Microbiological Quality and Safety of the Zambian Fermented Cereal Beverage: Chibwantu

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by

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Front page cover: Home prepared chibwantu beverage. Photo taken during data collection on the production and utilisation of indigenous cereal based fermented food products in Lusaka province, Zambia. Photo by Mercy Mukuma Mwale, March 2012.

To my grandfather ***Isaac Ndaba Hara*** *for being a mentor in my life*

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DECLARATION

I the undersigned, declare that the dissertation submitted hereby by me for the Ph.D. degree at the University of the Free State is my own independent work and has not been previously submitted by me at another university or faculty.

Mercy Mukuma Mwale		
Name	Signature	Date

List of abbreviations and symbols

ANOVA	Analysis of Variance
BCN	Biodiversity Community Network
BGLB	Brilliant Green Lactose Bile
BLAST	Basic Local Alignment Search Tool
CFU	Colony Forming Units
^{o}C	Degrees Celsius
CO_2	Carbon Dioxide
CSO	Central Statistics Office
e.g.	For Example
EHEC	Enterohaemorrhagic *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FAO	Food and Agriculture Organization
FAOSTAT	The Statistical Division of the Food and Agriculture Organization
Fig.	Figure
g	Gram
HIV/AIDS	Human Immunodeficiency Virus/Aquired Immunodeficiency Syndrome
Hr /hrs	Hour/ Hours
Km	Kilometer
LAB	Lactic Acid Bacteria
µg	Micrograms
ml	Milliliters
MoH	Ministry of Health
NCBI	National Center for Biotechnology Information
NFNC	National Food and Nutrition Commission
MRS	De Man, Rogosa and Sharpe
nm	Nanometers

PCA	Plate Count Agar
PLHIV	People Living with Human Immunodeficiency Virus
RBCA	Rose Bengal Chloramphenicol Agar
rRNA	Ribosomal Ribonucleic Acid
SD	Standard Deviation
Sq.	Square
µl	Microliters
VRBA	Violet Red Bile Agar
VRBA- MUG	Violet Red Bile Agar- 4-methylumbelliferyl-ß-D-glucuronide (MUG)
v/v	Volume/ Volume
WHO	World Health Organization
w/w	Weight/ Weight
ZDHS	Zambia Demographic and Health Survey
%	Percentage

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Chapter 1

Introduction

Chapter 1

1 INTRODUCTION

Around the world, particularly the tropics, the fermenting of raw materials in the preparation of foods has a long history (Guyot, 2012). In Africa, fermentation has been used as an effective and inexpensive means to preserve the quality and safety of foods (Mensah, 1997; Nout and Motarjemi, 1997; Anukam and Reid, 2009). Fermented foods constitute a significant component of the diet and the major raw materials fermented include cereals, legumes, tubers, and milk (Mensah, 1997). Others are fruits and vegetables, meat and fish (Guyot, 2012). Particular attention has been paid to starchy raw materials such as cereals because they are more widely utilised as food in African countries, than in the developed world (FAO, 1999) thereby contributing more to the human energy intake (Guyot, 2012). In addition, a diversity of indigenous cereal based fermented food products such as porridges, breads, pancakes and beverages are prepared in Africa (Steinkraus, 1996, FAO, 1999; Marshall and Mejia, 2011).

The process of fermentation improves the nutritional value of these foods; such as through increased availability of some essential amino acids, vitamins and minerals (Wang, 1987; FAO, 1999; Tamang *et al*., 2012), reduces anti nutritional factors such as phytate and tannins (Svanberg and Lorri, 1997; Kayode *et al*., 2006) and also enhances their highly appreciated sensory properties. Furthermore, the process of fermentation prolongs the shelf life of foods, which is a very important and crucial aspect in the reduction of the risk of food borne illnesses, particularly in the developing countries where economic problems pose a major barrier to ensuring food safety (Holzapfel, 1997; Odunfa and Oyewole, 1998; Gadaga *et al*., 1999; Caplice and Fitzgerald, 1999; Motarjemi, 2002; Jespersen, 2003). Hence, in areas where preservation techniques such as cold storage (refrigeration) cannot be used due to lack of facilities and resources, fermentation becomes an important food preservation technique. In addition, since the 90’s more people have realized the nutritional and therapeutic value of fermented foods and drinks, and this has made fermented foods even more popular (Farnworth, 2003). There is a growing scientific interest in indigenous fermented foods and their importance in the nutrition and health of Africans as a result of which efforts are being made to industrialize some of the processing (Odunfa, and Oyewole, 1998).

Zambia is situated in the tropical belt of south central Africa, 8 to 18 degrees south of the equator and on a plateau 900 to 1,500 meters above sea level (Mwila *et al*., 2008). It covers 752, 629 sq. km (Mwila *et al*., 2008). The climate is tropical, and is divided into three distinct seasons: warm and wet from December to April, cool and dry from May to August and hot and dry from September to November (Export Board of Zambia, 1995). The country has ten (10) provinces; Southern, Western, North western, Lusaka, Central, Copperbelt, Eastern, Northern, Muchinga and Luapula, with an estimated total population of 13 million as of 2010, with 7.9 million (61%) living in the rural areas (FAOSTAT (FAO), 2004; Zambia Population census, 2010). Zambia produces a variety of staple crops with maize being one of the most important contributing about 70% of the total dietary calorie-intake of the population (FAO, 2001). Maize meal is the major staple food for the majority of Zambians and is important to food security. The other staple crops grown are cassava, sorghum, millet, rice, peanuts, and beans.

There are a number of different types of fermented food products that contribute to human diet in Zambia. However, were there is information on these products, it is generally inadequate. The products are prepared from different types of raw material such as cereals, milk, fruits and in some parts of the country fish and meat. Cereal raw materials include maize, sorghum, millet and rice which are mainly staples. Chibwantu and munkoyo are fermented beverages from maize grit and maize meal respectively and the root *Rhynchosia insignis,* and/or *Rynchosia heterophylla* (Zulu, Dillion and Owens, 1997) or *Rhynchosia venulosa* (Simwamba and Elahi, 1986).

The root is generally called munkoyo by the local people. The munkoyo root (*Rhynchosia* species) is from a sub shrub found as an under storey plant in the woodlands particularly the miombo woodlands. The densely forested miombo woodlands cut across southern central Africa. In Zambia it covers about 47% of the total land area of Zambia (Sekeli and Phiri, 2002). Consequently, the munkoyo roots are found in different parts of the country. The munkoyo root is tuber-like and fibrous and it is debarked, beaten into fibrous strands, dried and stored uncovered prior to use. In Zambia, there is no published information (to the authors' knowledge) on the classification of the edible species of munkoyo (*Rhynchosia*) roots and on which *Rhynchosia* species give yellow and white munkoyo roots and on the species of *Rhynchosia* roots that are suitable for preparation of chibwantu and munkoyo beverages, particularly the effect of the munkoyo roots on the microbiology of the beverages. The edible varieties are recognized in one way by the blooming flowers of the munkoyo plant. Literature on edible wild foods by Malaisse, 2010 highlights various types of munkoyo plants of South Katanga, Democratic Republic of Congo, which include *Rhynchosia insignis*, *Eminia holubii*, *Eminia harmsiana*, *Eminia antentennulifera* and *Vigna nuda*.

The process of preparation for Munkoyo and Chibwantu is the same, i.e. thick or thin maize porridge is prepared and the roots (Munkoyo) and/or extract added to the warm porridge and left to ferment spontaneously due to naturally occurring microorganisms, primarily lactic acid bacteria and yeasts. The microorganisms are inherent in the raw materials, utensils used in the preparation of the beverages such as metal and plastic buckets and drums and calabashes (*insupa*) and the surrounding environment. Fermentation occurs at ambient temperatures (25 – 30 ^{o} C) in 24 - 48 hrs (Lovelace, 1977; Simwamba and Elahi, 1986; Zulu *et al*., 1997).

