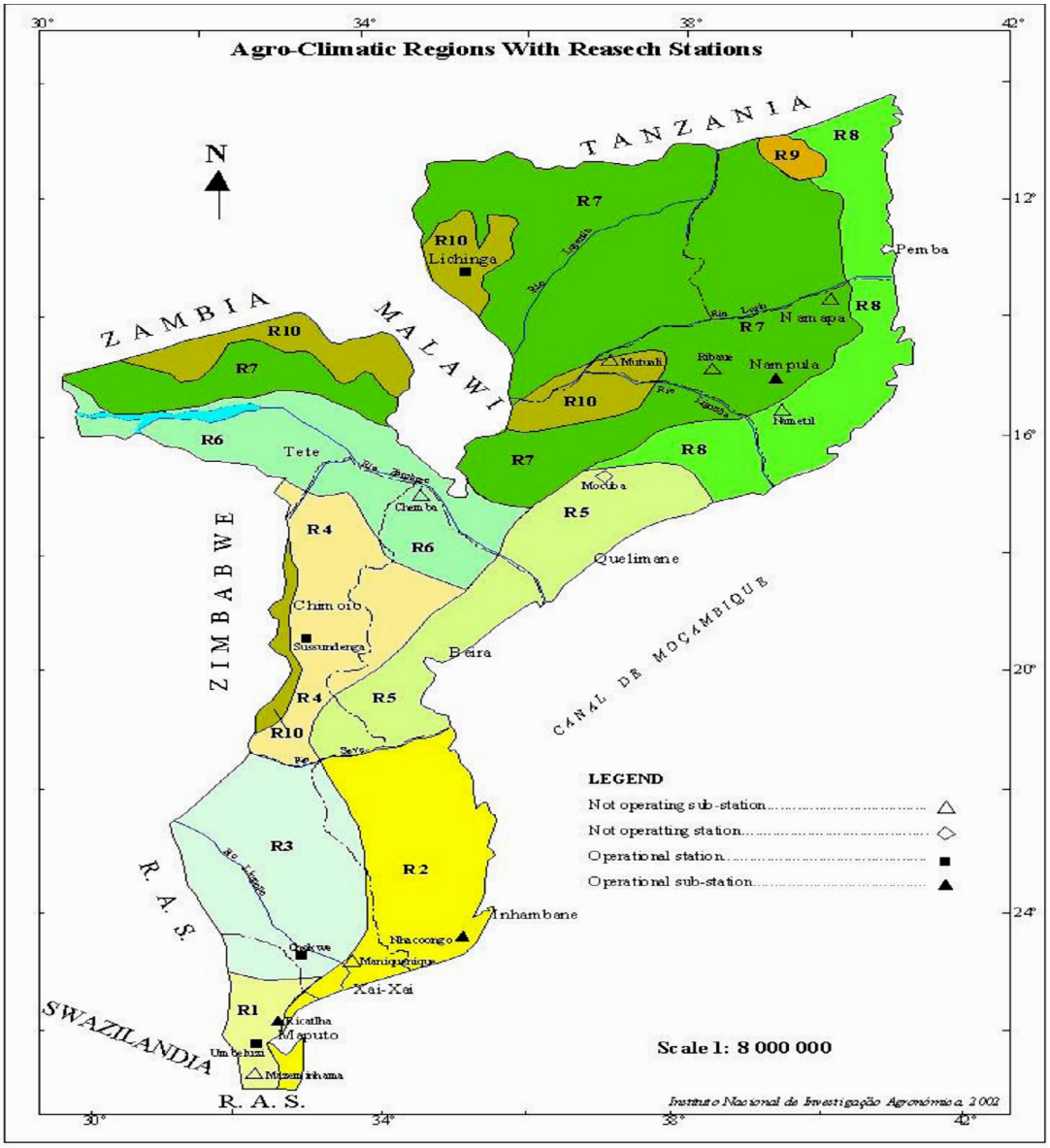

Figure 2.3: The 10 agro-ecological zones of Mozambique.[35]

Cassava production improved between 2002 and 2008, when the average annual production, was estimated as six million tons. It rose further to nine million tons in 2010[18,36] Overall, between 2005 and 2012, the yield of cassava in hectogram per hectare increased from 43.155 to 131.804.[37] By 2012, the annual production had escalated to 10.05 million tons.[37] Cassava is mainly grown in the Southern Region of Inhambane Province (8.80%), the Central Region of Zambezia Province (26.76%) and the Northern Region of Cabo Delagado and Nampula Provinces with 18.68% and 29.27% respectively (Fig 2.4). [33]

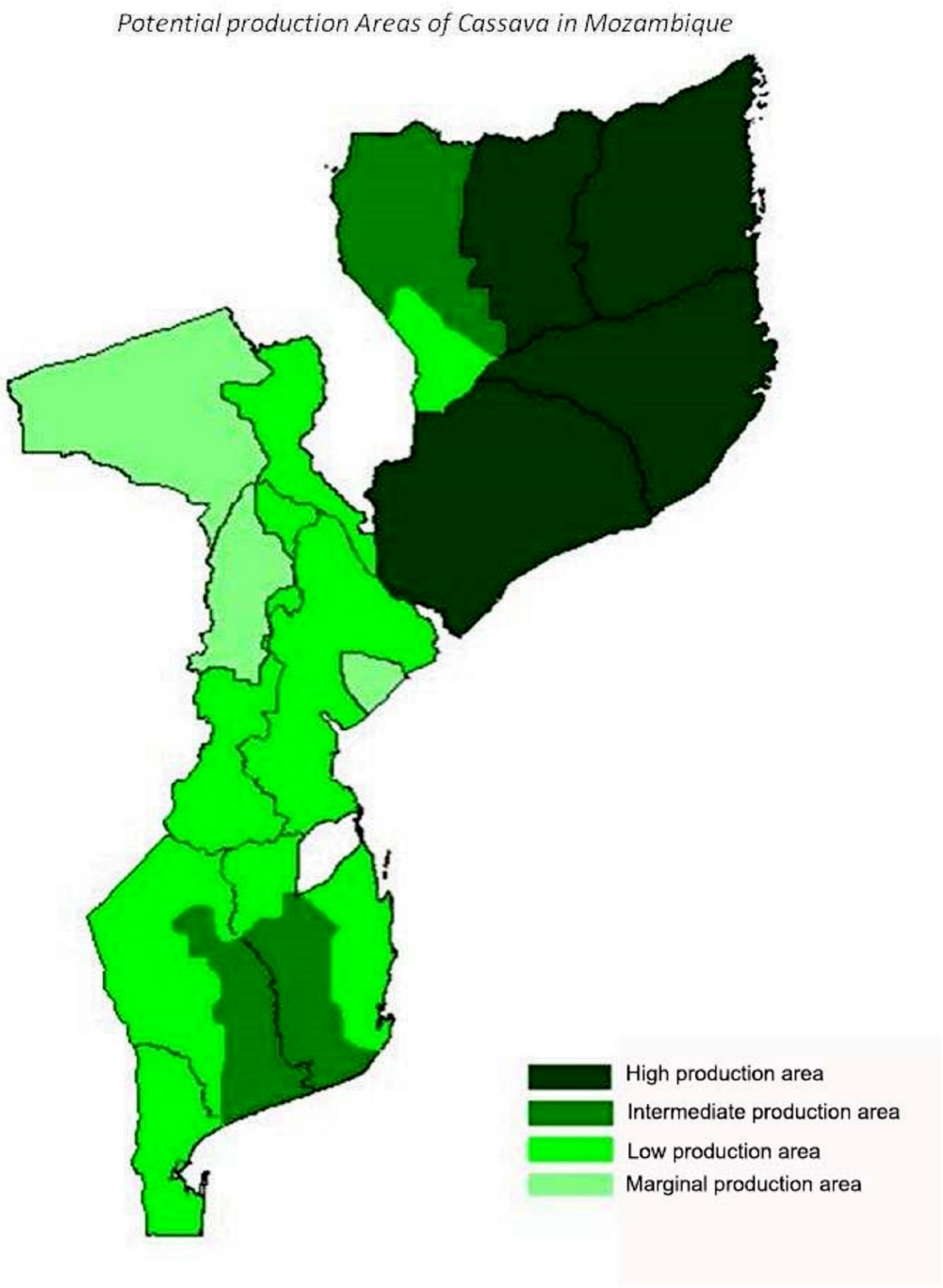

Fig 2.4: Potential production area of cassava in Mozambique.[38]

There are approximately 109 varieties of cassava that have been identified in 31 districts across Mozambique, of which the five most common are listed in Table 2.1.

Table 2.1: Varieties of cassava cultivated in Mozambique by region.[5]

Variety	Region	Type
Munhaça	Southern	Sweet
Calamidade	Northern (Nampula Province)	Sweet
Tomo e Cocorro	Northern (Nampula Province)	Bitter
Inciricano	Central (Zambezia Province)	Bitter
Bedo	Central (Zambezia Province)	Bitter

Although there are many varieties, cassava is divided into two major types: sweet and bitter.[5] The bitter type is highly toxic due to the presence of cyanogenic glycosides. However, this type predominates due to its high yield, multiple-year in–ground storage potential, as well as pest and drought tolerance[5,23] Farmers appreciate the pest resistance, although the bitter cassava requires greater care in processing. The sweet type is low in cyanogenic glycoside content and is mostly consumed fresh or is processed by a smaller proportion of producers.[22,23,39] Using marketed volumes, as an alternative for total cassava production, bitter cassava accounts for about 90% of national cassava production in Mozambique, with the sweet varieties making up the rest .[40] Cassava is the main source of food security in rural areas of Mozambique for the following reasons:[6]

(i) it adapts to poor soil;

(ii) it does not demand expensive planting implements, irrigation or fertilizers;

(iii) it resists droughts and locust damage;

(iv) in comparison to other crops it has a relatively high yield at a low cost;

(v) it can be harvested as and when needed, with delays to harvesting for periods of 6 to 48 months without major changes to the composition and quality; and

(vi) it can be planted at any time during the year.

The FAO/FAOSTAT,[41] reported that approximately 94% of total cassava in Mozambique is utilized for human consumption, 4% for animal feed, and the remaining 2% for industrial use. Cassava alone provides the largest source of calories in Mozambique. However, consumption has gradually decreased due to an increase in urbanization, change in life style and preference for prepared and convenience foods. Over the past 45 years, cassava's contribution to food consumption has decreased from 46% of national calorie availability in the early 1960's to 30% in the late 2000's.[41] In Mozambique, cassava is consumed fresh, boiled, baked (Nsima, bread, and pastry products), dry, dry roasted or semi-processed. The leaves are also consumed as a vegetable in most parts of the country.[5] Most households located in the cassava growing zones, eat the leaves as well as the roots.[8] Currently, Nampula Province is experimenting with production of packaged cassava leaf powder and frozen cassava leaves. Fresh cassava is favored in the central and southern parts of Mozambique, where it composes 10% of total cassava consumed. About 20% of cassava is consumed in the form of *rale* (roasted cassava), by households of southern Mozambique. Inhambane Province is the center of *rale* production.[42] Cassava flour, made from milled, dried cassava chips at farm level, comprises 90% of the total cassava consumption and is consumed by both rural and urban households.[43]

Mozambican farmers market about 11% of their total cassava production, while the rest is consumed locally.[42] The northern farmers sell a greater proportion (13%) of their cassava crop compared to only 3 to 4%, marketed by central and southern farmers. Farmers in northern Mozambique account for 85% of national cassava production and over 90% of marketed volumes.[42] Cassava roots are composed mainly of carbohydrates. Seventy percent of the cassava root consists of moisture; 24% is starch and fibe, 1% is protein, while mineral and other components comprise 3%.[44] Table 2.2 illustrates the nutrient composition of cassava.

Table 2.2: Proximate nutrient composition of cassava roots and leaves.[45-49]

	Unit	Raw cassava	Cassava roots	Cassava leaves
Proximate composition 100 (g)				
Food energy	kcal	160	110 - 149	91
Food energy	kj	667	526 - 611	209 - 251
Moisture	g	59.68	45.9 – 85.3	64.8 – 88.6
Dry weight	g	40.32	29.8 – 39.3	19 – 28.3
Protein	g	1.36	0.3 – 3.5	1.0 – 10.0
Lipid	g	0.28	0.03 – 0.5	0.2 – 2.9
Total carbohydrate	g	39.06	25.3 – 35.7	7 – 18.3
Dietary fiber	g	1.8	0.1 – 3.7	0.5 – 10.0
Ash	g	0.62	0.4 – 1.7	0.7 – 4.5
Vitamins				
Thiamin	mg	0.087	0.03 – 0.28	0.06 – 0.31
Riboflavin	mg	0.048	0.03 – 0.06	0.021 – 0.74
Niacin	mg	0.854	0.6 - 1.09	1.3 – 2.8
Ascorbic acid	mgç	20.6	14.9 - 50	60 - 370
Vitamin A	µg	-	5.0 – 35.0	8300 – 11800[e]
Minerals				
Calcium	mg	16	19 - 176	34 - 708
Total phosphorus	mg	27	6 - 152	27 - 211
Ca/P		0.6	1.6 – 5.48	2.5
Iron	mg	0.27	0.3 – 14.0	0.4 – 8.3
Potassium	%	-	0.25 – 0.72	0.35 – 1.23
Magnesium	%	-	0.03 – 0.08	0.12 – 0.42
Copper	ppm	-	2.00 – 6.00	3.00 – 12.0
Zinc	ppm	-	14.00 – 41.00	71.00 – 249.0
Sodium	ppm	-	76.00 – 213.00	51.0 – 177.0
Manganese	ppm	-	3.00 – 10.00	72.0 – 252.0

The protein content of cassava root is low.[9] Cassava leaves are richer in protein than the roots, with the leaves additionally containing essential amino acids.[10] Cassava leaves are considered a good source of protein, minerals, and vitamins B1, B2 and C and carotenoids.[8,11,50]

2.3.2. Cyanogen in cassava

The roots and leaves of cassava possess potential toxicity due to the presence of natural nitrile (-CN) compounds known as cyanogenic glycosides or cyanogens, which are in the form of linamarin (alphahydroxyisobutyrº3onitrile-beta-D-glucopyranoside) (93%) and lotaustralin (methyl-linamarin) (7%).[51] These are beta-glycosides of acetone cyanohydrins and ethyl-methyl cyanohydrins, respectively.[51]

Cassava cultivars with less than 100 mg/kg cyanogenic glycosides (fresh weight) are referred to as sweet, while cultivars with 100-500 mg/kg cyanogen glycosides (fresh weight) are referred to as bitter.[52] The total concentration of cyanogenic glycosides depends on the cultivar, environmental conditions, cultural practices and plant age.[53] The range of cyanogen concentration in cassava falls between 15 and 400 mg HCN/kg fresh weight.[54] To avoid acute toxicity in humans, the maximum level of cyanide in cassava allowed is less than 10 mg equivalent/kg dry matter, according to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).[55] It is estimated that the level of cyanide from cassava, consumed per person in Mozambique, is 14 to 70 times higher, based on bodyweight[12], than this safety limit.[55]

Cyanide or hydrocyanic acid in cassava can be produced through an enzymatic reaction, which occurs when plant cells are damaged, grated or sliced or when degradative enzymes come into contact with each other.[53,56,57] Linamarin is converted into hydrogen cyanide (HCN) by the enzyme linamarinase. This may generate the equivalent of 0.2-100 mg of HCN per gram of fresh cassava following cellular lysis.[58] Hydrogen cyanide is extremely toxic to a wide spectrum of organisms, including humans, due to its ability to link with metals (Fe^{2+}, Mn^{3+} and Cu^{2+}), which are functional groups of many enzymes, inhibiting

processes like the reduction of oxygen in the cytochrome respiration chain, electron transport in photosynthesis and the activity of catalase or oxidase.[59]

The primary effect of cyanide poisoning in humans is the impairment of oxidative phosphorylation, whereby oxygen is utilized for the production of essential cellular energy sources in the form of adenosine triphosphate (ATP). The necessary part of this process is the transfer of electrons from nicotinamide adenine dinucleotide (NADH) supplied during the Krebs Cycle, via a series of electron carriers. These are catalyzed by the cytochrome oxidase enzyme system in the mitochondria, and the impairment arises from the inhibition by cyanide of cytochrome oxidase a_3.[60,61] This in turn arises from the high binding affinity of cyanide to the ferric ion found in the heme moiety of the oxidized form of this enzyme. The chemical combination results in loss of the structural integrity and hence effectiveness of the enzyme; as a result tissue utilization of oxygen is inhibited with rapid impairment of vital functions. Other metabolic processes continue and the rate of glycolysis is increased markedly, however, the pyruvate so produced can no longer be utilized via the impaired Krebs Cycle, as it is reduced to lactate, resulting in metabolic acidosis. It has been shown that cyanide significantly decreases brain ATP and increases brain lactate levels.[62]

It can also result in pulmonary arteriolar and/or coronary artery vasoconstriction, decreased cardiac output and in extreme cases cardiac shock. Pulmonary oedema has also been observed in chronic cyanide poisoning, although it is thought that this may be more related to left ventricular failure than capillary endothelial damage or neurogenic causes.[63]

Chronic poisoning following long-term consumption of cassava roots with high cyanogenic glycoside content has been reported in countries, including Mozambique, where cassava is a staple food.[14-16,64-66] Cyanide resulting from cassava consumption is found in rural populations also suffering from severe undernourishment, marasmus and kwashiorkor.[64] In Mozambique, acute poisoning from cassava consumption has been reported in the Nampula Province.[13] Chronic intoxication from cassava can manifest as tropical neuropathy[67], glucose intolerance, konzo,[15-17] goitre and cretinism.[68]