Since fermentation is natural, and uncontrolled, fermentation time, product quality and stability varies. Most of African cereal based fermented foods deteriorate rapidly and become unacceptable to consumers within one or two days of production (Lorri and Svanberg, 1995; Mugula, Nnko and Sorhaug, 2001) which is also the case with chibwantu and munkoyo. Chibwantu and munkoyo beverages are consumed while actively fermenting (Zulu *et al*., 1997). Additionally, the level of hygienic practices during production determines the chances of contamination by spoilage and pathogenic microorganisms, further contributing to the low quality and unsafe beverages.

Among the rural people in Zambia, chibwantu and munkoyo are the favorite indigenous beverages produced at household level with traditional techniques. The beverages are consumed as major breakfast for the whole family, in between meal, during ceremonies such as marriage and funerals, and also during field works such as planting, weeding and harvesting. The beverages are also utilised as weaning and/or complementary foods for children. There are also claims among the rural populations that the beverages increase milk production in lactating mothers.

Peri-urban is a name given to grey area which is neither entirely urban nor purely rural in the traditional sense (Groppo, 2000). For the purpose of the present study, the peri-urban were transitional zones, on outskate of the urban areas were rural and urban activities are juxtaposed. In the peri-urban and some urban areas, chibwantu and munkoyo beverages are also consumed as breakfast and in between meals. The beverages are also prepared in homes by those who know how to prepare them and sold in the local markets, in used bottled water bottles as an alternative cheap energy drink compared to others on the market that in Zambia are considered as energy drinks (e.g. Coca-Cola company drinks).

Chibwantu and munkoyo are very important food products in Zambia, but at the moment, there is limited information on the microorganisms involved during fermentation to produce these beverages, and also on the microbiological quality and safety of these traditionally fermented products. And also information on the effects of the Munkoyo roots on the microbiology of these fermented beverages is nonexistent. The knowledge on fermentation including effects of the munkoyo roots, quality and microbial safety of these beverages is essential for the development of improved products for increased consumption, commercial production and marketing and development of standards and guidelines for commercial production.

The present research work aimed at evaluating the microbiological quality and safety of chibwantu beverage.

The specific objectives were to:

1. Gather information on the production and utilisation of indigenous cereal based fermented of food products in Lusaka and Chongwe districts of Lusaka province to help establish the relevance of these foods in households and the country at large – with special emphasis on munkoyo and chibwantu beverages.
2. Isolate and characterize the microorganisms associated with the Munkoyo Roots and Maize grit used during the preparation of chibwantu.
3. Isolate and characterize the essential microorganisms involved during the fermentation of chibwantu.
4. Study the survival of selected food borne enteropathogenic microorganisms during fermentation of chibwantu
5. Investigate the effect of munkoyo roots (*Rhynchosia insignis, Rhynchosia heterophylla* and/or *Rhynchosia venulosa*) on growth of selected microorganisms.

A review of literature related to cereal based fermentations was carried out to give some background information on these foods of Africa, their fermentation, dominant microorganisms involved during the fermentation process and the beneficial effects the fermentation process impart on the cereals and also the role these foods play in Africa. Additionally, some examples of non-alcoholic fermented cereal based foods and beverages, spoilage and safety of fermented cereal based food products including the ones that are street vended were also examined. The antimicrobial potential of plant products is also included since the beverage of concern for this study is prepared using munkoyo roots which are plant products and finally the future of cereal based fermented foods is discussed.

Chapter 2

Literature review- *Indigenous Cereal Fermented Foods of Africa*

Chapter 2

2 LITERATURE REVIEW - Indigenous Cereal Fermented Foods of Africa

Abstract

Fermentation is one of the oldest skills used for food preservation. Indigenous cereal based fermented food products are prepared in Africa using various techniques and raw materials and the food products form a vital part of the diet of many communities. The food products are highly esteemed because of their flavor and taste and the keeping quality under ambient conditions; thereby contributing to food security. Several indigenous cereal based fermented foods and beverages are prepared at household level in Zambia and the foods are consumed by members of the household including toddlers and children. The beverages include chibwantu, munkoyo, thobwa, katata, katubi, gankhata (seven days), kachasu and some commercial opaque alcoholic beverages such as chibuku. Literature on the microbiology of these foods such as the dominant microorganisms involved during fermentation, nutrition and probiotic potential is not available. Therefore, the objective of this review is to highlight some of these cereal based fermented foods prepared in Zambia with emphasis on the beverages and document the information known about them and examine some of the similar non-alcoholic beverages prepared in Africa. Since the focus of the current study is on Zambian cereal based fermented foods prepared using the munkoyo roots; which are plant products and also the fact that the beverages are sold in local markets, the antimicrobial potential of plant products as well as the spoilage and safety of cereal based fermented foods, including the street vended was examine. The documented information would be valuable in devising scientific means of improving their quality and optimizing their production.

Key words: Cereal fermented foods and beverages, food quality and safety

2.1 Background

Fermented foods are an essential part of diets of many people around the world (Hesseltine and Wang, 1980; Tamang, 1998). These fermented foods are prepared using various techniques, raw materials and microorganisms which vary from place to place (CI-ROAF, 2002).

Fermented foods have a long history in Africa and there is substantial evidence that these food products were used, from pottery material (ceramic pots) excavated in central Sudan in the eighties, used for sorghum based foodstuffs, porridges and beer and from engravings on Egyptian tombs (Haaland, 2007; Lyons, 2007) and have long been a traditional part of African culture (Haaland, 2007). However, it is difficult to trace their origin due to the poor writing culture in most of Africa (Odunfa, 1988; Lyons, 2007).

A diverse types of indigenous fermented foods are prepared in many parts of Africa in part as an expression of culture and lifestyle and the substrates for their preparation include cereals, legumes, root tubers, fruits (wild and domestic), dairy, meat and fish. Their preparation techniques may have been derived from the need to improve the sensory properties, preserve the food and also improve the safety before consumption and been passed on from one generation to the other without much documentation. Perhaps the most documented of the fermented foods is sour milk (Odunfa, and Oyewole, 1998).

In many instances indigenous foods are considered to be of poor quality thereby viewed as inferior (Vinceti *et al*., 2013) or food for the poor (Cloete and Idsardi, 2012; Matenge *et al*., 2012). In addition, the study by Matenge *et al*. (2012) found that the young adults (in the 20-29 year old age group) associated indigenous foods with a lifestyle that was too traditional and old fashioned. The perceptions are mainly due to the fact that the raw materials for preparation of indigenous foods are freely collected from the forest and/or the simplicity of the production technology, coupled with poor packaging and storage. The positive aspect is that such products are consumed for nutrition and health purposes by consumers of these foods (Matenge *et al*., 2012).

The peculiar nutritional and sensory properties of indigenous cereal based fermented foods and beverages (both alcoholic and non-alcoholic) are derived from the fermentation of specific raw materials. The fermentation techniques vary from the simple spontaneous fermentation that is complete within few hours to a day, to the very complex and sometimes long fermentation which can take anything from few days to several months (CI-ROAF, 2002). The preparation of many of these cereal fermented foods and beverages is still a traditional art in homes, villages and small-scale industries as it serves as a low cost method of preserving food for improved quality and safety. Therefore fermented foods are very important from a nutritional and health perspective and overall food security to most African populations (Oyewole, 1997; Odunfa, 1985; Odunfa, 1988; Odunfa and Oyewole, 1998; Lei, 2006).

2.2 Fermentation process

Fermented foods are defined as animal or plant tissue that has been subjected to the action of selected microorganisms and/or enzymes to give desirable biochemical changes and significant modification of food quality resulting in an acceptable product for human consumption (Tamang, 1998). Africa is one of the lowest cereal producers globally; however, cereals are more widely utilised in African countries, than in the developed worlds (FAO, 1999; Steinkraus, 2002; Kohajdova and Karovicova, 2007). The major cereals in Africa include maize, sorghum, millet and rice, and a large proportion of these cereals are processed by fermentation prior to consumption. In Africa fermented foods and beverages constitute a major portion of the people’s diet. A large number of food products prepared from cereals are well known and are generally used as refreshing beverages, weaning foods, breakfast or light meal foods and some as main foods in the diet. The food products can be classified on the basis of their texture as

1) Liquid (gruel) e.g. chibwantu, munkoyo, ogi, mahewu, pito, uji.

2) Solid (dough) and dumplings e.g. kenkey, mawe
3) Dry (bread) e.g. kisra, injera

(Oyewole, 1997; Odunfa and Oyewole, 1998; FAO, 1999; Nout, 2009).

Fermentation of indigenous foods is spontaneous (or natural) thus; no fermentation inoculation is used, such as starter cultures. The fermentation is carried out by lactic acid bacteria, yeasts, filamentous fungi or a mixture of these, through their role in biochemical changes within the substrates, to give the desirable tastes and flavor (Vicki, 2006; Nout, 2009). The fermentation typically results from the competitive activities of these different microorganisms; strains best adapted and with the highest growth rate dominate during particular stages of the process (Holzapfel, 1997). The microorganisms are naturally occurring on raw materials, utensils used in preparation and in the environment of the production site (Zulu *et al*., 1997; Tamang, 1998; Gassem, 2002). The fermentation conditions for production such as temperature, humidity and aeration are often not optimized (Odunfa, 1988) for the reason that, the preparation of many of these cereal fermented foods and beverages is still a traditional art in homes, villages and small-scale industries (Jespersen, 2003). This may also result in the proliferation of undesirable microorganisms that would convert e.g. lactic acid to undesirable end products that can adversely affect the taste and texture of the fermented food products.

Uncooked cereals are fermented first and then cooked prior to consumption. Cooking after fermentation and immediately before consumption offers some advantage, since pathogenic microorganisms are inactivated and thus increase the safety of the product. Examples of uncooked cereals that are fermented then cooked include sour porridge from Zimbabwe, mawe from Benin, uji from Kenya, ogi from Nigeria, kenkey from Ghana, kisra from Sudan and injera from Ethiopia (Simango, 1997; FAO, 1999; Ohenhen, and Ikenebomeh, 2007). Some cereal though are cooked and then fermented prior to consumption. (Jespersen, 2003; Gadaga *et al*., 1999; Lund *et al*., 2000). The consequence of cooking first is that endogenous grain enzymes (amylases) are inactivated and therefore no auto-amylolysis occurs (Lund *et al*., 2000). A source of fermentable carbohydrates, the right type of functional microbiota e.g. for mahewu and togwa (Mugula *et al*., 2001), or a source of amylolytic enzymes e.g. for munkoyo (Zulu *et al*., 1997) must be provided. Examples of cereals that are cooked and then fermented prior to consumption include chibwantu and munkoyo from Zambia, maheu (amahewu, mahewu) from Southern African countries, bushera from Uganda, and togwa from Tanzania (Zulu *et al*., 1997; Simango, 1997; Gadaga *et al*., 1999; Mugula *et al*., 2001; Muyanja 2003; Mugula *et al*., 2003b). The substrates added after the cooking are a source of concern particularly in terms of product quality and safety, since after the fermentation process most of the products undergo no further food processing before consumption such as cooking or pasteurization. In addition the fermentation processes are usually poorly controlled therefore increasing the chances of survival of pathogenic bacteria, production of bacterial toxins and also possible production of mycotoxins if inoculants contained fungi, in the food products.

Nonetheless, there are some fermented African foods whose production has been remarkably developed and these include kaffir beer and mahewu of South Africa, and ogi of Nigeria (Odunfa, 1988, Steinkraus 1997).

The categories of food fermentation include alcoholic, lactic acid, acetic acid and alkali fermentation (Blandino *et al*., 2003). Alcohol fermentation results in the production of ethanol and yeasts are the predominant microorganisms responsible (e.g. beers). Lactic acid fermentation (e.g. milk and cereals) is mainly carried out by lactic acid bacteria and during acetic acid fermentation alcohols are converted to acetic acids in the presence of excess oxygen. *Acetobacter* species are the main bacterial producers of acetic acid. Alkali fermentation is not common in cereals, but fish and seeds (Blandino *et al*., 2003). Other compounds formed during fermentation include other organic acids, aldehydes and ketones and carbonyl. Some of the products formed are volatile and contribute to flavors of fermented foods. In lactic acid fermentation the process is mainly by lactic acid bacteria present in the environment, present on raw materials, utensils or those derived from a starter culture. In such fermentation endogenous grain amylases generate fermentable sugars that serve as a source of energy for the lactic acid bacteria, since lactic acid bacteria (and many types of yeast) are generally poor degraders of starch, due to their lack of amylolytic enzymes (Zulu *et al*., 1997; Lund, *et al*., 2000; Nout and Motarjemi, 1997). The fermentation process can be accelerated by addition of a lactic acid bacteria starter culture through addition of some already fermented material (the practice referred to as back-slopping) or malt such as is a case in the preparation of togwa a Tanzanian traditional beverage, were germinated millet or sorghum grain are used as a source of amylase and/or starter culture (Nout and Motarjemi, 1997; Holzapfel, 1997). In case of the Zambian fermented beverages chibwantu and munkoyo, roots of *Eminia, Rhynchosia*, and *Vigna* species known locally under the generic name munkoyo are used as source of amylase (Zulu *et al*., 1997). However, there is no documentation as to whether the roots are also a source of starter cultures for the fermentation.

Chibwantu and munkoyo beverages are very similar fermented food products from maize grit and maize meal respectively and the munkoyo root. Munkoyo is common in many parts of the country. Chibwantu is common in the Southern, Central and Lusaka Provinces of Zambia. The process of preparation for chibwantu and munkoyo is the same, i.e. thick or thin maize porridge is prepared. Fresh munkoyo roots are pounded and the barks removed. The roots are then soaked in a small amount of water for up to 1hr during which time the water becomes yellow and takes on a characteristic munkoyo beverage flavor (Lovelace, 1977). The munkoyo roots and/or extract is then added to the warm porridge and left to ferment spontaneously due to naturally occurring microorganisms, primarily lactic acid bacteria and yeasts at ambient temperatures (25 – 30 ^{o} C) in 24 - 48 hrs (Simwamba and Elahi, 1986; Zulu 1997).

In Zambia, there are a number of other non-alcoholic and alcoholic cereal based fermented products that are prepared mainly using maize, sorghum and millet, however, there is not much documentation on these products like is the case in other African countries, example Nigeria (Chinyere and Onyekwere, 1996). Other non-alcoholic fermented products of Zambia on the eastern part of the country include, thobwa which is similar to the togwa prepared in Tanzania and mteteka which is similar to the sour porridge prepared in Zimbabwe (Gadaga *et al*., 1999) and is very common on the eastern part of the country where maize is pounded to remove the hull and then fermented before it is prepared for grinding into maize meal. The sour water from the fermentation process is generally called mteteka and is used in the preparation of the sour porridge. Sour porridges are quite common throughout Africa, particularly south of the Sahara (Odunfa, 1988). The alcoholic fermented food products include katata, katubi and kachasu; which is the same as the one brewed in Zimbabwe (Gadaga *et al*., 1999).