In Mozambique, konzo is the most frequently reported disease, caused by cassava poisoning. The first case of konzo in the country was reported in 1981.[14] Population groups found to be most vulnerable were women of child bearing age and children up to two years of age.[16] All cases reported in the country have been related to consumption of bitter cassava which was poorly processed, collected during a famine period and civil war or a diet deficient in sulfur amino acids.[14-17] Patients with konzo have been found to have a high level of thiocyanate in their urine.[14,16,17] Thiocyanate remains in the body as a result of the detoxification of cyanide. It is stored in the stomach and patients with konzo are also at risk of developing stomach cancer.[69,70]

Processing cassava by peeling, soaking, cooking, drying and fermentation reduces or eliminates the cyanogenic glycosides. For sweet cassava, cooking is sufficient to eliminate all toxicity. Processing cassava is also necessary for improving palatability and as a means of preservation.[71-75]

2.3.3. Cassava fermentation

Fermentation is the most common method of processing cassava foodstuffs in Africa.[76] More than 90% of cassava for human consumption is processed using fermentation.[77] Examples of fermented cassava foods worldwide are: *gari, fufu* and *lafun* (Nigeria); *kumkum, myiodo* and *atangana* bread (Cameroon); *agbelima* and *akyeke* (Ghana); *foofoo* (Congo); *tapioca* and *puba* (Brazil) and *rale* and *karakata* (Mozambique).[22,25,78-81]

One of the methods for effective fermentation of cassava products is by a lactic acid fermentation process.[82] The method of fermentation varies from one location to another and is fermented using two processes, solid or sub-merged.[83] The solid fermentation process is characterized by the growth and/or cultivation of microorganisms under controlled conditions in the absence of free water for the production of the desired products.[83-86]

The typical microorganisms which grow during solid-state fermentation are yeasts and fungi. Aeration and agitation is used to remove carbon dioxide and for temperature control. Due to the small amount of water present, the biomass

levels are lower and the heat generated per mass tends to be much greater than in submerged state fermentation.[87]

Submerged fermentation uses a dissolved or solid substrate, suspended in a large amount of water to form slurry.[84] Bacterial and yeast cells are thus evenly distributed throughout the medium, but due to the high water content, bacterial cells are predominant and the process requires high oxygen concentrations.[87]

Both spontaneous fermentation and starter culture fermentation can be used. However, spontaneous fermentation is neither predictable nor controllable, although it is typically utilised at household level, while starter culture is implemented during small scale or industrialized processing.[88,89]

The microbial populations isolated during cassava fermentation, are mainly lactic acid bacteria (LAB) although yeast and moulds have also been identified. Lactic acid bacteria involved in cassava fermentation include: *Leuconostoc spp*., *Bacillus spp*., *Corynebacterium spp*.[90,91] *Lactobacillus plantarum*, *L. perolans*, *L. brevis*.[92] *L. fermentum* [93,94,95] *L. casei, and L. delbrueckii*.[81]

During fermentation of cassava roots, LAB are responsible for digestion of starch to lactic acid, resulting in a drop in the pH.[76,78,96] This results in the typical characteristics of fermented foods such as smell, flavor, visual appearance, and consistency. Fermentation of cassava by LAB results in the removal of cyanogenic glycosides and development of antimicrobial substances and bacteriocins.[97-99]

Fermentation by LAB can improve the nutritional value through production of essential amino acids and vitamins, including vitamin B, folate and cobalamin as well as bio-active substances.[100,101] During fermentation the LAB facilitate the breakdown of numerous complex substances forming simple and easily digestible compounds.[102,103] Fermentation of cassava can also raise the bioavailability of minerals like calcium, iron and zinc.[104-106]

Yeasts such as *Saccharomyces cerevisiae, Candida spp, C. krusei, C. tropicalis, Pichia saitoi, P. anomala* and *Zygosaccharomyces bailii* , have been reported as fermenting cassava products.[91,97,107,108] Yeast fermentation of

cassava roots is known to improve nutritional content and organoleptic properties as well as decreasing the levels of cyanide.[97,107,108]

Moulds act on cellulose processing, which results in the hydrolysis of cassava roots. Species of moulds like *Penicillum sclerotiorum, P. citrinum, P nodulum, Geotrichum candidum* and Basidiomyces spp., have been found to play a role in cassava fermentation.[107]

Microorganisms such as bacteria, yeasts and fungi,[109,110] found in fermented foods and beverages, including cassava; have health promoting benefits and biological importance. This includes the production of antioxidants and omega-3 polyunsaturated fatty acids, therapeutic value and immunological effects.[111-113]

2.4. *Mahewu*

Mahewu is a non-alcoholic fermented beverage made from carbohydrate staple foods in Africa and some Arabian Gulf countries.[114] In South Africa it is commonly made from maize and consumed by indigenous people. It is recognized by several names according to the ethnicity and language of the consumers. In Zulu it is known as "*amahewu*", in Xhosa it is "*amarehwu*", in Swazi, "*maphulo*", in Pedi "*matogo*", in Sotho "*machleu*", while in Venda it is called "*maphulo*".[88,115-117]

Maize *mahewu* is consumed by people of all ages and in African countries is often consumed as a part of social ceremonies.[118] It plays an important role in infant feeding and is used to wean children, normally being introduced to children between 4 and 48 months of age.[119] Due to its elevated nutritional density *mahewu* is the preferred drink during the working day and is consumed by farmers, schoolchildren, miners and manufacturing workers. Social development organizations have also used it in nutritional programs.[118]

Although *mahewu* is classified according to the raw material used in the manufacturing process (cassava, sorghum, rice and sweet potato); the most common is made from maize gruel, which is mixed with water, then fermented sorghum, millet malt or wheat flour is added to initiate fermentation.[120-122] Alternatively *mahewu* can be made by crushing left over maize porridge into

slurry followed by fermentation.[123] The major solid substrate in aqueous suspension is between 8 and 10% with about 0.4-0.5% lactic acid, corresponding to an average pH of 3.5.[116,124]

The fermentation of *mahewu* occurs naturally, although starter culture can be used to rapidly initiate the process of fermentation.[120,121] Natural fermentation is neither predictable nor controllable, when prepared at household level, while starter culture is more reliable and can be used in small scale or industrialized processing.[88,89,125] Fermentation of *mahewu* at family level is carried out at room temperature over a period of two or three days.[120-122]

Commercial production of maize *mahewu* has been carried out in African countries such as Botswana, South Africa and Zimbabwe.[88,121,124-126] At commercial level maize *mahewu* has been enriched with proteins, minerals and vitamins to improve the nutritional value.[127,128] Also the flavour is improved by adding different fruits at later stages of fermentation.[89,125] Commercially maize *mahewu* exists as a powdered formula, which is mixed with water at the time of consumption and becomes a ready to eat product.[121]

The microorganisms responsible for fermentation of *mahewu* are LAB and yeast; which are also responsible for organoleptic properties and acidity.[102,126,129] The lactic acid bacteria are reported to be the predominant micro-flora.[89,130] Species of lactic acid bacteria reported to ferment *mahewu* include: *Lactococcus lactis* subsp. *lactis*, *Lactobacillus bulgaricus, L. delbrueckii, L. brevis*, and *Streptococcus lactis*.[89,130,131]

It has been reported previously, that the LAB in fermented *mahewu* raise the protein concentration and bioavailability of amino acids.[132] *Mahewu*, like other non-alcoholic fermented foods such as yoghurt, has been reported as functional food, which can sustain health.[117,133-135] Bactericidal and bacteriostatic substances have also been reported in maize *mahewu*.[123,136]

2.5. Micronutrients and anaemia

2.5.1. Micronutrients

Micronutrients are vitamins and minerals required in very low amounts, which should be continually consumed, as part of a balanced diet, to ensure cellular growth and metabolism.[137] Micronutrient deficiencies develop when a variety of foods are absent or when the diet depends on staple food alone, as is the case of cereal or tuber-based foods including cassava;[28] or where individuals do not have enough to eat.[138] The number of people affected by micronutrient deficiency globally is estimated to exceed 2 billion. The three most important deficiencies are iron, vitamin A and iodine. Combined, these affect at least one third of the total population of the world most of whom live in developing countries.[139,140] It is estimated that in Africa, 46% of the total population is anaemic, 43% have insufficient iodine intake and 49% of preschool children are vitamin A deficient.[141,142] In Mozambique, the diet is extremely poor in micronutrients; the prevalence of under-nutrition in 2005-2007 reached 38%.[18] Throughout the country, 43% of children younger than five year of age are moderately under-nourished, 20% chronically under-nourished and 8% acutely under-nourished.[19]

The World Bank estimated that the total once a year cost of micronutrient deficiencies in the world was around 3.03% of the gross national products (GNP) in 2010.[143] The peak expenditure was in unindustrialized countries, with 5 % of the GNP of these countries. In Mozambique, the cost is estimated at 4.76% of the GNP.[143]

2.5.2. Anaemia

Anaemia is defined as a substantial decrease in hemoglobin concentration, hematocrit or the number of red blood cells in circulation, at a level under that which is considered standard for age, gender, biological state, and altitude, without considering the cause of deficiency.[142] Approximately one-third of the global population are anaemic.[144] Assessments in high-risk people propose that overall anaemia occurrence may be between 50% and 80%, with as many as 10% to 20% having moderate to severe anaemia.[145] In 2010 it was reported that

anaemia was responsible for 8.8% of the entire disease burden from all disorders. In the same period, South Asia had 37.5% of total anaemia cases in the world; sub-Saharan Africa 23.9%; whereas in the rest of the developed world the prevalence of anaemia was lower than 25%.[146]

The global prevalence of anaemia by population groups according to WHO is:

i. Preschool children (0 - 5 years) (47.4%),

ii. School age children (5 – 15 years) (25.4%)

iii. Pregnant woman (41.8%),

iv. Non pregnant woman (15 – 50 years) (30.2%),

v. Men (15 – 60 years) (12.5%),

vi. Elderly (both sexes >60 years) (23.9%) and

vii. Total population (24.8%).[147]

From the above it can be seen that preschool children and pregnant women are the populations most as risk for anaemia. Anaemia can arise from both nutritional and non-nutritional factors. Non-nutritional factors include diseases, parasites and genetic propensity.

The most prevalent reasons include iron deficiency which is responsible for 50% of cases of anaemia,[142] hookworm, sickle cell disorders, thalassemias, schistosomiasis, and malaria.[146] Non-nutritional causes include infection, chronic diseases, and pernicious anaemia.[148]

(i) Nutritional anaemia

Nutritional anaemia is defined as an illness in which the hemoglobin content of the blood is lower than normal, as a consequence of an insufficiency of one or more essential nutrients, regardless of the cause of such insufficiency.[149] Insufficient consumption of micronutrients including vitamin A and B12, folate, riboflavin, and copper can raise the risk of iron deficiency anaemia.[147] There is a close relationship between anaemia and under-nutrition.[150] This relationship is

explained by the fact that people with deficient calorie intake are more likely to be lacking in micronutrients, particularly iron.[151,152] According to the WHO, the most important nutritional deficiency linked to anaemia is iron.[142,153,154] Iron deficiency is also ranked as the 15th most important cause of preventable death and incapacity.[155] Nutritional iron deficiency mainly results in iron deficiency anaemia.[156] It is a consequence of a wide diversity of factors, but most of them co-occur.[147] Reduced absorption of iron from food with a high phytate and phenolic content during times when high iron content is required (growth and pregnancy) are considered the main risk aspects.[147] On the other hand the absence of knowledge of nutritional requirements and nutritive density of different foodstuffs also contributes.[157]

Secondary causes of iron deficiency anaemia include substantial blood loss as consequence of menstruation or parasite infections such as hookworm, ascaris and schistomiasis;[158] tuberculosis and HIV.[159-161]

Globally, in approximately 41% of females and 27% of preschool children with anaemia, the disease is caused by iron deficiency.[155] Iron deficiency anaemia increases the risk of maternal morbidity and death and it affects the development and health of infants. In preschool children, it impairs motor development and growth, reduces school performance, reduces immune function and increases susceptibility to infections. In adults it decreases responsiveness and activity; increases body tension and fatigue and decrease the physical capacity for work performance.[155,158,162]

(ii) Anaemia due to chronic disease

Anaemia due to chronic disease is ranked second after that caused by iron deficiency and arises in patients with severe or chronic immune stimulation.[163,164] This illness has also been called “anaemia of inflammation”.[163] Illnesses frequently related with anaemia of chronic disease are severe. The consequences can include continuous infections caused by viruses (including HIV) bacteria, parasites and fungi;[165-167] neoplasia, including hematologic and solid tumors;[167-170]autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus, connective-tissue diseases, like vasculitis, sarcoidosis

and inflammatory bowel disease;[167,171-173] chronic rejection after solid-organ transplantation[174-176] and chronic kidney disease and inflammation. [177-179]

Anaemia of chronic diseases is frequently immune determined. Cytokines and cells of the reticule endothelial system cause irregularities in iron homeostasis, synthesis of erythroid precursor cells, production of erythropoietin and reduction in the life span of red cells; all of which result in the pathogenesis of anaemia.[180] Erythropoiesis is weakened by certain chronic diseases, including the intrusion of tumor cells or microbes into bone marrow, HIV infection, hepatitis C and malaria.[181,182] Other risk factors which exacerbate anaemia linked to chronic diseases include blood loss events, vitamin shortages (e.g. cobalamin and folic acid), hypersplenism, autoimmune hemolysis, renal weakness, as well as radio-activity and chemotherapeutic mediations.[183,184]

(iii) Anaemia due to infection

Infections are considered to be the most common reason of anaemia in developing countries, predominantly in susceptible groups (expectant mothers and preschool-aged children).[185] In unindustrialized nations these include malaria and parasitic infections.[186] Anaemia is one of the secondary diseases present in individuals infected with HIV and has been associated with a quick disease evolution and death.[187,188]

In Africa it is suggested that almost 50% of children with malaria have severe anaemia.[189] In Sub-Saharan Africa, it is estimated that between 200,000 and 500,000 expectant mothers develop severe anaemia as a result of malaria.[145] *Plasmodium falciparum* infection during gestation is the major cause of up to 10,000 maternal anaemia-related deaths in Sub-Saharan Africa per annum.[190] Helminthes such as flukes, hookworm and whipworm cause continuing blood loss, and subsequently iron loss, which results in the development of anaemia.[191-193]

In Sub-Saharan African countries about 6.9 million women of childbearing age are infected with hookworm.[193] It has been observed that 51% of anaemic children unindustrialized countries are iron deficient. If hookworm could be

reduced by 25%, it would decrease iron deficiency anaemia by 35% and reduce anaemia by 75%.[194,195]

(iv) Pernicious anaemia

Pernicious anaemia is described as a common sign of cobalamin (Vitamin B12) deficiency. It is due to the absence of intrinsic factor (IF), a glycoprotein secreted by gastric parietal cells which is responsible for vitamin B12 captivation at the end of the ileum.[196] The reasons for the absence of IF include degeneration of the gastric mucosa, autoimmunity in contrast to the gastric parietal cells that secrete IF, and/or autoimmunity contrary to IF itself.[196-198] Other causes of vitamin B12 deficiencies include poor dietary intake, mainly due to diets composed of vegetarian origin, because vitamin B12 is only found in food of animal origin or food fortified with this vitamin.[199-201] Deficiency of vitamin B12 is frequently observed in developing countries; because pregnant woman and breastfeeding mothers are mostly deprived of food of animal origin which results in lack of vitamin B12 in their children.[202,203] In these countries approximately 40% of women of childbearing age are vitamin B12 deficient.[200]

The occurrence of vitamin B12 differs according to the age group. In the age group of 20 to 39 years the prevalence is estimated to be less to or equal to 3%; 40 to 59 years approximately 4% and ≥ 70 years it is estimated at about 6%[204]. The prevalence of vitamin B12 deficiency increases with age.[205,206]The deficiency of vitamin B12 in infants is related to lower mental acquittal.[196] Cognitive disorders in children with a deficiency of vitamin B12 such and include: slower response time in neuropsychological trials of perception, memory and cognitive, academic problems including inferior school performance, attention difficulties and aberrant performance as well as neural tube defect.[207]

(v) Genetic propensity

Sickle cell anaemia is due to a genetic change in hemoglobin and red cell structure; which results in lifelong hemolytic anaemia. Annually nearly 300,000 infants in Africa are born with hemoglobin sickness and more than 200,000 with sickle-cell anaemia.[208,209]

2.5.3. Anaemia as a public health concern

The classification of anaemia as a public health concern, is based on occurrence of hemoglobin values under the population-specific hemoglobin onset.[142] Ranking as a public health concern depends on prevalence (Table 2.3.)