2.3 Beneficial effects of fermentation on cereals

Cereal grains are considered one of the most important sources of energy, dietary fiber, proteins, vitamins and minerals required for human health all over the world (Blandino *et al*., 2003). However, the nutritional quality of cereals and sensory properties of cereal products are sometime poor due to low protein content, deficiency in certain essential amino acids (e.g. lysine, methionine and tryptophan), course nature and presence of anti-nutritional factors such as phytic acids, tannins and polyphenols (Lopez *et al*., 2000; Kohajdova and Karovicova, 2007).

Several methods have been employed with the aim of improving the nutritional quality of cereals. These include genetic improvement (Munck, 1972; FAO, 1992; Shewry, 2007), amino acid supplementation with protein concentrates (Bressani *et al*., 1960) or other protein rich sources such as grain legumes (Mbata *et al*., 2009) or defatted oil seed meals of cereals (Blandino *et al*., 2003). Additionally technologies such as sprouting, milling, cooking and fermentation have been practiced, with fermentation probably being one of the best (Blandino *et al*., 2003).

Fermentation offers a lot of beneficial effects on the cereals which include the following:

- Improvement of flavor and texture
- Prolong shelf-life
- Reduce loss of raw materials; unfermented foods spoil easily compared to the fermented foods
- Reduce cooking time and fuel requirement
- Improvement of protein quality and carbohydrate digestibility
- Detoxification and destruction of undesirable factors present in raw foods such as phytates, tannins and polyphenols and Improved bioavailability of micronutrients
- Inhibition of mycotoxin producing moulds and degradation of mycotoxins
- Probiotic effects and reduced levels of pathogenic bacteria

(Kohajdova, and Karovicova, 2007)

2.4 Role of indigenous cereal fermented foods in Africa

2.4.1 **Nutrition**

Nutrition is defined as the provision, to cells and organisms, of the materials necessary (in the form of food) to support life providing the body with nutrients it needs to function properly at optimal levels. Food choices can influence the body’s health positively or negatively and many common health problems can be prevented or alleviated with good nutrition. Food choices (or intake) are shaped by diverse factors including pleasure, culture, tradition, religion and other social and economic reasons (Tepper *et al*., 1997; Blaylock *et al*., 1999).

Nutrients can be divided into two broad categories. Macronutrients are those that the body requires in large amounts and Micronutrients those we need in very small, but critical amounts. Macronutrients are fat, protein, carbohydrates and water and they exclude fiber, provide energy, measured in kilocalories, often called "calories" which is required to maintain basal metabolism and vital body functions, while vitamins and minerals are micronutrients necessary for other reasons such as co enzymes, co catalysts and buffers in the miraculously watery arena of metabolism. The body's nutritional health is determined by the sum of its nutritional status with respect to each required nutrient. The two categories of nutritional status are;

- Optimal (desirable) nutrition- adequate diet that provides sufficient energy and all the essential nutrients and fiber in amounts sufficient to maintain a healthy body

- Malnutrition- failing health resulting from dietary practices that do not coincide with nutritional needs. It includes over nutrition and under nutrition. Under nutrition - results from insufficient nutrient intake that does not meet the body's nutritional needs / deficiencies.

2.4.1.1 Nutritional Challenges in Africa

Protein Energy Malnutrition (PEM) is one of the major nutritional challenges in Africa, particularly in children (infants, pre-school and primary school going). Unlike many nutritional deficiency diseases, PEM is a macronutrient deficiency (energy and protein), not a micronutrient deficiency caused by insufficient food intake due to factors such as inappropriate weaning practices, staple diets that are often of low energy density and infections (viral, bacterial and parasitic) which may hinder nutrient absorption and utilisation (Nnakwe, 1995; WHO, 2006). The severe forms of PEM are Kwashiorkor-protein deficiency and Marasmus-protein and energy deficiency (Latham, 1997).

Micronutrient deficiencies are also due to inadequate food quantities and/or poor dietary quality. Main nutritional problems associated with deficiencies include anaemia - due to deficiencies of iron, folate (folic acid), vitamin B12 and other minerals, xerophthalmia - due to vitamin A deficiency, rickets - due to vitamin D deficiency and generally poor physical development - due to Zinc deficiency (Latham, 1997; Rivera *et al*., 2003).

Although the foods (diet) particularly the cereal based, may contain high amounts of minerals, these foods are associated with poor mineral bioavailability especially of iron and zinc (Šimić *et al*., 2009; Afify *et al*., 2011). Bioavailability is the proportion of the total amount of mineral element that is potentially absorbable in a metabolically active form (Poutanen *et al*., 2009; Šimić *et al*., 2009).

2.4.1.2 Consequences of Iron and Zinc Deficiencies

Iron and Zinc are very important in human nutrition. Iron deficiency anaemia is the most widespread nutrient deficiency in the world particularly in preschool children and women and is very common in Africa with the prevalence of 40.7% (WHO, 2008). Iron is a vital component of haemoglobin, myoglobin and many enzyme systems. In children, Iron deficiency anaemia is associated with decreased physical development, impaired immune function, poor growth, decreased physical activity and increased vulnerability to infections (Latham, 1997; Stolzfus, 2003; Davies and O'Hare, 2004).

Zinc influences the catalytic properties of many enzyme systems and intracellular signaling, thereby playing a central role in cellular growth, differentiation and metabolism. In infants and children, zinc deficiency is associated with decreased growth and development, impaired immunity and increased morbidity and mortality from infectious diseases (Latham, 1997).

2.4.1.3 Effects of fermentation on nutritional value

Fermentation is known to improve the nutritional value of raw materials improving the nutrient density and increase the amount of and availability of nutrients (Svanberg and Lorri, 1997; Lopez *et al*., 2001). A wide variety of indigenous foods are prepared in different parts of Africa and by far the largest group of these types of foods is the fermented foods where both the plant and animal based raw materials are used. Plant raw materials; cereals form the largest part, making them the principal source of energy and nutrients (CI-ROAF, 2002). Cereal based foods have been shown to improve in nutritional value when fermented (Svanberg and Lorri, 1997; Lopez *et al*., 2001; Chelule *et al*., 2010).

Microorganisms associated with the fermentation of food produce desirable amounts of enzymes which may degrade undesirable factors (including anti-nutritive compounds) (Lopez *et al*., 2000; Elyas *et al*., 2002) thereby converting the raw materials (which sometimes are inedible in their unfermented state) into foods with improved nutritive quality and enhanced flavor and aroma (Steinkraus, 1995) due to several volatile compounds that are formed. Fermented cereals produce a flavor much different from those of cooked unfermented foodstuffs.

Microbial fermentation also leads to a decrease in the level of carbohydrates as well as some non-digestible oligo and polysaccharides. This latter reduces the side effects such as abdominal distension and flatulence (Lei, 2006). In cereal fermented foods certain essential amino acids may be synthesized and the availability of B group of vitamins is improved compared to the unfermented foods (Blandino, 2003).

A study by Simwamba and Elahi, 1986 showed that during preparation of munkoyo beverage using munkoyo roots (yellow and white types), the munkoyo roots contain and contribute nutrients (sugars, protein and/or amino acids, and minerals) to the fermented beverage. Both types of munkoyo roots contain essential amino acids, however, the yellow munkoyo root contained more total and individual amino acids (which included lysine deficient in maize) than the white munkoyo root, contributing to the improved nutritional value of the beverage.