Table 2.3: Ranking of anaemia as public health concern.[142]

Prevalence of anaemia (%)	Type of public health concern
≤ 4.9	No public health concern
5.0 – 19.9	Minor public health concern
20.0 – 39.9	Moderate public health concern
≥ 40.0	Severe public health concern

Taking into account all population groups, anaemia is a public health concern in every country in the world. Anaemia in expectant mothers is a reasonable to severe public health concern in more than 80% of the countries studied.[147] In Mozambique anaemia is a severe public health concern; more than 40% of preschool children, expectant mothers and non-pregnant women are anaemic.[147] According to the National Demographic and Health survey report, approximately 69% of children younger than five years of age are anaemic; with about 26% being minor anaemic, 39% moderately anaemic and 4% severely anaemic.[19]

Various schemes and interventions have been suggested to deal with micronutrient deficiencies and anaemia. However, these vary according to the region and country as well as between specific population groups.[210] The main ones include supplementation or fortification of food stuffs, addition of balancing foods, dietetic diversification, promoting food with highly absorbable vitamins and minerals, together with nutrition training and control of parasitic infections.[147,210]

Supplementation consists of providing a high dose of micronutrients in a pill, capsule, suspension or tablet.[42,211] Food based plans are important factors in a long term global scheme for control of micronutrient deficiencies and anaemia. They include a variety of procedures with the aim of increasing micronutrient status by increasing the production and intake of micronutrient rich food as well as the increase in bioaccessibility of micronutrients. Another method includes promotion of food fortification.[42]

2.6. Iron and iron fortification of food

2.6.1. Iron

Iron is an important element in body functions of metabolic progression such as oxygen transference, deoxyribonucleic acid (DNA) synthesis, and as an electron vehicle.[212,213] In the human body iron exists as heme and non-heme substances which include hemoglobin and myoglobin; transferrin and ferritin.[213]

The portion of iron captured as a proportion of consumed food is estimated to be low (only 15% to 35%) and depends on the situation and category of iron.[212] Various factors have been found to influence iron absorption throughout the gastrointestinal tract. The low pH of the gastric acid in the proximal duodenum increases the solubility and absorption of iron.[214] Also, ascorbic acid and citrate act as chelators, which solubilize the iron in the duodenum and increase iron absorption.[215]

As briefly mentioned earlier, anti-nutritional substances in food such as phytates, polyphenols and calcium are inhibitors of iron absorption. These substances are mostly found in a diet of plant origin.[216] Heme iron, which is found in food of animal origin, is highly bioavailable; which means that the anti-nutritional factors have no or very little influence, While non-heme iron from plant based foods is severely affected by anti-nutritional substances accounting for Its poor bioavailability.[216] Thus, the bioavailability of iron varies considerably, according to its dietary source.

Iron needs vary according to age (Table 2.4).[217] Iron losses occur during various metabolic actions such as peeling of cells from skin and mucosal shells,

including the liner of the gastrointestinal area;[218] menstruation[219] and increase of body mass during neonatal and infantile growth.[220] These iron losses are normally substituted through food consumption.[221]

Table 2.4: Iron needs of individuals in terms of absorbed iron[a] according to age and sex. [221]

Age/sex	mg/day[b]
4 – 12 months	0.96
13 -24 months	0.61
2 – 5 years	0.70
6 – 11 years	1.17
12 – 16 years (girls)	2.02
12 – 16 years (boys)	1.82
Adult men	1.4
Pregnant women[c]	
First trimester	0.8
Second trimester	6.3
Lactating women	1.31
Menstruating women	2.38
Postmenopausal women	0.96

[a]Absorbed iron is the fraction that passes from the gastrointestinal tract into the body for further use. [b]Calculated on the basis of median weight for age. [c]Requirements during pregnancy depend on the woman's iron status prior to pregnancy.

2.6.2. Food fortification with iron

Food fortification is the addition of one or more nutrients to food. The main objective of food fortification is to increase essential nutrients in the diet and thereby improve nutritional status of a given population. The reasoning behind food fortification is to prevent micronutrient deficiency in order to avoid the occurrence of disorders that lead to death or disability and socioeconomic disadvantages.[222,223]

Fortification of food is reported to be one of the most useful, cost effective and durable procedures to prevent and control micronutrient deficiency. Reports indicate that fortification of food with iron and iodine is successful.[224-226] Food fortification is classified as mass fortification or universal, targeted, household or community fortification.[209]

In universal fortification, specific micronutrients are added to foods; which are consumed by the majority of the population in a specific country. Targeted fortification is addition of specific micronutrients to foods which are consumed by high risk population group. Community and household fortification refer to the addition of micronutrients to food regularly consumed at family level.[209,227]

Application of powder to fortify Fortification of food for children immediately before eating, with a powder containing an assortment of minerals including iron and vitamins, has shown good results at household level.[228-230] The use of this multi-nutrient powder has been described to have various benefits: packets can contain several micronutrients (vitamins and minerals), packets are not heavy and can easily be carried, kept and circulated, multi-nutrient powder is inexpensive, it does not interfere with normal dietary habits of the community, and the possibility for nutrient overload is low.[229]

Constraints on iron fortification of food include: undesired properties due to iron reacting with other substances in the food eaten,[231] poor solubility,[232] and concern about the safety of iron food fortification in malarial regions.[233] As yet no negative effects have been reported after iron fortification, in a non-malarial iron deficiency region.[233] In areas where genetic disorders such as thalassemia and hemochromatosis occur, fortification of food with iron is not practical due to

the possible occurrence of iron overload.[234] Iron deficiency and malaria are endemic in the whole of Mozambique and a study carried out on the use of nutritional iron prophylaxis in pregnant mothers, did not report negative effects.[20]

Foodstuffs and condiments have also been fortified with iron in many developed countries.[235-237] This method of fortification has been found to decrease the occurrence of iron deficiency.[237-239] In contract, fortification of food with essential micronutrients has only recently been introduced in developing countries.[240] In South-East Asian countries, consumption of iron fortified foods has been found to increase the level of iron and reduce the occurrence of iron deficiency anaemia.[241] In West Africa a multi-sectorial programme for fortifying foodstuffs with micronutrients, including iron is well established; with more than 80% of families consuming fortified food.[242] Due to the success noted in the multi-sectorial food fortification program of West Africa; international organizations have declared this program a model for the implementation of public-private food fortification partnership programs in other African countries including Mozambique.[240]

2.6.3. Iron compounds for food fortification

Iron sources suggested for fortification of food include ferrous sulfate, ferrous fumarate, ferric pyrophosphate, and electrolytic iron powder.[209] These iron sources are categorized according to their solubility: water soluble; poorly soluble in water yet soluble in diluted hydrochloric acid; and, insoluble in water and poorly soluble in diluted hydrochloric acid.[243-245] Table 2.5 illustrates the iron sources proposed for food fortification with their respective bioavailability.

The amounts of iron suggested per iron source in Table 2.5, are based on wheat flour fortification, as it is the oldest staple food that has been fortified. It is also based on organoleptic properties acceptable after fortification.[246] However, according to the WHO, each country should estimate the amount of iron required for fortification, which would provide the iron that is lacking in the most commonly consumed traditional foods.[223]

Table 2.5: Iron sources usually used to fortify foods. [236,243-245]

Compound	Fe (%)	RBV[a]	RBV (man)
Water-soluble iron salts:			
Ferrous sulphate heptahydrate, dried	20.33	100	100
Ferrous gluconate	12	97	89
Ferric ammonium citrate	18	107	-
Water-soluble iron chelates:			
Sodium iron EDTA	13	-	200
Ferrous bisglycinate	19	-	200
Poorly water water-soluble iron salts (soluble in dilute hydrochloric acid):			
Ferrous fumarate	33	95	100
Ferric saccharate	10	92	74
Ferrous citrate	24	76	74
Ferric citrate	17	73	31
Insoluble in water (poorly soluble in dilute hydrochloric acid):			
Ferric pyrophosphate	24	45-58	21-74
Carbonyl iron	98	27-66	5-20
Reduced iron	97	12-54	12-148

[a]Relative bioavailability with respect to ferrous sulphate (100), RBV: relative bioavailability

Fortification with ferrous fumarate, rather than ferrous sulfate is reported to cause fewer undesired side effects.[247] Encapsulated forms of both ferrous sulfate and ferrous fumarate are not associated with oxidation of lipid during storage of the fortified food.[248] Neither of these electrolytic iron powders affects the organoleptic properties of fortified food.[243] Food vehicles that contain a high content of inhibitors such as phytates, are normally fortified with ethylenediaminetetraacetate (EDTA) a constituent of NaFeEDTA; because this iron source is reported to increase the uptake of both intrinsic iron in the food as well as fortified iron. Also, NaFeEDTA prevents lipid oxidation during the storage period.[249]

2.6.4. Iron overload

Iron overload is defined as an excess total body iron. It is a condition that manifests as a result of both hereditary and environmental factors. Hemochromatosis is a hereditary disorder found in people from Northern European descent.[250] Iron overload can be related to hereditary iron anaemias and blood transfusion dependent illnesses, include thalassemia and other blood conditions. These conditions are common in individuals who reside in the Mediterranean region, Southwest and Southeast Asia and India.[251] In sub-Saharan Africa iron overload appears to be related to intake of beverages prepared in iron-rich pots or due to genetic origin.[252,253] With the toxic effects of iron overload in mind, it would be important to evaluate the level and bioavailability of iron in a particular foodstuff, prior to fortification.

Iron overload related to food fortification has not yet been reported.[254,255] However, accumulation of iron has been reported in individuals who have taken oral iron medication for prolonged periods.[256] Intake of fortified food by individuals with genetic or other acquired conditions where impaired iron absorption is present may result in increased levels of iron. Fortunately however, the increase in the body iron load in the majority of these individuals is reported to be small.[257]

Iron that is taken in parenterally, or as oral medications has resulted in unwanted side-effects related to immunity and infections.[255] In countries where

malaria is not endemic, the link between iron fortification and infections or disease development has not been established.[258] A weak relationship between oral iron supplementation and the effect on immunity and infection has been reported in some endemic malarial regions.[258,259]

2.7. Summary

The following were the main features found during the review of the literature that led to the formulation of research questions pertinent to this study.

In Mozambique both soil and climate are suitable for cassava cultivation and it is widely grown, yet data related to the mineral content of soil where cassava is cultivated is scarce. It is also likely that the mineral content of soil in Mozambique varies according to geographic location[34] however, it is not known if the levels of minerals in the soil are related to the levels in cassava roots grown in that soil. Cassava is an important, widely consumed staple energy food in Mozambique due to its high content of starch, which makes it an ideal candidate for fortification, yet it appears the roots are low in minerals, especially iron and the bioavailability has not yet been tested.

The main cause of micronutrient deficiency is deficient micronutrient intake from the diet. Iron deficiency is the most common micronutrient deficiency.[140,153,154] Iron deficiency and resulting anaemia are major public health concerns.[19,147] Fortification of food with micronutrients has only recently been implemented in developing countries.[240] Due to the high levels of anaemia, particularly in mothers and pre-school children in Mozambique, it is likely that iron fortification could at least assist in alleviating the 40% of anaemia cases resulting from dietary deficiencies. Cassava is a widely consumed staple in Mozambique and therefore a possible candidate, in terms of what WHO recommends, for iron fortification.

Yet, as seen from the literature, it is not only iron deficiency that causes anaemia; other micronutrients like B vitamins and even protein, contribute to prevention. Fermented staples are known to have higher nutrient density[105,106,108] and the popularity of *mahewu* made from cassava as a base for iron fortification, to alleviate dietary anemia, has not been explored previously. It

has been established that fermentation decreases the toxic properties of cassava root,[82,83,86] which would add the advantage of increasing food safety as well as dietary anaemia if the fortification is feasible. From the literature survey, however, it also appears that no previous work has been published on the bioavailability of iron added to *mahewu*. The following chapters describe how each of these problem statements and research questions deduced from an in depth literature review, have been answered.

2.8. Limitations and possible constraints

Mozambique lacks infrastructure and it is difficult to access cassava plantations in remote rural areas to sample soils and roots. There are also financial constraints to examining large numbers of samples. There may therefore be limitations associated with sample size during this study.

In addition, there is very little information on the parameters of *mahewu* fermentation and standardization for cassava and no information on iron fortification of cassava. Therefore empirical research will have to be done to standardize *mahewu* fermentation and determine the optimal time for fortification as well as bioavailability after addition of iron.

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CHAPTER 3
MINERAL CONTENT OF SWEET AND BITTER VARIETIES OF CASSAVA ROOTS AND SOIL FROM FOUR DISTRICTS OF MOZAMBIQUE

3.1. Abstract

Dietary anemia and malnutrition are considered to be serious public health problems in Mozambique, especially in young children and women of child-bearing age. Cassava is a main staple and it is known that the roots, although high in starch, are deficient in minerals, including iron. However, it is not known if the mineral content in the roots is related to the amount of mineral in the soils of Mozambique. The aim of this study was to assess whether the mineral content of cassava roots was influenced by soil composition or the variety or type of cassava. The concentrations of aluminum, calcium, copper, iron, manganese, phosphorus, lead and zinc in cassava roots and soil, collected in four districts of Mozambique, were determined using an Inductively Coupled-Plasma Optical Emission Spectrometer (ICP-OES), after microwave digestion. The mineral content of soils from the four districts were found to differ significantly ($p < 0.05$). There was also a significant difference ($p < 0.05$) in the mineral concentration of cassava roots between varieties, but no difference between sweet and bitter types. The mineral concentration was found to be significantly higher in soil, than in the roots. This was greatest for Fe, where the concentration in root samples was not detectable, although soil concentration was as high as 24.78 mg/kg. It was concluded that in Mozambique the levels of minerals in soil did not influence the level in cassava roots. It is therefore unlikely that using fertilizers in soils would increase the concentration of essential minerals to meet the nutritional needs of communities where cassava is the main staple. Consequently it is recommended that fortification of cassava roots should be investigated as a means of improving the level of essential minerals, especially iron, in the diet of vulnerable populations in Mozambique.

Key words: cassava, mineral concentration, Mozambique, soil type.

3.2. Introduction

In Mozambique, agriculture is the key sector for social and economic growth.[1] Approximately 80% of the rural population depend on agriculture for survival.[2] Small-holder farmers cultivate 95% of the total land area, which is estimated to be 5 632 781 ha.[3] Mozambique is ranked 5th with regards to cassava production in Africa.[4] Cassava is cultivated in an area spanning approximately 43% of the total cultivated land, with small-holder farmers producing 94% of total cassava without addition of any fertilizer.[3] However, the cultivation of cassava with addition of fertilizers to the soil has been practiced elsewhere in the world.[5,6]

The optimal temperature for cassava cultivation ranges from 25 to 35°C.[7] In Mozambique the climate is tropical and humid; the annual ambient temperature varies between 23 and 26˚C for the coastal zones of southern and northern Mozambique with a mean annual rainfall of approximately 1200 mm.[8] The country is divided into ten agro-ecological zones, based on altitude, rainfall, temperature, humidity index and soil type.[9] Although cassava is cultivated in all ten agro-ecological zones, only four are considered intermediate to high cassava production areas while the others are low or marginal.[9]

Cassava is mainly cultivated in the following provinces: Nampula (29.27%), Zambezia (26.76%) and Inhambane (8.80%).[3] Often it is cultivated in combination with other crops such as maize, peanuts and legumes.[10] The majority of cassava produced (> 90%) is used for human consumption.[11,12] It contributes to food security, particularly in rural communities[13] and it is also considered a famine reserve crop in areas where drought occurs.[10] In 2012 the production of cassava was estimated at 10.05 million tons.[14]

Cassava varieties are divided into two types, bitter and sweet.[13] The bitter type has high cyanogenic glycoside content.[15] It is more common in Mozambique than the sweet type, since it is pest and drought tolerant and produces higher yields.[16,17] Although the peel of cassava roots contains calcium, copper, iron and zinc,[18] it is generally removed prior to processing or cooking for human consumption. The concentration of minerals differs between the peel and

roots[19] and depends on the physiology and mechanism of accumulation of the plant.[20,21] There is data on the mineral content of cassava cultivated in various parts of the world (Table 3.1), however, limited data exists for Mozambique.

Table 3.1: Mineral content of cassava roots.

Mineral	Content	Reference and country or part of the world
	15 -35 mg/100g	[28,] Humid tropics of West Africa, [29] North Thailand
Calcium	16 mg/100g	[30] United States (National Nutrient Database for Standard References)
	0.076%*	[29] North Thailand
	0.1 mg/100g	[30] United States (National Nutrient Database for Standard References)
Copper	5.8 mg/kg	[29] North Thailand
	8 - 24 mg/kg	[31] Mozambique (Mozambique Agricultural Research Institute)
Iron	0.27 mg/100g	[30] United States (National Nutrient Database for Standard References)
	17.1 mg/kg	[29] North Thailand
	21 mg/100g	[30] United States (National Nutrient Database for Standard References)
Magnesium	0.105%*	[30] *United States (National Nutrient Database for Standard References)*
	0.384 mg/100g	[30] United States (National Nutrient Database for Standard References)
Manganese	1.4 mg/kg	[29] North Thailand
	27 mg/100g	[30] United States (National Nutrient Database for Standard References)
Phosphorus	0.165%*	[29] North Thailand
	271 mg/100g	[30] United States (National Nutrient Database for Standard References)
Potassium	1.172 mg/kg	[29] North Thailand
	14 mg/100g	[30] United States (National Nutrient Database for Standard References)
Sodium	129.2 mg/kg	[29] North Thailand
	8 - 19 mg/kg	[31] Mozambique (Mozambique Agricultural Research Institute)
Zinc	0.34 mg/100g	[30] United States (National Nutrient Database for Standard References)
	7.5 mg/kg	[29] North Thailand

*percentage in dry matter

The mineral content of soil is known to influence the mineral content of certain crops,[22] but there is no information on whether soil composition influences mineral concentrations in cassava roots. Minerals such as calcium (Ca), copper (Cu), Iron (Fe) and zinc (Zn) are important micronutrients in human nutrition and health,[23,24] whereas aluminum (Al), manganese (Mn) and lead (Pb) can be toxic.[25-27] The aim of the present study was to assess whether the concentrations of these minerals in cassava roots, was influenced by the mineral concentrations in soils from four different areas of Mozambique, or by the variety and type of cassava. This information would provide an indication as to whether fertilization or fortification would be required to increase the nutritional value of cassava roots intended for human consumption.