2.4.1.4 Anti-nutritional factors and change in nutrient content

Cereals contain anti-nutritional factors such as phytates, polyphenols and trypsin inhibitors. In addition, cereals such as maize have low levels of vitamin A and vitamin

B12 as well as essential amino acids such as phenylalanine, lysine, and methionine (Belitz *et al*., 2009; Chelule *et al*., 2010).

Phytates are charged and normally found in the form of complexes with minerals (polyvalent cations, such as iron, zinc, calcium, magnesium), starch and protein in cereals and other foods such as legumes, seeds, nuts reducing the bioavailability of minerals in the digestive tract (Svanberg and Lorri, 1997; Lopez *et al*., 2000; Blandino *et al*., 2003) and thereby limiting the nutritive value of these foods. Polyphenols such as tannins are rich in phenolic hydroxyl groups. Polyphenols also form complexes with minerals and protein and are known to inhibit iron absorption (Svanberg and Lorri, 1997).

Phytases which hydrolyze phytates are present in most cereals (Reale *et al*., 2007; Belitz *et al*., 2009) and are believed to be activated during the germination (sprouting) and fermentation processes (Svanberg and Lorri, 1997). Fermentation provides optimum pH conditions (5.0- 4.5) enzymic degradation of phytates by endogenous cereal phytases (Reale *et al*., 2007), thereby increasing the amount of soluble minerals. In addition, a wide range of fermenting microorganisms possess phytase activity which also contribute to the reduced phytate contents of fermented cereals and the activity is enhanced by the optimal temperature known to be in the range of 35^{o} C to 45^{o} C provided by the fermentation process (Kohajdova and Karovicova, 2007). Phytate degradation through lactic acid fermentation of maize or sorghum can change a diet of low iron bioavailability into a diet of intermediate to high iron availability (Svanberg and Lorri, 1997).

The study by Lopez *et al* (2000) showed that during lactic acid bacteria fermentation of sour dough, lactic acid bacteria destroyed phytate and increase calcium and magnesium solubility. The fermentation process lowered pH conditions for phytate degradation. Also during lactic acid fermentation in Ogi, phosphate is released from phytic acid (Odunfa, 1988) and this is similar with other studies (Lopez *et al*., 2001; Kayode *et al*., 2006).Therefore degradation of some anti-nutritional factor during the fermentation process makes some minerals more available such as iron, zinc (Svanberg and Lorri, 1997; Kayode *et al*., 2006), magnesium and calcium (Lopez *et al*., 2000). Reports also indicate that B vitamins such as thiamin, riboflavin and niacin content increase significantly during natural fermentation of sorghum, pearl and finger millet (Svanberg and Lorri, 1997; Elyas *et al*., 2002).

The study by Elyas *et al*. (2002) indicated that natural fermentation of pearl millet had no effect on tannins. However, polyphenols and phytate decreased significantly, protein content increased and elimination of phytate improved protein digestibility of the fermented millet. Similar results on protein content increase of cereals and improved quality (digestibility) were indicated by Antony *et al*. (1996), El Hag *et al*. (2002) and by Chelule *et al*. (2010).

2.4.1.5 Nutrient density

The staple cereal foods such as maize, millet and sorghum in Eastern and Southern parts of Africa are commonly prepared as a thick porridge for adults and older children or thin liquid gruel for the younger children (Svanberg and Lorri, 1997). Thin gruel maybe more easily consumed by the young children, however, its energy density is too low to meet the energy requirement of the young children (Svanberg and Lorri, 1997; Nout, 2009). Weaning foods will be reviewed in the next section. Fermentation involving use of germinated cereal grains (malt) decrease the viscosity of porridge due to low pH (3.6-3.8) and/or the amylase activity developed by the microorganisms. Malt contain active amylolytic enzymes that could degrade the starch components in the gruels and thus make them more liquid (Svanberg, and Lorri, 1997; Kayode, *et al*., 2006). Munkoyo root contains amylolytic enzymes that contribute to the quick liquefaction of the maize-based munkoyo beverage (Simwamba and Elahi, 1986; Zulu *et al*., 1997). The decreases in viscosity increase the nutrient density of the gruel.

In conclusion, improvement in nutritive value of a raw material is important particularly for the developing countries were majority of the people cannot afford commercially available and expensive fortified and expensive foods (Tamang, 1998).

Increasing nutrient density and nutrient (mineral and vitamin) bioavailability of cereal based foods through malting and fermentation is an approach that can be accessible to many populations particularly in rural areas. The process can therefore contribute to addressing nutrient deficiencies in a sustainable way. By using fermented foods in the diet; the nutritional status of the consumer can be improved.

2.4.2 **Weaning**

2.4.2.1 Weaning and breast feeding

Weaning is the process of gradually replacing breast milk or formula milk with solid foods as the main source of nutrients and energy. Maternal breast meal is recognized as the best food for an infant. It is nutritionally balanced and generally free from pathogens and other substances that may be hazardous to health. Breast feeding has also been shown to protect infants from infectious diseases particularly diarrhea (Adams, 1998). It does this both directly, through shielding the child from contaminated sources of food and water and more directly through the anti infective properties of milk itself. For these reasons breast milk alone is recommended as the best possible food and drink for the baby during its first 4-6 months of life and that breast feeding should continue way into the send year of a child's life and for longer if possible (Adams, 1998), as it has been shown to reduce morbidity and mortality in countries with a high prevalence of infections (Michaelsen and Friis, 1998).

Infants in developing countries are growing reasonably well during these first 6 months when most are predominantly breast fed (Michaelsen and Friis, 1998). Most mothers are unable to produce nutritionally sufficient breast milk to sustain adequate growth and development when a child reaches 4-6 months. At this stage during the so called weaning period breast milk is replaced or supplemented with other foods (Adams 1998; Davies and O'Hare, 2004). Cereal-based porridges are introduced in infant feeding during this weaning period and sometimes before the age of 4 months (Michaelsen and Friis, 1998; Onyango, 2003; Kayode *et al*., 2006).

Traditional complementary and/or weaning foods in sub Saharan Africa are thin porridges usually prepared from cereal grains i.e. sorghum, millet or maize the local staples, or other starchy foods such as cassava, potato or plantain, with little vegetables and no animal products. The problem with such diets is that they are bulky, of low nutrient and energy density and might make it difficult for the young children to take in enough nutrients and energy for growth and development. The diets also have a high content of anti-nutrients; phytates, tannins and polyphenols (Svanberg and Lorri, 1997; Michaelsen and Friis, 1998; Nout, 2009). Anti-nutritional factors such as phytate reduce the bioavailability of dietary minerals especially iron and zinc. Consequently the deficiencies are associated with reduced growth and development, impaired immunity, increased morbidity and mortality (Thapar and Sanderson, 2004). Infants and children weaned to these kinds of diets and at an early stage on life are at a high risk of developing malnutrition.

The weaning period in many economically poor countries is the most dangerous period in early childhood, through its association with particular diseases such as gastrointestinal infections often leading to high mortality (Davies and O'Hare, 2004).

2.4.2.2 Weaning and malnutrition

Malnutrition in infants and young children is one of the most serious problems in the developing world. Thirty-two percent of children under 5 years old suffer from being under weight and thirty-nine percent from stunting. Malnutrition is associated with impaired growth due to delays in motor and mental development, reduced immune function resulting in increased morbidity and mortality (Michaelsen and Friis, 1998). Other than early weaning before the age of four months on cereal based weaning foods that are bulky and low in nutrient and energy density, cereal based weaning foods and water used for their preparation are contaminated with microorganism (Kunene *et al*., 1999). In addition, early weaning may increase the risk of diarrheal diseases due to the introduction of pathogens.

Reports confirm that infections causing diarrheal diseases are the major cause of child morbidity and malnutrition (as infections may lead to reduced intake, absorption as well as utilisation of nutrients) in developing countries and indigenous weaning food, drinking water, and water used for food preparation as sources of the diarrhea (Svanberg *et al*., 1992; Motarjemi *et al*., 1996; Simango, 1997), an indication of an underlying food safety problem (Motarjemi, 2002). The etiological agents responsible for the food borne diseases include bacteria, viruses and parasites and the major ones are stated to be toxin producing strains of *Bacillus cereus* and *Clostridium perfringens* and infectious bacterial pathogens such as *Escherichia coli* (enterotoxigenic *Escherichia coli* (ETEC), enterohaemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *Escherichia coli* (EPEC) strains), *Salmonella, Shigella, Campylobacter, Vibrio cholera*, parasites such as *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium* and viruses such as rotavirus. The microorganisms are often the result of faecal contamination of foods (Svanberg *et al*., 1992; Holzapfel, 1997; Kunene *et al*., 1999; Motarjemi, 2002).

Raw foods are frequently implicated as sources of contaminants, as some foods naturally habour pathogens or derived from infected animals. A study by Kunene *et al*., (1999) reported contamination of sorghum based weaning foods from rural and informal settlements of South Africa with spore forming bacteria such as *Bacillus cereus* and *Clostridium perfringens*. However, during handling and preparation food contamination may occur through diverse sources including contaminated (polluted) water, flies and pests, domestic animals, dirty utensils, food handlers (e.g. soiled hands) or human and human excreta in the environment. One of the major causes of the food borne disease is the time-temperature abuse during food preparation, leading to survival and/ or growth of pathogens or production of toxins to disease causing levels. Two practices lead to time-temperature abuse; storage of prepared foods at temperatures that favor growth of pathogenic bacteria and/ or formation of toxins (mainly due to lack of facilities for cold storage such as refrigerators) and insufficient cooking or reheating of food prior to consumption (Nout and Motarjemi, 1997; Motarjemi, 2002).

2.4.2.3 Fermented weaning foods

There is growing interest however, in the use of traditional fermented and malted cereal foods for weaning with a view of increasing their energy and nutrient density by reducing the high bulk of unfermented products by reducing the viscosity of the cereal gruel (Kayode *et al*., 2006), decreasing the anti-nutritional factors particularly phytate and reducing the pathogenic contamination (Simango, 1997; Kayode *et al*., 2006) thereby enhancing nutritive value and food safety. Besides, various fermentation technologies have been used traditionally to prepare and also preserve foods. Examples of some of the traditionally fermented weaning cereal foods in Africa are ogi in West Africa (Nigeria), uji (Kenya), mawe (Benin), magai (Tanzania) (Kunene *et al*., 1999).

Fermentation has been associated with improvement of the nutritional value of the food: improved energy and nutrient density, removing or reducing anti-nutritional factors, thereby improving the bioavailability of minerals and improving digestibility of proteins, as reviewed in the preceding section on nutrition.

Traditional household technologies such as germination or sprouting; the process usually used in malting, can reduce viscosity of cereal based porridges and thereby increase the energy density. In addition germination has a profound effect on the nutritional value of cereals (Michaelsen and Friis, 1998). The study by Kayode *et al*. (2006) showed that germination decreased the viscosity of the sorghum porridge whereas the fermentation processes had a minor role, and further suggested that the decrease in viscosity was due to alpha-amylase that is activated during the grain germination. Protein availability is improved and the amount of anti-nutritional factors in particular phytates is decreased due to endogenous phytase activity which, are increased in germinated cereals (Michaelsen and Friis, 1998). Flour from the germinated cereal can be added to ordinary flour to initiate starch degradation and thereby reduce viscosity. Soaking and germination, however, increase the risk of contamination with pathogenic bacteria and moulds.

Fermentation by lactic acid bacteria and yeast of cereal for complementary foods has many potential benefits. The process can be spontaneous, accelerated by back-slopping (adding leftovers from previous fermentation) or initiated by starter cultures. Lactic acid fermentation has attracted attention in reducing pathogenic contamination because of the well established ability of lactic acid to inhibit the growth and survival of other microorganisms, especially gram negative bacteria, by lowering the pH. The storage time and safety of the fermented food product is increased significantly. Observations on the inhibition of food borne pathogens in fermented gruels have been reported by Mensah *et al*. (1988), Svanberg *et al*. (1992), Simango *et al*. (1992) and Kunene *et al*. (1999). The study by Annan-Prah *et al*. (1997) showed that pathogens get reduced, but are not eliminated. Inhibition depended on microorganism involved and initial size of inoculums where there is contamination.

Cereal based fermented foods have probiotic potential and feeding young children with these foods may have positive microbial and immunological benefits as reviewed in the next section. The fermenting microorganisms might pass the stomach and colonize the intestines. In addition, these microorganisms or the substances they produce might affect the composition of the gastrointestinal microbiota (control intestinal colonization by pathogens) (Kingamkono *et al*., 1999), improve gut permeability and reduce the risk of diarrhea and malnutrition.

2.4.2.4 Weaning and HIV/AID (Human Immunodeficiency Virus/Aquired Immunodeficiency Syndrome)

The risk of mother to child transmission of HIV through breast feeding poses a difficulty dilemma for HIV-positive women in low income settings. Avoidance of breast feeding can prevent postnatal HIV transmission. However it can also substantially increase morbidity and mortality from diarrheal, respiratory and other infectious diseases and may contribute to malnutrition. Morbidity and mortality resulting from persistent diarrhea and malnutrition in children are still high in sub Saharan-Africa, in part due to the HIV/AIDS epidemic (Amadi *et al*., 2001). People with HIV have an increased risk of malnutrition because of reduced food intake, reduced nutrient absorption, and reduced nutrient utilisation. Malnutrition weakens the immune system, which worsens the effects of HIV.

Reports indicate that exclusive breast feeding reduce mother to child transmission of HIV, (Coovadia *et al*., 2007; Charurat *et al*., 2009) however, once breast feeding ceases to be exclusive, new HIV infections begin to accrue among breast fed infants (Charurat *et al*., 2009). Exclusive breast feeding with abrupt weaning at the time when complementary foods are required in order to minimize any period of mixed breast feeding are being sort as alternative strategy. There may be acute health risks for both mother (e.g. mastitis) and child (e.g. diarrhea) (Thea *et al*., 2004). However, reports indicate exclusive breast feeding up to 6 months not being the norm in many African countries.

The study by Amadi *et al*. (2001) also showed that in children with persistent diarrhea and malnutrition, Salmonellosis and Cryptosporidiosis were the dominant intestinal infections. Therefore weaning with local (traditional) fermented safe foods with Probiotic potential would possibly minimize the morbidity and mortality due to diarrheal diseases. In addition, information on survival of these *Salmonella* and *Cryptosporidium* in fermented foods like chibwantu and munkoyo beverage would be very important in promoting use of these foods particularly for people from the low income areas of Zambia, as complementary foods made from locally available foods.

Table 1 shows some fermented cereal foods and beverages prepared in different parts of Africa.