3.3. Materials and methods

3.3.1 Study area

Samples of soil and cassava roots were collected from four districts of Mozambique (Figure 3.1).

The districts are located in the agro-ecological zones R2, R5, R7 and R8, which coincide with the intermediate and highest production areas of cassava.[9] The agro-ecological zones R2, R5 and R8 together produce 60%, whereas R7 produces 20% of total cassava in Mozambique. Agro-ecological characteristics for R2, R5 and R8 zones include an altitude between 0 and 200 m, rainfall ranging between 800 and 1400 mm and temperature between 24 to 26°C. In the R7 zone the altitude ranges between 200 and 500 m, the temperature ranges from 20 to 25 °C and rainfall is from 1000 to 1200 mm.[9]

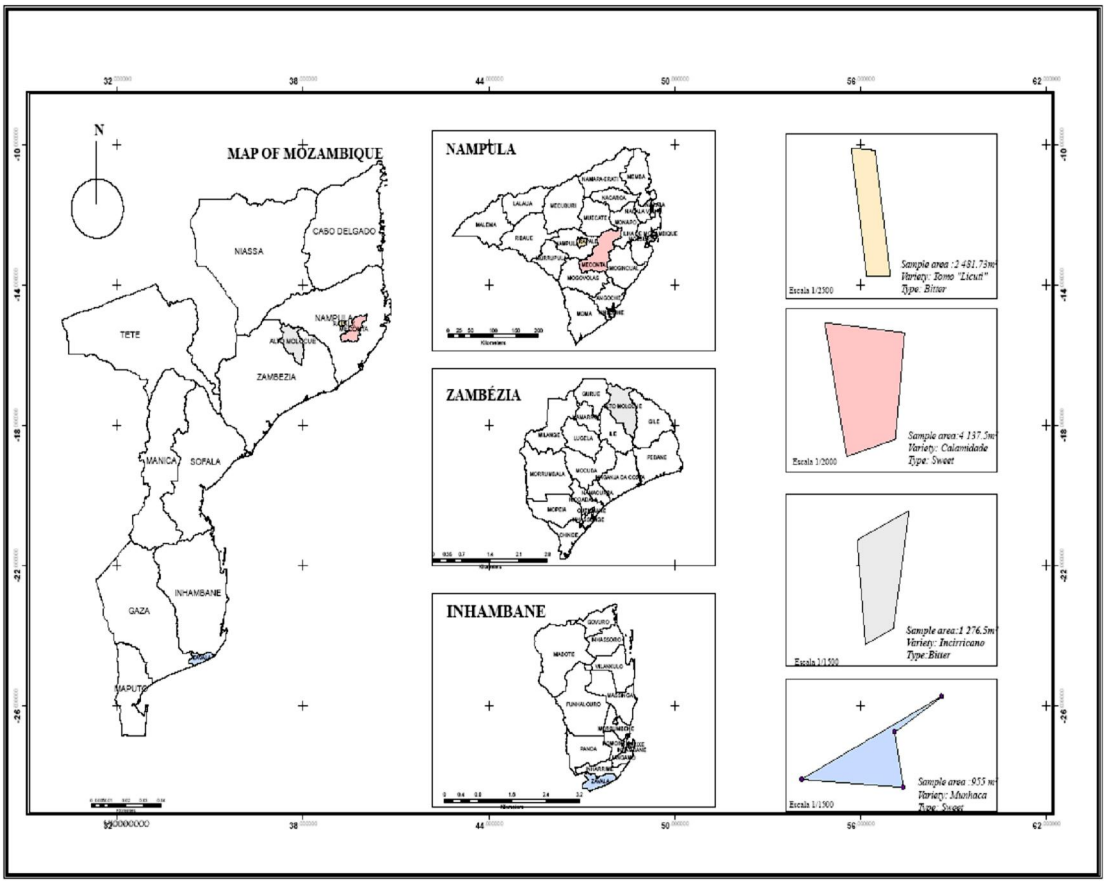

Figure 3.1: Map of Mozambique, indicating the four districts in which the samples were collected.

3.3.2. Sample collection

Cassava cultivation has been described in Chapter 2. Only mature plants were used for sampling and all cassava samples were collected from small-holder farmers. Samples of cassava root and adjacent soil (five per district) were collected using the non-probability judgement method.[32] The perspective of judgement includes the variety (the most cultivated), type (bitter or sweet) and region/area of cultivation (high and intermediate). Five samples per variety of bitter cassava roots (*Tomo* and *Incirricano*) were collected from Rapale and Alto Molocue districts in the Northern region. A further five samples per variety of sweet cassava roots (*Calamidade* and *Munhaça*) were collected from the Meconta Central and Zavala Southern regions. In each area where roots were sampled, three points were chosen randomly around the plants sampled and soil samples were taken at a depth of 20 cm (Figures 1 and 2, Appendix 9), and

then mixed together in equal ratios, to compose a representative sample. All samples were kept in plastic bags and identified by number, location, variety and type. Cassava root samples were hand peeled using metal free instruments, washed and stored at 4°C until determination of mineral concentration.

3.3.3. Sample digestion

Each sample was digested in triplicate. Soil (0.250 g) was weighed in a digestion vessel. Three milliliters of ultrapure nitric acid (65%, Merck, Darmstadt, Germany) and 2 mL hydrofluoric acid (40%, Sigma Aldrich, Johannesburg, South Africa) were added to the sample. The vessels were capped and then subjected to digestion using the Microwave Digestion Accelerated Reaction System – MARS (CEM Microwave Technology Ltd, Buckingham, UK). The instrument was set to KA Soil-X Press method at 75% of 1200 W, control temperature of 180°C, ramping and holding time of 10 min and cooling down time of 15 min. After digestion, vessels were opened using the capping station, and 2 mL boric acid (70%, Sigma Aldrich) was added. Vessels were re-capped and re-digested. The sample was transferred to a 50 mL volumetric flask and deionized water (<18.2 MΩ cm) was added to the full capacity level of the volumetric flask.

Grated fresh cassava root was processed in an oven at 210°C for 3 h. Then the grated cassava root, in triplicate for each sample, was weighed (0.750 g) into a digestion vessel and 4 mL nitric acid (65%) and 2 mL hydrogen peroxide (30%, Merck, Darmstadt, Germany) added. The vessels were capped and triplicate samples were subjected to digestion, as described above. Each sample was analytically transferred to a 25 mL volumetric flask and deionized water (<18.2 MΩ cm) was added to the full capacity level of the volumetric flask.

3.3.4. Chemical analysis

All digested samples were analyzed in triplicate for mineral concentration, using a SPECTRO ARCOS model (Spectro Analytical Instruments, Kleve, Germany) inductively coupled plasma-optical emission spectrometer (ICP–OES). Multi-element standard solutions were prepared by dilution of stock standard

solutions (1000 mg/L, Merck, Darmstadt, Germany) to the desired concentration. The ranges of the calibration standards were selected to match expected concentrations for the elements Al, Ca, Cu, Fe, Mn, P, Pb and Zn in the samples. Operating conditions for the ICP-OES are listed in Table 3.2.

Table 3.2: ICP-OES operating conditions for SPECTRO ARCOS.

Method	Parameters
RF power (w)	1400
Coolant flow rate (L/min)	12.00
Nebulizer flow rate (L/min)	1.00
Auxiliary flow rate (L/min)	1.00
Pump speed (rpm)	30
Rinse time (s)	30
Replicate read time (s)	15
Element	**Emission line (nm)**
Al	394.401
Ca	317.933
Cu	219.958
Fe	238.204
Pb	283.305
Mn	257.611
P	214.914

3.3.5. Statistical analysis

Samples were analyzed in triplicate using STATA version 12 (Copyright 1985 - 2011 StataCorp LP, statistics/data analysis, Lakeway Drive, MP-Parallel Edition, College Station, Texas 77845 USA). The normality of the data was assessed using the Shapiro-Wilk method. One-way ANOVA® was performed to test the variance and Bonferroni was used to compare means.

3.4. Results and discussion

3.4.1 Mineral concentration in soils

Table 3.3 presents the concentrations of minerals determined in the soil samples. It is evident from Table 3.3 that the mineral concentration of the soil samples between the districts differed significantly ($p < 0.05$). The concentration of Al in the soil samples, ranged from 1.21 to 2.6 mg/kg, Maria and Yost[33] reported a 0.18 to 0.38 cmolc/kg for Al in soils in Mozambique. The concentrations determined were much lower than those reported by Burt el al.[34] in the USA, which ranged from 0.5 to 142 g/kg, with a median of 46 g/kg. Cassava tolerates relatively high levels of Al in the soil, with saturation estimated at less than 75% in soils suitable for cultivation.[35-38] Thus, based on the previous reports, the concentration of Al found in the present study would not impact negatively on cassava cultivation.

Calcium is an essential micronutrient for maintaining bone health and strength. Ca concentration in soil samples of Alto Molocue district was lower than the detection limit of the instrument (0.004 µg/g). In the other three districts, Ca was found in a much higher concentration (Table 3.3).

A previous survey of soil fertility in Mozambique (Nampula Province) showed that the level of extractable Ca ranged between 2.33 and 4.34 cmolc/kg.[33] For optimal growth cassava requires 0.25 to 1.0 me/100 g of Ca; based on this finding, except in Alto Molocue district, the soil tested provided enough Ca for cassava cultivation.

Table 3.3: Mineral concentration (mg/kg) of soil in four districts of Mozambique where cassava roots were sampled.

	Mineral content mg/kg (n =15)							
Soil (district)	Al▲	Ca	Cu▲	Fe▲	Mn▲	P▲	Pb	Zn
Rapale♦	1.83 ± 0.09	2.76 ± 0.10	0.005 ± 0.000	2.6 5 ± 0.10	0.11 ± 0.00	LDL(0.05)•	0.02 ± 0.00	0.01 ± 0.00
Alto Molocue♦	1.21 ± 0.03	LDL (0.004)•	0.026± 0.003	24.8 ± 0.66	0.49 ± 0.02	0.30 ± 0.02	0.04 ± 0.00	0.01 ± 0.00
Meconta♦	2.60 ±0.09	2.70 ± 0.08	0.010 ± 0.001	6.45 ± 0.10	0.2 4 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.01 ± 0.00
Zavala♦	2.54 ± 0.13	2.54 ± 0.05	0.006 ± 0.000	9.22 ± 0.16	0.16 ± 0.01	LDL (0.05)•	0.04 ± 0.00	0.02 ± 0.00
Range	1.21 - 2.6	2.54 - 2.76	0.005 – 0.026	2.55 – 24.8	0.11 - 0.49	0.05 - 0.30	0.02 – 0.04	0.01 – 0.02
Mean	1.60	2.67	0.012	10.78	0.25	0.17	0.03	0.01
SD	0.66	0.12	0.010	9.72	0.17	0.18	0.00	0.00

▲Values in the same column with the superscript after the element are significantly different ($p < 0.05$)
♦Values at the same row with the superscript after the soil (district) are significantly different ($p < 0.05$)
LDL – Limit of detection of instrument (mg/kg)
•Values of limit of detection of instrument

The concentration of Cu in the soil samples ranged between 0.005 mg/kg and 0.026 mg/kg, with soil from Alto Molocue, showing more than double the concentration of the other three districts. The mean concentration of Cu (0.012 mg/kg) in the soil samples was found to be much lower than the mean concentration (1.25 mg/kg), previously found in unpolluted Nigerian soil where cassava was cultivated. [39]

Soil samples from the four districts tested, were significantly different ($p < 0.05$) in Fe concentration. The concentration of Fe in the soils was found to be higher than those of other minerals tested. These results are similar to those of Nubé and Voortman[40], who reported Fe as the most abundant mineral in the soil. The Fe concentration range was also higher than the range of 0.28 to 0.48 mg/kg, reported by Maria and Yost[33] in soils from Mozambique. It was also higher than that found in Zambian soil (median 0.8 mg/kg), in areas where cassava was cultivated.[41]

A significant difference ($p < 0.05$) in Mn concentration was found between soil samples from the different districts assessed. The concentration of Mn in the soil samples tested in the present study was lower than the 3 mg/kg considered normal for soil, by Davies[42].

Soil from Rapale and Zavala districts, contained P concentrations which were below the detection limit of the instrument. This probably indicates a deficiency in P the soils of Rapale and Zavala districts. This is supported by finding of previous researchers, who reported P deficiency in the soils of Mozambique.[33] However, in Alto Molocue and Menconta district, the concentration of P differed significantly ($p < 0.05$).

It was found that Pb concentration in the soils sampled, differed significantly ($p < 0.05$).The mean concentration (0.03 mg/kg) was considerably lower than that found in Zambian soils (11 mg/kg) where cassava was cultivated.[41] This is a positive finding, as Pb is a toxic element and its presence in soil can affect the health of communities.[25-27]

The mean concentration of Zn (0.01 mg/kg) in soils analysed, was found to be very low and its range was narrow (0.01-0.02 mg/kg). This low concentration

was in agreement with Cakmak[43], who reported Zn as the most prevalent mineral deficiency in soils globally. According to rather extensive surveys the levels of Zn in the soils range from 10 to 300 ppp.[44] The mean Zn concentration in the soils sampled, was also lower than that described for soil used to cultivated cassava (median = 15 mg/kg) in Zambia.[41]

3.4.2. Mineral concentration in cassava roots

The concentration of minerals in the roots of four varieties of cassava analyzed is presented in Table 3.4. Al concentration differed significantly ($p < 0.05$) between the Tomo and Incirricano varieties (Table 3.4). However, the mean concentration of Al ($0.04x10^{-3}$ mg/100 g) in the roots of cassava varieties analysed was low in comparison to published values.[43] This may indicate that cassava does not absorb Al from the soil. It was also noted that the concentration of Al in the root samples did not differ significantly between the types of cassava, better or sweet.

A significant difference ($p < 0.05$) in Ca concentration between the root samples of four varieties of cassava was observed. The mean concentration of Ca (9.29x10-3 mg/100 g) in the roots sampled was lower than that found in previous studies: 1.05 mg/100 g,[45] 120 mg/100 g[46] and 70 mg/100 g.[47] However, results indicated that there was considerable variability between different varieties, which was not related to whether that variety was a bitter or sweet type (range 19.5 to 188.90 mg/100 g). This may be related to the bioavailability of Ca.

The concentration of Cu in the root samples was found to be significantly different ($p < 0.05$) but this was not correlated with whether the variety was bitter or sweet. The mean concentration of Cu (0.1x10-3 mg/100 g) was lower than that reported by other authors: Oluyemi et al.[45] 0.034 mg/kg; Adeniji et al.[47] range 0.46 to 1.09 mg/100 g. In contrast, Ayodeji[46] could not detect Cu, in samples of cassava root from Nigeria. Copper, like iron, is an essential micro-nutrient and the low levels in a staple food are important as they will affect the nutrition of a large proportion of the population.

Table 3.4: Mineral concentration (mg/100 g) of cassava root varieties cultivated in four of districts Mozambique.

	Mineral content (mg/100 g) $x10^{-3}$ n =15							
Cassava variety	Al	Ca▲	Cu	Fe	Mn▲	P▲	Pb	Zn
Tomo BT (Rapale)	0.08 ± 0.00	139.5 ± 1.9	0.20 ± 0.00	LDL (0.052)•	1.10 ± 0.10	135.2 ± 2.4	LDL (0.084)•	0.70 ± 0.00
Incirricano BT (Alto Molocue)	0.03 ± 0.00	23.90 ± 1.30	0.10 ± 0.00	LDL (0.052)•	0.30 ± 0.00	42.30 ± 1.90	LDL (0.084)•	0.20 ± 0.00
Calamidade SW (Meconta)	0.04 ± 0.00	188.9 ± 2.6	0.20 ± 0.00	LDL (0.052)•	1.40± 0.00	127.10 ± 2.6	LDL (0.084)•	0.60 ± 0.00
Munhaça SW (Zavala)	0.02 ±0.00	19.50 ± 0.70	0.10 ± 0.00	LDL (0.052)•	0.30 ± 0.00	49.30 ± 2.20	LDL (0.084)•	0.20 ± 0.00
Range	0.02 – 0.08	19.50 – 188.9	0.10 - 0.20	LDL (0.052)•	0.30 – 1.14	42.30 -135.2	LDL (0.084)•	0.20 – 0.70
Mean	0.04	9.29	0.10	LDL (0.052)•	0.80	88.50	LDL (0.084)•	0.40
SD	0.03	8.49	0.10	LDL (0.052)•	0.60	49.50	LDL(0.084)•	0.20

▲Values in the same column with the superscript after the element are significantly different ($p < 0.05$)
BT-Bitter,
SW-Sweet,
LDL- Limit detection of instrument,
•Value of limit of detection of instrument in µg/g

The concentration of Fe in root samples from all four varieties of cassava was lower than the detection limit of the instrument. Extremely low concentrations of Fe in cassava root, has been reported previously and is ascribed to its unavailability to crops.[48] Although the Fe concentration in roots sampled during the current study was low, there was great variability reported in the literature, for instance, 0.53 mg/100 g,[45] 10 mg/100 g,[46] 11.73 mg/100 g[47] and 40.51 ppm.[39] It was also found during a previous study in Nampula Province Mozambique, that the concentration of Fe in cassava roots was 8 to 24 mg/kg.[31]

The concentration of Mn in cassava root was found to be significantly different ($p < 0.05$) between the varieties of cassava root sampled. However, these differences did not correlate with whether the root came from a sweet or bitter type (Table 3.4). The mean concentration of Mn (0.80×10^{-3} mg/100 g), was low compared to that of other studies: 0.05 mg/kg[45] and 2.45 to 2.82 mg/100 g.[47] Ayodeji[46] did not report the presence of Mn in cassava root.

It was further found that the concentration of P in the roots was significantly different ($p < 0.05$) between the four varieties of cassava. The mean concentration of P in bitter and sweet types appeared to be significantly different; however, the range was similar. The concentration range for P (42.30×10^{-3} to 132.20×10^{-3} mg/100 g) was lower than that reported in previous studies. For instance, Ayodeji[46] reported a range from 50 to 70 mg/100 g and Adeniji et al. [47] a range from 60 to 120 mg/100 g.

Although no Pb was detected in the roots sampled (the concentration was below the detection limit of the instrument), a previous study reported a concentration of 0.7 mg/kg,[41] while another found 159.3 mg/kg.[39] Other studies have also reported Pb in cassava roots to be 113.6 mg/kg in Nigeria[39] and 1.8 mg/kg in Zambia.[41] The significance for human health is that this reflects no risk of Pb poisoning for those consuming cassava as a staple.

Different root samples showed a significant difference ($p < 0.05$) in Zn concentration. The mean concentration of Zn (0.40×10^{-3} mg/100 g) and range (0.20×10^{-3} to 0.70×10^{-3} mg/100 g) was different to earlier studies, where concentrations found were: 0.082 mg/100 g[45]; range between 210 and 260

mg/100 g;[46]; 4.80 mg/kg;[41] and range 9 to 19 mg/kg.[31] The results indicated that there was also a significant difference ($p < 0.05$), in Zn concentrations between different types of cassava (bitter or sweet).

According to the findings of this study and the findings reported in earlier studies, the mineral concentration in cassava roots varies. The differences may be due to differences in geographic localization and cultivar.[31,38] According to Howeler[49], cassava roots absorb low levels of minerals such as Fe, Mn, Cu and Zn, moderate amounts of Ca and large amounts of P.[49] The later statement probably explains the difference in mineral concentration for cassava from the different locations and soil types shown in Tables 3.3 and 3.4. As it is difficult to compare concentrations where different units are used, mineral concentration of soil and cassava root samples, are compared in Table 3.5, using two units (mg/kg and mg/100g).

Table 3.5: Mineral concentration of soil compared to that of the roots.

	Mean concentration in soil		Mean concentration in roots		
Minerals	mg/kg	(mg/100 g) x 10^{-3}	mg/kg	(mg/100 g) x 10^{-3}	Ratio (Soil/root)
Al	1.60	160	0.004	0.04	4000
Ca	2.67	267	0.093	9.29	28.7
Cu	0.012	12.0	0.010	0.10	120
Fe	10.8	108	LDL	LDL	NA
Mn	0.25	25.0	0.008	0.80	31.3
P	0.17	17.0	0.089	88.5	0.19
Pb	0.03	3.00	LDL	LDL	NA
Zn	0.01	1.00	0.040	0.40	2.5

LDL: low detection limit of the instrument
NA: not applicable.

Yokel and Florence[50] have indicated that Al toxicity in humans could be caused by consumption of food staples high in Al, such as soya. According to the WHO,

approximately 1 mg/kg of body weight per week is considered the limit that can safely be consumed.[51] The chemical form of Al determines its toxicity (Personal communication, Prof H Rõllin). The bioavailability of Al consumed as food staple has been reported to be around 0.1 to 0.3%.[52] Neurodegenerative disorders, metabolic bone disease, dyslipemia and even genotoxic activity have been associated with Al toxicity.[53] Consuming cassava roots in Mozambique, is unlikely to result in Al toxicity, as this mineral was found to be 4000 times lower in the roots than in the soil and it is probable that cassava roots do not bio-accumulate Al.

The concentration of Ca was found to be more than 25 times lower than the concentration in the soil. In Alto Molocue district Ca concentration in the soil, was lower than the limit of detection of the instrument. Calcium is an essential microelement in human nutrition and only available to the body through food consumption. It is particularly important in bone composition.[54] Although the requirement for Ca varies according to age, the range is between 1000 and 1500 mg/day.[55] As cassava roots are a main staple for the population of Mozambique, the very low levels found in the roots are a cause for concern.

The concentration of Cu in cassava roots was also found to be extremely low. Copper is an essential micronutrient as it forms part of several enzymes in diverse biochemical processes including respiration, ant-oxidative defense and iron metabolism.[56] The low levels of Cu in cassava roots would therefore indicate a possibility of Cu deficiency arising in rural communities where cassava is the main staple.

It is evident from Table 3.3, 3.4 and 3.5 that the Fe concentration in soils is far higher than in the roots. This finding indicates that either the roots of did not take up Fe, or the Fe in the soil was not bioavailable. It has been suggested that the uptake of minerals by plants is inversely proportional to the concentration in soil,[57-59] which may partly explain the low uptake of Fe in the cassava roots samples (Table 3.4).

The presence of minerals such as Fe, in soil does not ensure that these minerals will be taken up, by plants growing in that soil. The uptake of minerals

by plants is influenced by soil texture, pH, Eh, cation exchange capacity, organic matter, the presence of other metals and microbial transformations.[41] In addition, it has previously been reported that Cu, Mn, P and Zn inhibit Fe uptake by cassava.[40] Interactions and antagonism between minerals or other substances in the soil, humidity, pH and temperature have also been found to influence mineral uptake from soil.[40]

It is important to note that Fe is an essential trace element in human nutrition. The body of a well-nourished adult contains around 3 to 4 g of Fe.[60] The lack of Fe in cassava roots indicates that rural populations in Mozambique, consuming cassava as a main staple, are at risk of Fe deficiency and resultant dietary anaemia. Iron deficiency in human populations is mainly caused by a low concentration of Fe in the main foodstuffs consumed.[61] In Mozambique, Fe deficiency and its related anaemia are considered to be a serious public health concern, affecting almost 40% of the population.[62] People most at risk of diet related Fe deficiencies are preschool children and woman of reproductive age.[63] A woman of reproductive age needs to consume 18 mg/day of Fe.[64] Those children born to mothers suffering from Fe deficiency, show deficient cognitive development and lower intelligence.[60] Pregnant woman with Fe deficiency are at risk of untimely delivery, children with low birth mass and prolonged, difficult parturition.[65]

Manganese concentration was found to be more than 30 times lower in cassava roots than in the adjoining soil. Consumption of food deficient in Mn has led to signs of deficiency.[66] This can manifest as Mseleni's disease,[67] skin diseases and bone abnormalities.[68] A well-nourished human body contains between 200 to 400 µmol of Mn.[69] Manganese acts as an enzyme activator and forms part of pyruvate descarboxilase and manganese-superoxidase enzymes,[70] when linked to argenine, it also regulates pH in the human body.[71]

There was little difference in P concentration between cassava roots and soil. It was found to be only 0.19 times lower in roots, than soil (Table 3.5). However, the concentration of P, detected in the cassava root samples in this study, is lower than the norm of 21 mg/100g.[30] Phosphorus is an essential macronutrient

required for bone and teeth composition. The daily P dietary requirement varies according to age and sex. Between the age of 19 and 30 the approximate normal requirement is 580-700 mg for both men and woman.[72] This low level of P in a staple food could be significant in vulnerable rural communities in Mozambique, where there is little access to foods of animal origin which are rich in P, such as milk.[73]

The fact that Pb was not found in the root samples of cassava, could be considered beneficial, since foods containing Pb, can have negative implications for human health.[74,75] Health concerns related to bioaccumulation of Pb, include mental impairment in children,[76] colic, anaemia and renal disorders.[77]

Although Zn is considered an essential element in food, it was also found to be deficient in the cassava roots analysed. The main functions of Zn within human cells are catalytic, structural and regulatory.[78] A nutritional deficiency could result in skin disease, diarrhea and sore throats,[79] as well as poor growth, mental disorders and epileptic convulsions.[80]

Based on the results it was evident that roots of cassava grown in Mozambique are deficient in a number of micronutrients that could affect the health of vulnerable rural communities. Generally when minerals are found to be deficient in crops used for human consumption, they are added to the soil in fertilizers.[40,81,82] However, the availability of minerals depends on physicochemical and biological aspects which influence uptake.[83] The uptake of Fe from the soil is extremely complex and increasing Fe content of crops through enrichment of soil is not easily realized.[84] Unlike Fe, the addition of Zn to soil can improve the Zn concentration in crops.[43,85,86] The levels of Cu and Mn can be improved in food crops by the application of fertilizers containing these minerals.[87,88] Previous studies have reported an increase in mineral concentrations in soil and cassava, in certain areas of Nigeria when fertilization of soil was implemented[89] Currently cassava in Mozambique is grown without any soil fertilization, however in rural and subsistence areas fertilizer would be very expensive and probably not affordable. Fertilization would in any case not

necessarily improve the Fe concentration in roots. In Mozambique it was found that even in areas where the soil had a high Fe concentration, it was not bioaccumulated in the cassava roots tested.

3.5. Conclusions

The most important finding was the low levels of essential micronutrients: Ca, Cu, Mn, P and particularly Fe in cassava roots, as it is a main staple food in Mozambique. This was probably most important in the case of Fe, where it has already been recognized that dietary anaemia is a serious public health problem affecting almost half of the population, mainly young children and expectant mothers. The concentration of these minerals in cassava root, both in the samples analysed and in the literature, would not be sufficient for good nutrition, particularly in young children and women of reproductive age.

The concentration of minerals in the soil, between the four districts, as well as between the roots of the four varieties of cassava, was significantly different. In all cases, the concentration in roots was lower than that found in the adjacent soil samples. However, this was not linked to whether the variety was of a sweet or bitter type. Thus the type of cassava root did not play a role in the concentration of minerals, although the variety did. This means that varieties with better uptake of micronutrients could be selected to improve nutrition of rural consumers. Strategies to increase mineral concentration in cassava roots through fertilization of soil could be possible, as in Mozambique cassava cultivation is done without the application of fertilizers, but may not be affordable due to poor road infrastructure and long distances to rural areas. However, the very large difference in concentration between soil and roots indicates that uptake of minerals from soil may be a problem, especially with regards to Fe, which is very low in the roots, even in areas with high Fe concentration in soils.

It is recommended that the best solution to the low level of essential minerals, particularly iron, in a major staple food in Mozambique, would be fortification of cassava products traditionally consumed by those vulnerable populations affected by iron deficiency.

3.6. Limitations

The pH of soils plays an important function during the chemical processes taking place between plants and soils. The forms on which the minerals and other nutrients from the soil are available to the plant depend on the soil pH. Data related to pH of the soil was not collected during the field work. This together with limited published information about soils in Mozambique limited the discussion of the results in the present study.

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CHAPTER 4
CONTROLLED FERMENTATION OF TRADITIONAL CASSAVA MAHEWU IN MOZAMBIQUE TO DETERMINE THE STAGE FOR IRON FORTIFICATION

4.1. Abstract

In rural areas throughout Mozambique, non-alcoholic fermented cassava (*mahewu),* is prepared at subsistence level using indigenous technology. Fermentation is known to inactivate cyanogenic glycosides, which are highest in cassava roots of the bitter type; and is also known to improve nutritional value. Up to 40% of anaemia cases diagnosed in women and children in Mozambique are probably due to dietary iron deficiency. The WHO and FAO recognize fortification of staple foods as an effective method to remedy dietary deficiencies of iron. Analysis of cassava roots in Mozambique showed a very low level of iron, regardless of soil composition. The objective was to standardize *mahewu* fermentation, to enable iron fortification of sweet and bitter cassava and investigate if the type of cassava fermented, or the iron compound used for fortification affected the final product. Sweet and bitter varieties of cassava from four districts in Mozambique were therefore fermented under controlled conditions (45°C for 24 h) and the optimal stage for fortification with ferrous sulfate ($FeSO_4.7H_2O$) or ferrous fumarate ($C_4H_2FeO_4$) compared. The mean pH at the endpoint was 4.5, with 0.29% titratable acidity and a solid extract of 9.65%. Mesophilic aerobic bacteria, lactic acid bacteria (LAB) and yeast growth were not significantly different in *mahewu* fortified with either of the iron compounds. There was no significant difference between bitter or sweet varieties. It is recommended that fortification occurs at the end of traditional fermentation when done at household level. However, where flour is being milled in larger villages, it could be fortified prior to sale in informal markets. There is also the possibility of large-scale commercial applications.

Key words: Cassava; *mahewu*; fermentation; iron fortification; bioavailability, Mozambique.

4.2. Introduction

Mahewu is a non-alcoholic fermented beverage consumed traditionally in several African and Arabian Gulf nations.[1] It is usually made by fermenting maize or sorghum using millet malt or a wheat flour starter culture.[1,2] Spontaneous fermentation is due to lactic acid bacteria (LAB) and yeast,.[2] Maize *mahewu* has been produced commercially in Botswana, South Africa and Zimbabwe.[3-5] Commercially, maize *mahewu* has also been enriched with, minerals and vitamins to improve the nutritional value.[6] Studies have reported that the LAB in maize *mahewu* raises the protein concentration and bioavailability of amino acids as well as improving levels of the B group of vitamins.[7] *Mahewu*, like other non-alcoholic fermented foods such as yoghurt, has probiotic properties that can sustain health.[8,9]

In Mozambique, maize is scarce and expensive, as very little is grown locally, so *mahewu* is made from staples like cassava, sweet potatoes or rice. However, home-grown cassava is mainly used in rural areas as the other two staples are also expensive.[10] Unfortunately cassava contains cyanogenic glycosides and anti-nutritional substances.[11] Processing techniques, including fermentation, can reduce these to a safe level, or eliminate them entirely.[12]

Fermentation promotes the bioavailability of minerals such as calcium, iron and zinc.[1] No published information on cassava *mahewu* could be found, but it is likely that, as for maize, fermentation would increase the protein concentration and the bioavailability of amino acids and minerals. As fortification of maize *mahewu* has proved effective, it is possible that cassava *mahewu* could also be successfully fortified with iron.

Excessive iron in the diet is known to be toxic, however previous research found that the concentration of iron in cassava roots was much lower than the minimum daily requirement for dietary iron (See Chapter 2 and 3). Ferrous sulfate ($FeSO_4.7H_2O$) and ferrous fumarate ($C_4H_2FeO_4$) have been used for iron fortification of several food staples including maize flour in countries such as

Brazil, Mexico and Venezuela.[13] Ferrous sulfate is a water soluble iron source, which can cause sensory changes in the fortified food. It is recommended for the fortification of foods with a short shelf life. Ferrous fumarate is poorly soluble in water with fewer sensory effects in the fortified food.[14] The absorption of iron from the fortified food depends on various factors such as iron status of the consumer and the presence of enhancers or not.[15] When maize flour is fortified with iron without the addition of enhancers the absorption of ferrous sulfate and ferrous fumarate has been reported to be similar.[16] However, the absorption of ferrous sulfate was found to be higher than that of ferrous fumarate in young children and infants diagnosed with iron deficiency.[16] Iron fortification of food with ferrous sulfate has been shown to significantly increase serum ferritin and hemoglobin levels and decrease anaemia in women of reproductive age and pregnant women.[17] According to the WHO, the iron compounds recommended for fortification of wheat and maize include ferrous sulfate, ferrous fumarate, and sodium iron EDTA (NaFeEDTA).[18] However, during the current study, only ferrous fumarate and ferrous sulfate were commercially obtainable as they appeared to the compounds most generally used for fortification of staple carbohydrates.

It has been estimated that about 40% of anaemia cases are due to a deficiency of iron in the diet.[19] It was shown that cassava roots from four districts of Mozambique were very low in iron, even when grown in iron rich soils (See Chapter 3) and this may be a contributory factor. It is well recognized by WHO that iron fortification is valuable for vulnerable populations suffering from dietary anaemia.

The objectives of this study were to investigate and standardize the fermentation of cassava *mahewu* with a view to comparing the effect of fortification before or after fermentation, the effect of cyanogenic glycosides (sweet or bitter types) and the type of iron compound to be used.

4.3. Materials and methods

4.3.1. Cassava

Cassava roots were collected in May 2013 from small-scale farmers in four districts of Mozambique. Five samples of each variety were collected from each district. Sweet cassava roots (varieties *Calamidade* and *Munhaca*) were collected from Meconta and Zavala Districts located in Nampula and Inhambane Provinces, respectively. Bitter cassava roots (varieties *Tomo* and *Incirricano*) were collected from Rapale and Alto Molocue Districts situated in Nampula and Zambézia Provinces, respectively.

4.3.2. Cassava flour preparation

Cassava roots were peeled, washed with tap water, grated and sun-dried before preparing the flour (Figure 3 and 4, Appendix 9), as done traditionally in villages in Mozambique. This dried cassava root, was pounded with a pestle and mortar and then sieved to prepare the flour used for cassava *mahewu* (Figure 5 and 6, Appendix 9). All instruments and containers used for flour preparation were made from plastic or wood to prevent contamination with metals.