2.4.3 **Probiotic, Prebiotic and Synbiotic Potential**

Another important dimension of indigenous fermented foods is their contribution to health (therapeutic values). This has become a topical subject in recent years, particularly in the fermentation industry. The healing properties of indigenous fermented foods are well documented in Asia and Eastern Europe. The belief in the medicinal properties of indigenous foods is well known in South Korea, China and other Asian communities. However, the situation is different in Africa where the production of indigenous foods is not so well documented, although the existence of such products is known (CI-ROAF, 2002). In Africa fermented foods represent mainly improved flavor and taste, nutrition and digestibility, and prolonged shelf life of foods, especially in areas without refrigeration. There are some studies though that has been conducted on African fermented food products to identify health promoting properties (Kingamkono *et al*., 1999; Lei and Jackobsen, 2004).

Presently the use of foods that promote a state of well being, better health and reduction of the risk of disease have become popular as the consumer is becoming more and more health conscious, as well as the increase in health care which is becoming unaffordable for many (Mussatto and Mancilha, 2007; Kalui *et al*., 2010).

2.4.3.1 Probiotics

There is some evidence that fermented food products involving lactobacillus species and some other lactic acid bacteria have Probiotic potential. Research works have been carried out on spontaneously fermented cereal based foods with an aim of identifying predominant microorganisms involved in the fermentation process (Table 1). Spontaneously fermented cereal based food in Africa have a diverse number of bacteria with *Lactobacillus* species among the predominant ones and there is a possibility that some of these microorganisms could have probiotic attributes (Anukam and Reid, 2009). Most of these microorganisms are generally regarded as safe (GRAS) (Kalui *et al*., 2010). The probiotic potential of African spontaneously fermented cereal based foods has been extensively reviewed by Anukam and Reid, (2009) and Kalui *et al*. (2010).

The term probiotic is translated from the Greek language meaning ‘for life’. The term probiotic was earlier used by Lilly, and Stillwell, (1965) to describe ‘substances produced by one microorganism which stimulated another’ and Parker, (1974) defined probiotics as ‘organisms and substances which contribute to intestinal microbial balance’. However the term was revised by Fuller, (1989) to a popularly used definition ‘live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’. A probiotic may be defined as a product containing mono or mixed cultures of live microorganisms which when ingested exert beneficial (stabilizing or normalizing) influence on the GI tract (bacteria that work to maintain the host's health). This effect may be the direct or indirect result of the consumption of such a product and may be manifested by;

- Colonization of the intestinal mucosa
- Improved protection against harmful bacterial pathogens such as diarrheal pathogens
- Increased immuno- resistance against infectious diseases
- Reduced lactose intolerance
- Improved digestion and Elimination

(Motarjemi *et al*., 1996; Kigomkono *et al*., 1999; Blandino *et al*., 2003; Farnworth, 2003; Lei, 2006; Kalui *et al*., 2010). People with flourishing intestinal colonies are better equipped to fight the growth of disease causing bacteria (Kalui *et al*., 2010).

Examples of probiotics that have found application in probiotic products include some strains of *Lactobacillus* genera (*L. casei, L. plantarum, L. rhamnosus, L. acidophilus, L. reuteri, L. gasseri, L. lactis and L. amylovorus*); *Bifidobacterium* genera (*B. adolescentis, B. animalis, B. bifidum, B. breve, B. infantis, B. lactis, B. longum*); *Enterococcus* (*E. faecalis and E. faecium*) (Saarela *et al*., 2000; Desai, 2008; Kalui *et al*., 2010). Probiotics have been widely used in therapeutic applications including alleviation of constipation, urogenital diseases (candida vaginitis), protection against traveler's diarrhea, prevention of infantile diarrhea, control of inflammatory bowel diseases and irritable bowel syndrome, although some of these benefits are yet to be thoroughly proven (Douglas and Sanders, 2008; Ranadheera *et al*., 2010). Therefore, use of indigenous African fermented foods in treatment and prevention of diseases particularly diarrheal that are a challenge is a possibility (Lei *et al*., 2006).

2.4.3.2 Prebiotic

Associated with probiotics are prebiotics. Prebiotics are non-digestible food ingredients that have a beneficial effect on the host by selectively stimulating growth of health promoting bacteria (Kalui *et al*., 2010). For a food to qualify as a prebiotic, it: has to be non digestible by human enzymes; has to undergo selective fermentation by potentially beneficial bacteria in the colon; should allow specific changes both in composition and/or activity in the gastrointestinal microbiota that lead to a beneficial health of host (Kalui *et al.*, 2010). Examples of prebiotics include non-digestible oligosaccharides.

Non-digestible oligosaccharides (NDO) stimulate growth of beneficial bacteria in the colon. NDOs are also associated with a lower risk of infections and diarrhea and an improvement of the immune response. Moreover due to the decrease of the intestinal pH cause by their fermentation biota NDOs provoke a reduction of the pathogenic biota, an increase of bifidobacteria population and an increase of the availability of minerals such as vitamins of the B-complex (B1, B2, B6 and B12), nicotinic and folic acids (Mussatto and Mancilha, 2007).

Non-digestible oligosaccharides (NDO) are defined as food components or ingredients which affect the physiological function(s) of the body in a targeted way so as to have positive effect(s) which may in due course justify health claims. NDOs are classified as prebiotics because they stimulate the growth and/or metabolic activities of bacteria species benefic for health (Mussatto and Mancilha, 2007). Cereal processing through fermentation can also produce a large range of oligosaccharides such as galacto-oligosaccharides and fructo-oligosaccharides with prebiotic potential (Mussatto and Mancilha, 2007; Farnworth, 2003).

2.4.3.3 Synbiotic

The term synbiotic is used to describe the combination of a probiotic and a prebiotic (Farnworth, 2003). This combination confers benefits further than the benefits of the probiotic and prebiotic on their own. Since most indigenous cereal based fermented foods contain lactic acid bacteria with probiotic potential as well as non digestible oligosaccharides, in addition to fibers and resistant starch, they may therefore be potential synbiotic products.

2.5 Microbiology of Fermented Cereal Foods of Africa

Many food products prepared from cereals (millet, sorghum, maize, and rice) are well known in African communities and are generally used as refreshing beverages, weaning foods, breakfast or light meal foods and some as main foods in the diet. However, the microbiology of many of these fermented foods is quite complex and not known. The basic fermentation process of cereals is mostly spontaneous (natural) and involves the enzymatic activities of mixed cultures of bacteria, yeast and moulds. The bacteria involved in fermentation of cereals are mainly lactic acid bacteria which include species of *Lactobacillus, Leuconostoc, Pediococcus* and *Weissella*. The most dominant yeast species is *Saccharomyces* and others include *Candida* and *Issatchenkia.* Moulds are mainly of the species *Aspergillus, Fusarium, Penicillium and Cladosporium* (Blandino *et al*., 2003; Kohajdova and Karovicova, 2007). The microorganisms may participate in parallel, while others act in a sequential manner with changing dominant biota during the course of the fermentation.

2.5.1 **Dominant microorganisms involved in cereal fermented foods**

2.5.1.1 Lactic Acid Bacteria

Lactic acid fermentations are probably the simplest and often the safest way of preserving food A large number of cereal based fermented foods in Africa are predominantly lactic acid fermented (Oyewole, 1997; Holzapfel, 1997; Mugula *et al*., 2003; Ali and Mustafa, 2009; Yousif *et al*., 2010) because lactic acid fermentation methods of processing and preservation are of low cost, have low energy requirement for both processing and preparing foods for consumption, thereby contributing to safety, nutritional value, increased shelf life and acceptability of a wide range of these cereal based foods (Oyewole, 1997). Many of these indigenous cereal-based fermented foods have been investigated for their dominating microorganisms as shown in Table 2.1 and the major genera are *Lactobacillus, Pediococcus Leuconostoc* and to a lesser extent *Lactococcus* and *Weissella* (Holzapfel, 1997).