4.3.3. *Mahewu* preparation

Flour from each of the bitter cassava varieties, was mixed together in equal portions, to prepare a representative sample of bitter cassava and the same procedure was followed for sweet cassava flour.

Cassava flour was fermented in the laboratory under controlled conditions, following the method described by Mutasa and Ayebo[3] and Bvochora et al.[4] for maize *mahewu,* with minor modifications. A 20 g portion of cassava flour was mixed with 49 mL of distilled water, and then added to 150 mL of boiling water. The mixture was boiled on a magnetic stirrer/hotplat, (FMH Electronics, STR-MH, F1093-0207, 230V ± 1050 HZ 500 watt, Durban, South Africa) for 10 min to gelatinize the starch (Figure 7, Appendix 9). It was then set aside to cool to 25°C. This porridge was transferred to a 250 mL glass Erlenmeyer flask and

1.25 g of starter culture (freeze-dried cassava *mahewu* previously prepared in the traditional manner), was added and mixed thoroughly.

A pilot study showed that the end-point of approximately pH 4.5 was consistently reached after 24 hours at 45°C and thereafter the pH stabilized. Thus the broth was fermented in a Labcon Standard Incubator, (Forced Circulation incubator, FSIM, with temperature range +5°C to 90°C, Maraisburg, South Africa) at 45°C for 24 h (Figure 8, Appendix 9). During the fermentation period, changes of pH, titratable acidity (acid concentration) and total solids were measured at hour 0 and hour 24. At the same time (hour 0 and hour 24) samples were collected for microbial analysis (aerobic mesophylic bacteria, LAB and yeast). The schematic flow of cassava *mahewu* preparation is presented in Figure 4.1.

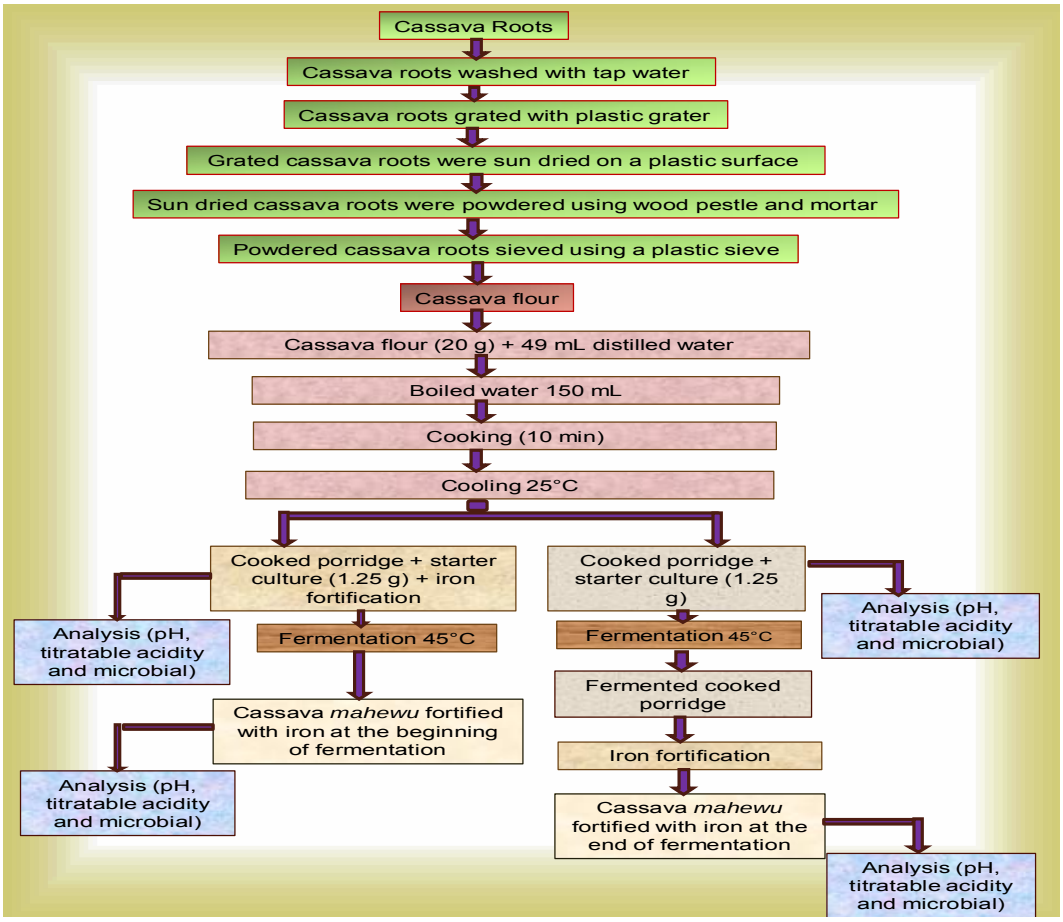

Figure 4.1: Schematic presentation of the preparation of cassava *mahewu* and indication of the stage at which iron is added.

4.3.4. pH changes, titratable acidity and total solids

The pH of *mahewu* was measured just after the addition of starter culture, at hour 0 and repeated at hour 24, the end of the fermentation period. The titratable acidity was determined by titrating 9.00 mL of the sample with 0.100 M NaOH and phenolphthalein as indicator. The concentration of acid was expressed as a percentage of lactic acid, according to the formula:

$$\% \text{ lactic acid} = \frac{\text{mL of 0.1 M NaOH} \times \text{normality of NaOH} \times \text{Mol wt of acid}}{\text{mL of sample} \times 10}$$

Total solids were determined using a freeze drying technique. Prior to freeze drying, the freeze drier cups were washed, dried and placed in a desiccator overnight. The mass of freeze drier cups were measured, *mahewu* was transferred and they were reweighed. The *mahewu* in the cups was freeze dried using a freeze drier (Air and Vaccum thechnologies, Intruvac 13 KL, V150120, final pressure 0.03 bar, Durban, South Africa) over 5 days, then reweighed. The following formula was used to express the results on a dry weight basis:

$$\% \text{ Total solids} = \frac{\text{Mass of dry sample}}{\text{Mass of sample before freeze drying} \times 100}$$

4.3.5. Microbial analysis

Fermented mahewu was serially diluted and 1 mL aliquots were plated onto Nutrient Agar (NA), Malt extract Agar (MEA) and de Man, Rogosa & Sharpe (MRS) Agar (Merck, Germany). The NA plates were incubated at 37°C for 24 h, to assess if mesophylic aerobic bacteria were present. The MRS agar plates were incubated at 37°C for 48 h under anaerobic conditions, to assess the growth of LAB. The MEA plates were incubated at 25°C for 5 days to assess if yeast was present. At the end of the incubation period, colony counts were performed (Figure 9, Appendix 9).

4.3.6. Iron fortification of *mahewu*

Cassava *mahewu* was fortified with two different iron sources, ferrous sulfate ($FeSO_4.7H_2O$) and ferrous fumarate ($C_4H_2FeO_4$) at two different stages: at the beginning (hour 0 when the starter culture was added) and at the end of fermentation (hour 24). Different stages of fortification were investigated to determine when cassava *mahewu* should be fortified, either at home or as a commercial product, see Figure 4.1.

The amount of iron compound added to cassava *mahewu* (68 mg/100 g) was based on the average of the prescribed range of iron used to fortify maize meal (2.9 - 5.7 mg/100 g).[20] The "overage", which is the extra amount of the fortificant added to the flour to compensate for storage and processing losses, was calculated as 1.2 mg. [20]

Ferrous sulfate (6.8 g) or ferrous fumarate (3.8 g) was dissolved in 100 mL of distilled water and 1 mL of the solution added to the *mahewu*. For fortification at the end of the fermentation process (24 h), 6.2 g of ferrous sulfate or 3.45 g ferrous fumarate, was dissolved in 100 mL distilled water and 1 mL of the solution added to the *mahewu* (Figure 10, Appendix 9).

Samples taken to compare the effects of adding iron at the beginning or end of fermentation were in each case taken following the addition of iron compound. That is to say sampling at 0 hours followed addition of iron and sampling at 24 hours, was also done after iron fortification, for each iron compound (Figure 4.1).

4.3.7 Data Analysis

Mahewu was prepared in duplicate for each variety of cassava (bitter or sweet). Samples for each repeat of the bitter or sweet variety of cassava were taken in duplicate for pH and titratable acidity. During microbial analysis serially diluted (10^{-1} to 10^{-6}) samples of each repeat were plated in duplicate on each medium (NA, MRS and MEA). All data was analyzed using STATA version 12 with ONE way analysis of variance (ANOVA) at 95% confidence level.

4.4. Results and discussion

Traditionally, *mahewu* takes approximately 48 h to ferment at room temperature. During the pilot phase of this investigation, standardization experiments were performed at different temperatures and it was found that increasing the temperature resulted in *mahewu* with a similar appearance in a shorter time. This shorter time and higher temperature would be more suitable for commercialization of cassava *mahewu*. Having specific controlled temperature-time parameters for standardized fermentation also would result in improved food safety and quality of *mahewu*, as it has been reported that uncontrolled temperatures could be responsible for the inhibition of LAB and growth of adverse microorganisms.[21]

Table 4.1 shows the variation in pH, titratable acidity and total solids during fermentation of sweet and bitter cassava. *Mahewu* was fortified using two different sources of iron, added either before or after fermentation. During the fermentation process, as the pH decreased, the acidity increased and the microorganisms proliferated. When *mahewu* made from bitter and sweet cassava were compared, there was no significant difference in pH changes and titratable acidity at the 95% confidence level. There was also no significant difference in pH changes and titratable acidity between *mahewu* fortified at the beginning of fermentation or fortified after 24 h ($p \geq 0.05$).

Table 4.1: Comparison of pH changes, titratable acidity and total solids using two iron compounds.

		$FeSO_4.7H_2O$ Fortification				$C_4H_2FeO_4$ Fortification				Control	
Hours	parameters	BTB[a]	SWB[b]	BTE[c]	SWE[d]	BTB[a]	SWB[b]	BTE[c]	SWE[d]	BT[e]	SW[f]
0	*pH	6.05 ± 0.02	5.87 ± 0.30	6.18 ± 0.06	5.82 ± 0.05	5.99 ± 0.06	6.22 ± 0.18	6.10 ± 0.12	6.28 ± 0.13	6.13 ± 0.06	6.13 ± 0.00
	*Acidity (%)	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.00	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.03	0.06 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
24	*pH	4.50 ± 0.01	4.53 ± 0.01	4.58 ± 0.06	4.51 ± 0.13	4.46 ± 0.21	4.42 ± 0.24	4.5 ± 0.04	4.51 ± 0.13	4.57 ±0.01	4.57 ± 0.01
	*Acidity (%)	0.33 ± 0.05	0.35 ± 0.04	0.27 ± 0.00	0.29 ± 0.00	0.26 ± 0.02	0.29 ± 0.04	0.24 ± 0.01	0.27 ± 0.04	0.25 ± 0.00	0.30 ± 0.17
*Total solids (%)		9.71	10.2	8.90	9.95	9.56	9.55	9.52	9.76	9.82	9.51

[a]Bitter variety fortified at the beginning
[b]Sweet variety fortified at the beginning
[c]Bitter variety fortified at the end
[d]Sweet variety fortified at the end
[e]Bitter variety
[f]Sweet variety
*Values with no significant difference $p \geq 0.05$.

Table 4.2: Microbial counts (Log_{10} CFU/mL) of iron fortified cassava *mahewu* at 0 h and 24 h fermentation.

		$FeSO_4.7H_2O$ Fortification				$C_4H_2FeO_4$ Fortification				Controle	
	Hour	BTB[a]	BTE[b]	SWB[c]	SWE[d]	BTB[a]	BTE[b]	SWB[d]	SWE[e]	BT[e]	SW[f]
Aerobic mesophylic bacteria	0	5.68 ± 0.02	5.70 ± 0.01	5.71 ± 0.06	5.56 ± 0.04	4.95 ± 0.11	4.80 ± 0.14	4.55 ± 0.01	4.80 ± 0.10	4.01 ± 0.00	4.10 ± 0.12
	24	7.96 ± 0.10	7.67 ± 0.22	7.69 ± 0.15	7.15 ± 0.15	7.83* ± 0.04	7.57** ± 0.07	7.57 ± 0.60	7.45 ± 0.21	7.12* ± 0.02	7.59** ± 0.01
Lactic acid bacteria	0	3.86 ± 0.42	4.06 ± 0.00	4.21 ± 0.44	3.88 ± 1.05	3.95 ± 0.17	3.8 ± 0.01	3.56 ± 0.70	3.30 ± 0.01	3.59 ± 0.65	3.84 ± 0.04
	24	7.72 ± 0.26	7.31 ± 0.01	7.11 ± 0.25	7.53 ± 0.10	7.52 ± 0.25	7.51 ± 0.03	7.51 ± 0.02	7.50 ± 0.12	7.22 ± 0.05	7.17 ± 0.08
Yeast	0	3.41 ± 0.00	2.93 ± 0.32	3.28 ± 0.87	3.48 ± 0.49	3.86 ± 0.88	3.18 ± 0.80	3.52 ± 0.32	3.40 ± 0.54	3.86 ± 0.21	3.76 ± 0.00
	24	6.84 ± 0.46	6.83 ± 0.30	7.04 ± 0.19	6.71 ± 0.72	7.43 ± 0.06	7.46 ± 0.32	7.51 ± 0.01	7.25 ± 0.45	5.91 ±0.51	6.82 ± 0.16

[a]Bitter variety fortified at the beginning
[b]Bitter variety fortified at the end
[c]Sweet variety fortified at the beginning
[d]Sweet variety fortified at the end
[e]Bitter variety
[f]Sweet variety.

The mean pH, with or without fortification, at the start of the fermentation process, was 6.08 (range 5.87-6.28) and at the end it was 4.52 (range 4.50-4.58). The average total solids in *mahewu* made from both bitter and sweet cassava, with and without iron fortification, was 9.65% (range 8.90-10.2%). The mean acidity at the beginning of fermentation was 0.07% (range 0.05-0.09%) and after 24 h 0.29% (range 0.24-0.35%).

Table 4.2 presents comparisons of microbial counts in *mahewu* from sweet or bitter cassava, with two different iron compounds used for fortification, at hour 0 and hour 24 as mean Log_{10} CFU/mL ± standard deviation. There was a significant increase in microbial counts of aerobic mesophylic bacteria, LAB and yeasts from 0 to 24 h ($p \geq 0.05$). The microorganisms mainly involved in fermentation were LAB and yeast. It can be seen that there was no significant differences in the microbial counts ($p \geq 0.05$) in *mahewu* made from bitter or sweet cassava. However, yeast counts after fermentation appeared to be slightly higher in the *mahewu* fortified with $FeSO_4.7H_2O$ or $C_4H_2FeO_4$, compared with controls, in both sweet and bitter cassava ($p \geq 0.05$) (Table 4.2). The relatively higher counts of yeast may have been because it used the iron sources for metabolism and growth. According to Pan et al.[22] yeast has the capacity to accumulate iron. This also agrees with earlier studies reporting iron as an essential mineral for yeast metabolism,[23] although LAB are able to grow well in an environment without iron.[24]

This increase in LAB and yeast during fermentation from hour 0 to hour 24 suggests that these microbes were mainly responsible for the fermentation of cassava *mahewu*.

The significance and participation of LAB and yeast in the fermentation of traditional foods, including cassava, have been described in previous studies.[5] The association of LAB and yeast as fermentation agents, found in cassava *mahewu* (Table 4.2*),* also agreed with what was previously reported for maize *mahewu*.[4,25] It is suggested that LAB and yeast facilitate the breakdown of numerous complex compounds, making them into simple and easily digestible substances that improve nutrient quality.[26]

The participation and roles of yeast in traditional fermented drinks have been described.[27] Yeast has also been related to fermentation of other cassava products such as *agbelima*[28] and *fufu*.[29] The presence of LAB and yeast in cassava *mahewu* probably also influences the organoleptic properties and enzymatic quality of traditional cassava *mahewu*. A previous work reported the involvement of LAB and yeast in development of typical characteristics including smell, taste, visual appearance, and consistency of fermented cassava foods.[27]

Although ferrous sulfate is more soluble in water,[16] both ferrous sulfate and ferrous fumarate appear to be good sources of iron for cassava *mahewu* fortification, although the World Health Organization suggests ferrous sulfate as the primary choice for this application.[30]

During cassava *mahewu* fermentation, as the LAB multiplied, the pH declined and acidity increased, probably due to the fermentation of sugar to form lactic acid. This favoured the growth of yeast, which proliferates at a lower pH. This finding is in agreement with that of Silva and Yang[31] who reported that pH is a key factor in the growth of microorganisms and the biosynthesis ratio of lactic acid. Cassava flour had been reported as being a good substrate for lactic acid production through fermentation.[32]

Table 4.3 shows the parameters of a standardization procedure for the preparation of iron fortified traditional cassava *mahewu* in Mozambique. The mean pH of fortified cassava *mahewu* found in this study, was 4.5 and the acidity was 0.29%. The mean solid content of standardized cassava *mahewu* fermented in this study, was found to be 9.65%.

The parameters found for cassava *mahewu* in the present study differed from those observed for South African indigenous maize *mahewu*, which has been reported as having a pH between 2.74 and 3.5, and acidity between 0.4 and 0.5% when produced under laboratory conditions by different researchers.[3,4,20,33] Several studies have reported that the best indigenous maize *mahewu* contained a solid substrate of 8 to 10%,[3,4] which was in agreement with the solid substrate found in the present study.

Table 4.3: Parameters for standardized preparation of iron fortified traditional cassava *mahewu* in Mozambique.

	Mahewu fortified with $FeSO_4.7H_2O$	*Mahewu* fortified with $C_4H_2FeO_4$
Amount of cassava flour	20 g	20 g
Total amount of water	200 mL	200 mL
amount of starter culture	1.25 g	1.25 g
Fermentation temperature	45°C	45°C
Cooking time	10 minutes	10 minutes
Iron added at beginning	6.8 mg	3.8 mg
Iron added at end	6.2 mg	3.45 mg
pH before fermentation	5.98	6.15
pH after fermentation	4.53	4.47
% lactic acid before fermentation	0.065	0.055
% lactic acid after fermentation	0.31	0.27
Total solids (%)	9.7	9.6

It can be seen from the literature reviewed above that parameters for maize *mahewu* vary. The difference between the present study on cassava *mahewu* and the literature on maize *mahewu* possibly lies in the quality and quantity of microorganisms in the starter culture not being identical, as present results using a standardized method were repeatable.

Cassava also has different types and proportions of carbohydrates to maize and these may not ferment in the same manner, resulting in a different pH and acidity to maize *mahewu*. Based on the above discrepancies found with maize mahewu, it was clear that there was a need for controlled conditions and established reproducible portions of ingredients, in order to obtain standardized parameters for the fermentation of cassava *mahewu* prior to fortification with iron (Table 4.3).

From the results of this study it is suggested that the end of fermentation is the best stage for iron fortification when traditional *mahewu* is prepared at home

from cassava roots, particularly in rural communities who would not buy cassava flour. Traditionally, home fermented cassava *mahewu* is sweetened by adding sugar, just prior to consumption and fortification could occur at that point. Home fortification using sachets containing several minerals and vitamins in powder form, which could be spread over or mixed into semi-solid foods have been reported to address micronutrient deficiencies, including iron deficiency, in young vulnerable children.[34] In contrast, the best stage for iron fortification of cassava *mahewu* made commercially would be at the beginning of the fermentation process. Cassava flour could be fortified during the milling process.

Mass fortification of flour has been reported to have favorable results for foods consumed by the public. Targeted fortification is used to raise the intake of a specific micronutrient for a particular group of people at risk.[30] Cassava is produced and consumed throughout Mozambique and mass fortification would work for urban communities, who would probably buy fortified cassava flour or commercially produced fortified cassava *mahewu*. However, in rural areas where *mahewu* is made from home grown cassava roots, a targeted approach would be better. It is suggested, that a flavoured, fortified sugar could be supplied for use with traditional cassava *mahewu*. Distributions to people that are known to have iron deficiencies, particularly young children and women of childbearing age could be through NGOs involved in rural clinics and schools.

4.5. Conclusions

Cassava *mahewu*, fortified with iron, was prepared using a standardized method which could be used for both traditional and commercial production, with iron fortification. Commercial application of the method could have a positive impact on the socioeconomics of Mozambique as well as improving the nutritional status of vulnerable communities.

The fermentation of cassava *mahewu* may also increase bioavailability of iron after fortification. Previous studies on cassava fermentation have reported an increase of the bioavailability of minerals such as calcium, iron and zinc.[35] Cassava *mahewu* is known to contain an organic acid (lactic acid), which could

also potentially increase iron uptake. According to Teucher et al.[36] fermented foods with high levels of organic acids are appropriate vehicles for iron fortification. This needs more investigation and will be tested in Chapter 5.

As a majority of the population in Mozambique consume cassava, iron fortified cassava *mahewu* could significantly improve food security and nutrition throughout the country. It is also very important that there was no statistical difference in *mahewu* produced from sweet or bitter cassava, as the bitter type is more easily grown at subsistence level, because it is more pest and drought resistant than the sweet type.[37] Irrespective of the stage at which the cassava *mahewu* is fortified with iron it would be beneficial to vulnerable rural populations in Mozambique, most of who consume this traditional beverage and also suffer from iron deficiency anaemia.

4.6. Limitations

The fermentation of cassava *mahewu* could also reduce or eliminate cyanogenic glycosides and other anti-nutritional substances found in cassava roots, due to increased acidity.[12] In the present study the cyanogenic glycoside content of fortified and unfortified cassava *mahewu* was not determined, as it was beyond the scope of the current investigation. The importance of fermentation of cassava to decrease the level of cyanogenic to safe levels for human consumption was based in previous studies on fermentation of cassava based food.

The microbial assessment on this study was done at a level that allowed only for the identification and quantification through counting Log_{10} CFU/mL of mesophylicaerobic bacteria, LAB and yeast. No attempt was made to identify individual species, as the primary objective was to evaluate whether there was a significant difference between sweet and bitter roots and two kinds of iron compounds, when *mahewu* was fermented and fortified.

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CHAPTER 5

IN VITRO BIOACCESSIBILITY OF IRON FORTIFIED CASSAVA *MAHEWU*: A NON-ALCOHOLIC FERMENTED BEVERAGE IN MOZAMBIQUE

5.1. Abstract

Fortification of the popular non-alcoholic beverage cassava *mahewu,* with iron could help alleviate the chronic dietary anaemia present in nearly half the population of Mozambique. Cassava roots from four areas in Mozambique were found to be consistently low in iron, despite high levels in soil. Therefore iron fortification of *mahewu*, a widely consumed fermented drink, was recommended. However, this iron must be bioavailable to the consumer. Inductively Coupled Plasma-Optical Emission Spectrometer and *in vitro* dialysability were used to assess total iron content and bioaccessibility in *mahewu* made from sweet and bitter varieties of cassava. *Mahewu* was fortified with either ferrous sulfate or ferrous fumarate at the beginning or end of fermentation. The proportion and concentration of bioaccessible iron was significantly higher ($p<0.05$) in *mahewu* made from bitter varieties of cassava, fortified with ferrous sulfate, although ferrous fumarate was more bioavailable in *mahewu* made from sweet varieties. The stage of fortification was found to affect neither the total iron concentration nor iron bioaccessibility. It was concluded that ferrous sulfate was the preferred iron source for fortification of *mahewu* made from bitter cassava varieties. At household level it was recommended that fortification takes place after fermentation, at the stage when sugar is traditionally added to *mahewu*. However, commercially, cassava flour could be fortified prior to fermentation.

Key words: Bioaccessibility, Cassava *mahewu*, Ferrous fumarate, Ferrous sulfate, Iron fortification.

5.2. Introduction

The prevalence of under-nutrition in Mozambique is approximately 38%, of which 43% of children younger than 5 years are moderately, 20% chronic and 8% acutely undernourished.[1] In Mozambique dietary anaemia resulting from iron deficiency is recognized as a public health concern, with an occurrence of approximately 40%.[2] The prevalence of anaemia in children younger than five years of age was estimated as 69%; of which 26% were mildly, 39% moderately and 4% severely anaemic.[1]

Cassava is an important staple food in Mozambique. It is widely cultivated across the country, mainly by small-scale farmers.[3] Approximately 94% of cassava production is consumed by humans, with 4% made available for animal feed and industrial use.[4] While cassava is an excellent source of carbohydrates, it contains low levels of protein and micro nutients.[5] As shown in Chapter 3, cassava roots from four areas in Mozambique were found to be consistently low in iron, despite high levels in soil.

It has been strongly suggested that communities dependent on plant-derived foods for their major dietary intake, should consider iron fortification.[6] The recommendation for fortification of cassava *mahewu* has been supported by reports that populations consuming cassava as their basic energy source are at risk of deficient iron intake.[7] To date cassava *mahewu* has not been fortified in Mozambique. Safe fortification and consumption of cassava could alleviate the iron deficiency of vulnerable populations such as pregnant and nursing mothers and young children.[8] Taking into account the results of analysis of cassava roots from different parts of Mozambique (see Chapter 3) iron fortification of frequently consumed cassava products was recommended.

Cassava is consumed in various forms: stiff porridge (*karakata*) and roasted cassava or *rale*;[9] bread and other baked products;[10] cooked cassava mixed with vegetable and peanuts (*xiguinha*) and *mahewu*.[8] Cassava *mahewu* is a non-alcoholic fermented traditional beverage frequently consumed in Mozambique and is made using both bitter and sweet cassava. Chapter 4 has described a standard method for fermentation of *mahewu* and shown that fortification with

either ferric sulfate or ferric fumarate, was successful both at the beginning or end of fermentation. At village level, just before consumption, sugar is added to the beverage in order to sweeten it and this offers an opportunity for fortification with iron. Maize *mahewu*, which has a similar fermentation process to cassava *mahewu*, is fortified with minerals at industrial level.[11] This suggests that fortification of cassava flour to be used for *mahewu*, could also be done prior to fermentation. However, literature on iron fortification emphasizes that the bioavailability (bioaccessibility) of iron in a particular product, is an essential component in deciding which iron compound should be used.[12]

The aim of this study was thus to assess the bioaccessibility and total iron content of *mahewu* made from sweet and bitter varieties of cassava and fortified with either ferrous fumarate or ferrous sulfate.

5.3. Materials and methods

The collection of cassava roots, flour and *mahewu* preparation and the fortification of were performed as described in Chapter 4 (4.3.1, 4.3.2, 4.3.3 and 4.3.4).

5.3.1. *Mahewu* iron fortification

Cassava *mahewu* was fortified with two different iron sources, ferrous sulfate ($FeSO_4.7H_2O$) and ferrous fumarate ($C_4H_2FeO_4$) at two different stages; the beginning (hour 0 when the starter culture was added) and end of fermentation (hour 24). The selection of different stages of fortification was to determine whether cassava *mahewu* should be fortified at home or at commercial level.

The amount of iron added to cassava *mahewu* (68 mg/100 g) was based on the average of the prescribed range of iron used to fortify maize meal (2.9 mg/100 g to 5.7 mg/100 g.[13] The “overage”, which is the additional amount of the fortificant added to the flour, to compensate for storage and processing losses, was calculated as 1.2 mg.[13] The ferrous sulfate (6.8 g) or ferrous fumarate (3.8 g) was diluted in 100 mL of distilled water then 1 mL of the solution added to the *mahewu* for the fortification at the beginning of fermentation (0 h). For fortification at the end of the fermentation process (24 h), 6.2 g of ferrous sulfate

or 3.45 g of ferrous fumarate, was dissolved in 100 mL distilled water and 1 mL of the solution added to the *mahewu*.

5.3.2. Iron content and *in vitro* bioaccessibility

Iron content of the cassava flour and fortified *mahewu* was analyzed using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Spectro Arcos, Spectro Analytical Instruments, Kleve, Germany). *In vitro* bioaccessibility of iron from fortified cassava *mahewu* was determined using the dialysis method described by Luten et al.[14] Digestive enzymes used were pepsin (P-7000), pancreatin (P-1750), and bile extract (B-8631) that were procured from Sigma (Johannesburg, South Africa). Dialysis tubing used was Spectra/Por 7 (Ø = 20.4 mm) with a molecular weight cut-off (MWCO) of 10 kDa (G.I.C. Scientific, Johannesburg, South Africa) (Figure 11, Appendix 9). The iron content of the dialysate was assessed using the ICP-OES. Working multi-element standard solutions were prepared by dilution of the stock standard solutions (1000 mg/l, Merck, Germany) to the desired concentration. The ranges of the calibration standards were selected to match expected concentrations of iron in the samples analyzed by ICP-OES (Figure 12, Appendix 9).

5.3.3. Statistical analysis

Samples of cassava *mahewu* were homogenized independently and duplicated at the gastric stage. At the intestinal stage the duplicated homogenized samples were replicated to a final five repetitions. All data was analyzed using STATA version 12 with one way and/or multifactor analysis of variance (ANOVA) at 95% confidence level.

5.4. Results and discussion

A significant (p<0.05) difference was found in acidity and pH at the beginning and end of fermentation of cassava *mahewu* (Table 5.1). These findings were supported by previous studies on maize *mahewu* fermentation.[15] The changes were ascribed to fermentation by lactic acid bacteria (LAB) and yeast, which converts the starch into sugars to produce lactic acid, leading to a decrease in

pH.[16] Both the decrease in pH and increase in acidity of cassava *mahewu* have been considered as factors that could affect the safety or quality of cassava *mahewu*. The reduction in pH has been reported to be the result of antimicrobial action in fermented food.[17] Also, the product of fermentation, lactic acid, has been shown to have antimicrobial activity.[18] Although the pH and acidity of mahewu changed during fermentation, the total solid content and end pH remained more or less the same, whether fortified with iron at beginning or end of fermentation, using either bitter or sweet varieties.

Table 5.1: Chemical characteristics of cassava *mahewu* before and after fortification with iron.

Independent variables		Acidity (%)	pH	Total solids (%)
Stage of fortification	Beginning	0.06[a]	6.0[b]	*
	End	0.30[b]	4.4[a]	9.6[a]
Type of cassava	Bitter	0.30[a]	4.4[a]	9.5[a]
	Sweet	0.30[a]	4.4[a]	9.6[a]
Iron source	Ferrous sulfate	0.30a	4.5[b]	9.5[a]
	Ferrous fumarate	0.31[a]	4.3[a]	9.6[a]
	Control	0.31[a]	4.3 [a]	9.4[a]

[a, b] – Values within the same independent variable with different superscripts differ significantly ($p \leq 0.05$).

*Main effects ANOVA did not include the % of total solids, as this was not measured at the beginning of fermentation.

Traditionally cassava *mahewu* is considered to be safe for consumption if consumed within two days after fermentation if kept at room temperature. Pasteurized maize *mahewu* (pH ~3.5) has been reported to be safe for consumption up to 21 days after production if stored at 4°C.[19]

It has been seen from Chapter 3 that iron concentrations in cassava roots used to make *mahewu* were below the limit of detection of the instrument. This finding implies that the cassava roots contained little or no intrinsic iron; which is in agreement with earlier reports for this plant.[20]

Table 5.2 shows the influence of stage of fortification, type of cassava (bitter or sweet) and iron source (Ferrous sulfate or ferrous fumarate) on the proportion and amount of bioaccessible iron. The total iron content of the different *mahewu* fermentations made from bitter or sweet cassava, fortified with ferrous sulfate or ferrous fumarate, was similar at both stages of fortification (beginning or end) of fermentation for each iron source (ferrous sulfate or ferrous fumarate). Ferrous sulfate showed a significantly higher % bioaccessibility in bitter cassava than ferrous fumarate. However the proportional bioaccessibility of ferrous fumerate was higher in sweet cassava than bitter cassava at the end of fermentation.

The total iron content of *mahewu* fortified with ferrous sulfate or fumarate was below 68 mg/100 g, the initial concentration of added iron (Table 5.2). This finding is in agreement with Ikpeme-Emmanuel et al. [21]who fortified cassava meals (*gari* and *fufu*) with 20 mg/100 g of ferrous sulfate ($FeSO_4$), iron (III) sulfate ($Fe_2(SO_4)_3$) and ferric alum ($NH_4Fe(SO_4)_2 \cdot 12\ H_2O$-FA). These were shown after fortification to have an iron content for gari of 10.70 mg/100 g ($FeSO_4$), 8.80 mg/100 g ($Fe_2(SO_4)_3$) and 12.40 mg/100 g (FA); and for *fufu* of 13.40 mg/100 g ($FeSO_4$), 14.76 mg/100 g ($Fe_2(SO_4)_3$) and 13.85 mg/100 g (FA).[21] This decrease in iron content may be ascribed to changes in the oxidation state of iron or loses of iron during the fortification stage or during the processing procedure.

Mineral bioaccessibility is expressed as the amount (mg/100 g, db) of bioaccessible (dialysable) iron in the sample and the percentage (%) bioacessibility iron in the dialysate relative to respective total iron content. A significant (p<0.05) difference was observed in the percentage bioaccessible iron in *mahewu* fortified with either ferrous sulfate or ferrous fumarate). A higher percentage of iron was bioaccessible in *mahewu* fortified with ferrous sulfate. The difference in bioaccessibility of ferrous sulfate and ferrous fumarate is well known[22] and the present results confirm the latter findings.

The difference in total iron content between *mahew*u made from bitter or sweet varieties of cassava fortified with ferrous sulfate or ferrous fumarate, may be due to the difference in nutritional and ant-nutritional substances found between the varieties.[23] This difference could also be partially due to the solubilities of ferrous sulfate and ferrous fumarate in water. In the present study fortification was carried out using aqueous solutions of sulfate and fumarate; which seems to have had an influence on the amount of iron added to the *mahewu*. Ferrous sulfate is reported to be more soluble in water than ferrous fumarate.[24] This may also have to be due to the fermentation process as it is known to cause changes in physicochemical and functional characteristics of cassava.[25]

Table 5.3 shows the effect of variety, iron source and stage of fortification on the recommended RDA and absolute requirements of vulnerable communities.

The percentage of bioaccessible iron when cassava *mahewu* was fortified with ferrous sulfate was significantly (p<0.05) higher if made from the bitter variety, whereas the percentage of bioaccessible iron after ferrous fumarate fortification was significantly (p<0.05) higher when made from the sweet variety. Ferrous sulfate fortification of m*ahewu* made from the bitter variety of cassava was found to be more bioaccessible than the other preparations (Table 5.2 and Table 5.3). The stage of fortification (beginning or ending) did not affect the iron content nor the amount or percentage of bioaccessible iron, in the edible portion. .

Table 5.2: The effect of iron fortification, stage of fortification and cassava variety on the total iron content, iron bioaccessibility and amount of bioaccessible iron.

Fortificant	Cassava type	Stage of fortification	Total Iron content (mg/100 g, db)	Iron bioaccessibility (%)	Amount of bioaccessible iron (mg/100 g, db)
Ferrous sulfate	Bitter	Beginning of fermentation	$10.0^{c} \pm 0.0$	$26.3^{e} \pm 1.8$	$2.63^{d} \pm 0.2$
		End fermentation	$11.4^{c} \pm 0.7$	$21.4^{d} \pm 1.8$	$2.43^{d} \pm 0.2$
	Sweet	Beginning of fermentation	$18.2^{d} \pm 1.5$	$4.9^{a} \pm 0.4$	$0.88^{c} \pm 0.04$
		End fermentation	$17.9^{d} \pm 2.6$	$5.2^{a} \pm 0.4$	$0.94^{c} \pm 0.04$
Ferrous fumarate	Bitter	Beginning of fermentation	$8.1b^{c} \pm 2$	$7.8^{b} \pm 1.1$	$0.63^{b} \pm 0.1$
		End fermentation	$6.1a^{b} \pm 2.4$	$4.4^{a} \pm 1.7$	$0.26^{a} \pm 0.2$
	Sweet	Beginning of fermentation	$4.0^{a} \pm 1.2$	$12.7^{c} \pm 1.1$	$0.50^{b} \pm 04$
		End of fermentation	$4.5^{a} \pm 2.3$	$10.5^{c} \pm 2.7$	$0.47^{b} \pm 0.3$

a, b, c, d – Values within the same column with different superscripts differ significantly ($p \leq 0.05$), db – dry bases

Table 5.3: The effect of cassava variety, iron source and stage of fortification on level and percentage of bioaccessible iron per 200 g of cassava *mahewu.*

Independent variables		Total iron content (mg/ 200 g edible portion)	Amount of bioaccessible iron (mg/200 g edible portion)	% bioaccessible iron
Stage of fortification	Beginning of fermentation (0 hr)	1.9[a]	0.17[a]	11.7[a]
	End of fermentation (24 hr)	1.9a	0.20[a]	9.4[a]
Type of cassava	Bitter	1.6[a]	0.25[b]	13.2[b]
	Sweet	2.1[a]	0.13[a]	8.3[a]
Iron Source	Ferrous sulfate	2.9[b]	0.30[b]	12.6[a]
	Ferrous fumarate	1.1[a]	0.09a	8.8[a]

[a, b, c] – Values within the same independent variable with different superscripts differ significantly ($p \leq 0.05$)

Although, the fortification of cassava *mahewu* could be done either at the beginning or at the end of the fermentation process, it is proposed that the fortification at the end of fermentation would be better for *mahewu* production at household level. The use of sugar based sachets containing micronutrients, which could be added to semi-solid foods has been reported previously.[26] At household level in Mozambique, sugar is usually added to *mahewu* after fermentation ends and just before it is consumed so it would probably be very easy to motivate rural communities to use fortified sugar.

At the commercial level, fortification at the beginning is recommended as cassava flour could be fortified and used for *mahewu* preparation or to make other cassava foods. Cassava flour has previously been fortified with iron in Brazil, where a reduction in the prevalence of anaemia in school children consuming food products from the fortified cassava flour was reported.[27]

During the present study, the size of an edible portion of fortified cassava *mahewu* was taken as 200 g. (the mass of approximately a cup full of this non-alcoholic fermented drink, which has the consistency of yoghurt). Based on recommended daily allowances of iron for women and children below five years of age,[28] the percentage contribution of iron has beens calculated and is displayed in Table 5.4.

These results indicate that fortification of cassava *mahewu* could be helpful in increasing the daily dietary intake of iron. The amount of iron in 200 g of fortified cassava *mahewu* would be sufficient to provide between 15 and 45% of the recommended daily allowance, with the major benefit in children younger than five years of age (Table 5.4). In a previous study it was found that 100 g of processed cassava used in food could contribute to between 6 and 14% of RDA iron.[29] It should be noted that the contribution of cassava meals to iron RDA varies according to the method of processing as well as the population consuming the product. It is feasible that a higher level of fortification may be considered for particular segments of the population.

Table 5.4: The possible contribution that bitter and sweet cassava *mahewu* fortified with ferrous sulfate and ferrous fumarate can make towards the iron RDA and absolute requirements of vulnerable populations.

Independent variables		% of iron RDA for women	% of iron RDA for children younger 5 Yr	% of highest absolute iron requirement for women	% of highest absolute iron requirement for children younger 5 Yr
Type of cassava	Bitter	9.5a	15.5a	9.3a	39.0b
	Sweet	11.7a	19.1a	19.2a	18.9a
Iron Source	Ferrous sulfate	15.2b	24.8b	22.5b	45.6b
	Ferrous fumarate	6.0a	9.8a	6.1a	12.3a

[a,b] – Values within the same dependent variable with different superscripts differ significantly ($p \leq 0.05$)

Highest RDA[28] and absolute[30] iron requirements for women and children were selected.

Recommendations for women; RDA – 18 mg/day and absolute requirements – 1.46 mg/day.

Recommendations for children <5; RDA – 11 mg/day and absolute requirements – 0.72 mg/day.

The difference in the percentage contribution that *mahewu* could make from bitter and sweet cassava to the iron RDA for children younger than five years was not significant (Table 5.4). In children younger than five years, the percentage contribution *mahewu* can make towards the absolute iron requirements is higher than that made from sweet cassava. It was estimated that *mahewu* fortified with ferrous sulfate provides approximately 2.5 times the RDA of iron to women and children compared to ferrous fumarate, which means it contributes almost 4 times more to the absolute iron requirement.

5.5. Conclusions

It is concluded that the nutritional value of cassava roots, where iron was so low it could not be detected, could be significantly improved by fortification of *mahewu*, meeting up to 45% of the daily requirements of children less than 5 years of age. Bitter varieties fortified with ferrous sulfate, delivered the highest amount of bioaccessible iron in *mahewu*.

Of importance is that bitter varieties of cassava are more highly cultivated and offer many advantages to the communities which include resistance to pests and drought as well as a higher yield when compared to the sweet varieties.[3] At household level, sugar fortified with ferrous sulfate could be used to fortify home-made *mahewu* after fermentation. At commercial level it is recommended that mass fortification of cassava flour from bitter varieties using ferrous sulfate is instituted. It is further recommended that studies be carried out to determine whether cooking, followed by fermentation to produce *mahewu,* reduces the cyanogenic glycosides found in bitter varieties, to a safe level.

5.6. Limitations

The study did not include factors which could affect the uptake of iron by consumers of cassava. The effects of phytic acid and tannic acid in inhibiting the uptake of iron are well known, but were not measured in this study. Ascorbic acid is recognised as to promote iron uptake. It is present in raw cassava roots, but is destroyed by cooking – a necessary stage in the preparation of *mahewu*. Addition of ascorbic acid during or after fermentation may have altered the bioavailability of the iron, but this was beyond the scope of the study, although it

would be a good topic for further investigation of the iron fortification of cassava products.

The *in vitro* digestion in the present study was carried out at pH 2, which is assumed to be that of the adult stomach. It may have been useful if the study had been repeated at pH 4 which is that of an infant stomach, as iron uptake has been reported to differ. However, bio-accessibility studies are expensive and funds were not available to investigate the extra number of samples needed to compare this.

The study did not take into account zinc fortification, although the results on assessment of zinc in cassava roots also revealed that the concentration was very low and it is an essential micronutrient. However the findings of the present study open opportunities for further research on fortification of indigenous cassava products and addition of micro-nutrients other than iron.

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CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

6.1. Overview

The main motivation for this study was the high level of dietary iron deficiency and related anaemia, in vulnerable populations in Mozambique. Cassava is the main staple for nearly half of the total population and is grown in peri urban backyards or harvested as part of mixed cropping by small-scale farmers. The plant is drought and insect resistant and can remain edible for a very long time if kept underground. It is easily harvested, processed and consumed at household level. As such it contributes to household food security throughout Mozambique and is the main staple food of the poor, while middle income families can also afford other staples such as maize, rice or wheat breads.

However, it contains toxic cyanogenic glycosides and this makes certain cassava products a threat to food safety as it has previously been consumed raw in times of conflict. Fortunately many of the traditional products made from this root are sun-died, fermented and/or cooked, all of which decrease the levels of these toxins. *Mahewu*, a fermented non-alcoholic beverage is traditionally consumed by young children, not only in Mozambique, but elsewhere in Africa and it has the advantage of being made from cassava root that is peeled, sun-dried, cooked and fermented. It has the additional advantage common to many other fermented foods of increasing the levels of B vitamins and amino acids in a carbohydrate staple. *Mahewu* made from maize has already been successfully fortified with iron therefore, cassava *mahewu* met many of the criteria needed for a staple food to be fortified. Cassava *mahewu* is already made in homesteads, so it is both affordable and accessible, as the root is grown in the backyards of the very population that are most at risk of malnutrition.

The main research question was therefore: “Could we standardize the fermentation of traditional *mahewu* and find out which iron compound could be used at which stage of fermentation? This question could not stand alone. Iron

in excess could be toxic. Although literature review indicated a low level of iron in cassava roots, it was unknown whether this was the case throughout Mozambique and whether soil with a high iron content could result in cassava roots with a similar iron concentration. It was also important to find out whether the iron fortification of *mahewu* would yield sufficient bioaccessible iron to meet the recommended daily dietary needs in the populations most affected, which are pregnant women and children under the age of five years.

Soils and cassava roots growing in those soils were sampled in four of the regions in Mozambique where cassava was mainly produced. The mineral levels were assessed using an ICP-OES and it was found that in all cases, the levels in cassava roots were too low to be detected, irrespective of the levels in the soils. The next problem was to standardize the fermentation of *mahewu* and for this; the method used for fermenting maize *mahewu* was adapted and found to work. During the pilot study it was found that the key to reproducibility was the use of a standard, freeze-dried starter culture made from traditional fermented *mahewu* and containing mainly LAB and yeast.

Once the method of fermentation was standardized it was possible to investigate whether the acidity, pH and total solids would be adversely affected by iron fortification. Although no standardized blinded tasting trials were done, the opinion of locals tasting the fortified *mahewu* was that it tasted the same as the products they were used to consuming. Although the literature listed four possible iron compounds for fortification, only two could be accessed in South Africa, iron sulfate and iron fumarate. As these are also commonly used for fortifying commercially processed foods, they were used for the study. Iron sulfate proved to be the most bioaccessible, particularly in bitter cassava roots. This was an advantage as the majority of cassava produced in Mozambique is of the bitter type: it is more droughts and pest resistant than sweet cassava and grows more profusely. The *mahewu* made from bitter cassava roots and fortified with iron sulfate was found to have sufficient bioaccessble iron to meet between 15 and 45% of the RDA for women of childbearing age in communities where dietary anaemia has been previously found to be about 40%.

6.2. Discussion

The decision to study whether cassava could be fortified with iron was initiated after a previous study had reported the risk of inadequate intake of iron, zinc and vitamin A in children who consumed cassava.[1] It was known that anaemia was a major public health problem in Mozambique and that there was an important link between iron deficiency and anaemia linked to undernutrition.[2,3] This finding was useful to frame the context of the problem of anaemia, undernutrition iron deficiency, all of which were known to be present in Mozambique. Other countries, notably South Africa, had already successfully combatted under-nutrition through the implementation of programs where commonly consumed staple foods were fortified with iron.[4-6] As a result of iron fortification cases of iron deficiency and anaemia had been found to decrease significantly.[6-8] Another study had also reported a successful program for fortification of food with micronutrients in other African countries.[9] Although cassava root was known to be low in iron, a study reported the fertility of agro-ecological zones of Mozambique[10] Burns et al.[11] also mentioned variation in the chemical composition of cassava cultivated in some regions of Mozambique. This provided the impetus for examining whether soil played a role in increasing iron concentration in cassava roots, if it did, iron fortification could be dangerous as perhaps too much iron would then be present in the diets of vulnerable populations in some parts of Mozambique. However, this was shown not to be a risk as despite high levels or iron in soils in some areas, the concentration in cassava roots was very low indeed. The outcome agreed with earlier studies.[11-13] The challenge to developing a standardized method for fermenting cassava *mahewu*, so that it could be fortified, was solved by amending the existing method for fermenting maize *mahewu*.[14,15]

6.3. Conclusions and recommendations

It was concluded that the research questions asked at the beginning of this study had all been met. In fact, they had also been welcomed in Mozambique, where at the end of the project a workshop was held demonstrating how iron fortified, flavored sugar could be added to cassava *mahewu* to feed preschool children at school and it is likely that this project will be adopted by a local NGO.

Negotiations to fortify cassava flour at commercial level are ongoing. Programs for mass fortification of food with vitamins and minerals, similar to those in place in South Africa, have recently been introduced in Mozambique and it is likely that the current research will result in the inclusion of cassava flour in this initiative.

Recommendations for further research include studies on the level of cyanogenic glycosides in cassava *mahewu*, the effects of the addition of other minerals such as zinc, and the addition of Vitamin C during or after fermentation to improve bioaccessibility.

6.4. Strengths and limitations

A strong point of this study was the fact that it was the first time that the mineral concentration in both soil and roots of cassava was related to the under-nutrition of communities consuming cassava root as a main staple. This was also the first time cassava *mahewu* was prepared under controlled conditions a standard method established for producing this indigenous traditional non-alcoholic beverage. It is also offers a practical way of fortifying cassava *mahewu* with iron at both household and commercial level. From this it not only increases nutritional density of a staple food at subsistence level, but opens up economic opportunities for the entire country though commercialization. By facilitating fortification at both household and commercial level, the effect on the population is broadened and the iron fortification could have a positive effect on the level of dietary anaemia throughout the population of Mozambique. The research thus has long term health and socioeconomic benefits.

One of the limitations in the study was that there was very little information on soil structure and composition in Mozambique. Although mineral concentrations were done on soils in different areas, a comprehensive evaluation of soil type and composition was beyond the scope of this research. It was not possible to do in depth soil analysis in a food laboratory, in order to explore further the reasons why cassava roots did not take up iron, even in iron-rich soils.

A further weakness of this study was that it focused only on the very low iron concentration in cassava roots and specific fortification of *mahewu* with iron, as well as subsequent assessment of bioassessibility. Although it also provided information on the low concentration of zinc in soil and cassava roots, it did not explore the possibility of Zn fortification or bioaccessibility. This is a weakness because consuming more soluble iron has been reported to impair the absorption of zinc.[16,17] However other studies have reported that in fortified food the interaction between iron and zinc does not occur.[18] It could have been interesting to investigate possible interactions between iron and zinc, but unfortunately it was beyond the scope of this study.

Another weakness was present in the bioaccessibility assessment, where the addition of ascorbic acid, which is well known an enhancer of iron absorption, was not investigated. The inclusion of ascorbic acid would have allowed a comparison of the bioaccessibility of iron with and without enhancer. Another weakness, as mentioned in Chapter 5, was related to choosing a pH of 4 during the gastric stage without repeating the experiment at pH 2.

Even with the limitations mentioned above which were mainly related to limited financial resources, this study offered sufficient information to enable the decision makers to start a program to fortify cassava *mahewu* and other cassava products with iron.

6.5. General recommendations

During the investigation it was noticed that information on soil types and composition in Mozambique was lacking, especially in peer reviewed journals. It is recommended that agricultural institutes and research centers should publish this information if they have it. If not they should plan field research on soil types as this could have a major long term effect on cropping and food security. At the very least, it would bring Mozambique into line with other countries where a great deal of information is available on soil types and composition in different areas.

A program of mass iron fortification of food in Mozambique has already started with the fortification of cooking oil. It is recommended that cassava should be

included in this program. In rural communities cassava is milled by small enterprises and there is a need to involve this economic class in the program so that iron fortification could be implemented at the milling point and serve as a source of iron for consumers who buy this flour.

The fortification of cassava flour would also benefit urban communities as in Mozambique cassava flour is incorporated as 15 to 20% of wheat flour used for making bread. Home fortification of cassava *mahewu* could be done via provision of small sachets of sugar containing ferrous sulfate. This could be added to ready to eat *mahewu* or home fermented *mahewu*. It is proposed that provision of iron in sugar sachets could be done by NGO's working at rural communities in the field of nutrition and/or nutritional security.

A recommendation that is already being undertaken in Zavala district of Inhambane Province district is the use of fortified cassava *mahewu* in school feeding and during maternity classes as a source of income, which will also improve the health of school-going children and expectant mothers. The *mahewu* will not only be fortified, but also be flavored with fresh fruits such as bananas, mangos, orange and pineapples, directly after fermentation. In this province fruit is easily obtainable so it becomes a feasible small scale enterprise for unemployed women.

6.6. Directions for the future

The findings of this study were shared (Appendix 5) during the Faculty Day ceremony at the Faculty of Health Sciences of University of Pretoria in August 2014 as a Poster presentation. In addition it was presented at a workshop as feedback to the communities involved in cassava production in the Zavala district of Inhambane Province in Mozambique, one of the places where the samples were collected. More about the research will be shared (Appendix 7) in the articles submitted to peer reviewed journals. The author of this study intends to do further original and collaborative research on the role of fortified cassava to promote health in rural communities. Research will be focused on the nutritional density of cassava *mahewu*, investigation of the levels of cyanogenic glycosides and anti-nutritional factors. Investigation of fortification of cassava

mahewu with other essential micronutrients including zinc and vitamin A, will also be undertaken.

6.7. References

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