Lactic acid bacteria (LAB) is the term used to describe a broad group of Gram positive bacteria united by a constellation of morphological, metabolic and physiological characteristics. LAB are generally catalase negative, non sporing rods and cocci, usually non motile, that utilizes carbohydrates (hexoses) fermentatively and form lactic acid as their major end product (Kandler, 1983; Axelsson, 1993; Oyewole, 1997; Onilude *et al*., 2005). Recent taxonomic revisions suggest that lactic acid bacteria comprise of the following genera: *Aerococcus, Carnobacteruim, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus* and *Vagococcus.* The classification of lactic acid bacteria into different genera is based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced and acid or alkaline tolerance. For some of the newly described genera of lactic acid bacteria, additional characteristics such as fatty acid composition and motility are used as the basis for classification (Axelsson, 1993).

The pathways by which the carbohydrates (hexoses) are metabolized divide the lactic acid bacteria into two groups; homo-fermentative or hetero-fermentative as shown in figure 2.1. Homo-fermenters such as *Pediococcus* produce lactic acid as major end product of glucose fermentation through glycolysis (Embden-Meyerhof pathway) and hetero-fermenters such as *Leuconostoc* produce lactic acid, acetic acid, carbon dioxide and ethanol from glucose fermentation through the 6-phosphogluconate/phosphoketolase pathway (Brock, 1979; Axelsson, 1993; Caplice *et al*., 1999). Various growth conditions may significantly alter the end product formation by some lactic acid bacteria.

The lactic acid bacteria strains generally lack amylolytic, proteolytic and lypolytic enzymes thus have limited biosynthetic ability and therefore require simple sugars, preformed amino acids, purines and pyrimidine bases and B vitamins for growth. LAB grow anaerobically, however, most are not sensitive to oxygen and therefore can grow in its presence as well as absence (aero tolerant anaerobes) (Brock, 1979). Although lactic acid is the major end product of fermentation, it can be further catabolized to acetic acid and carbon dioxide via pyruvate under aerobic conditions by some lactic acid bacteria. Acetic acid and carbon dioxide may thus have an effect on the flavor and texture of the fermented product.

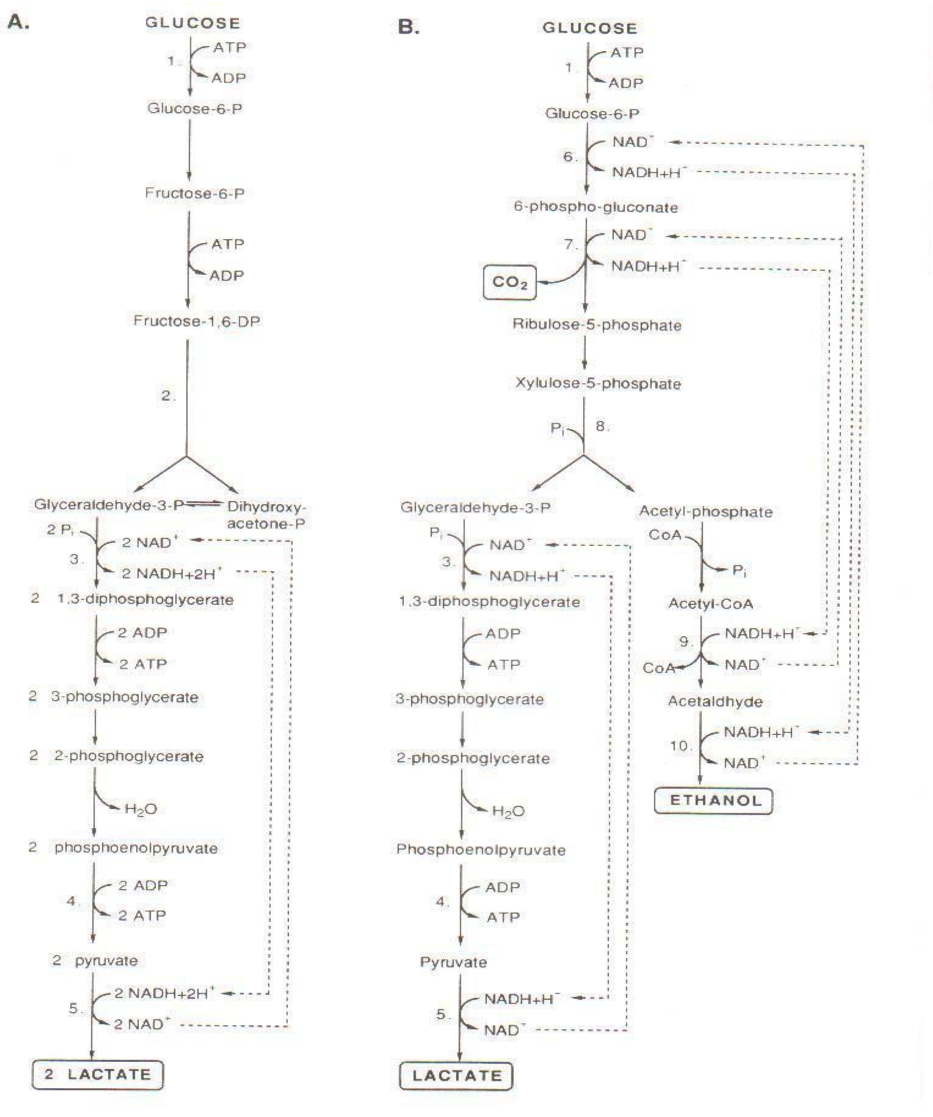

Figure 2-1: Schematic representation of the major pathways of carbohydrate (hexose) fermentation in lactic acid bacteria (A) Homolactic Fermentation and (B) Heterolactic Fermentation (Source: Axelsson, 1993).

***Lactobacillus* species**

The genus *Lactobacillus* is by far the largest of the genera included in LAB. It is very heterogeneous, encompassing species with a large variety of phenotypic, biochemical and physiological properties. The heterogeneity is reflected by the range of mole guanine +cytosine (% G+C) of the DNA of species included in the genus which is 32-53%, twice the span usually accepted for a single genus (Axelsson, 1993). *Lactobacillus* species are the most acid tolerant of the lactic acid bacteria, can lower pH to about 3.5 before inhibiting their own growth (Steinkraus, 1983). *Lactobacillus* species are also associated with the oral cavity and the gastrointestinal tract of humans and animals.

Lactobacillus species play a very significant role in most lactic acid fermented foods as can be seen in Table 2.1. *Lactobacillus* species are the most predominant microorganisms in cereal based fermented foods particularly the species *L. plantarum* which occurs frequently especially when the food is based on plant material (Farnworth, 2003) such as in the following African indigenous cereal based fermented foods: Togwa (Mugula, 2003b), ogi (Banigo, 1983), Sorghum based weaning foods (Kunene, 1999), uji (Mbugua, 1983) and bushera (Muyanja, 2003) and the other is *L. fermentum* isolated as the fermenting and dominant microbiota of indigenous cereal based foods such as koko (Lei and Jakobsen, 2004), kisra (Ali and Mustafa, 2009) and mawé (Agati *et al*., 1998).

Some species of lactobacillus are heterofermenters such as *Lactobacillus fermentum* and *Lactobacillus plantarum* while others are homofermenters such as *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus* and *Lactobacillus Lactobacillus*.

***Leuconostoc* species**

Leuconostoc are obligate hetero-fermenters and can lower pH to about 4.0-4.5 before inhibiting their own growth and share many characteristics with the genus *Lactobacillus* (Collin *et al*., 1993).

***Pediococcus* species**

Pediococci are homo-fermenters widely distributed in fermenting plant material (Kandler, 1984) and have been identified in many African indigenous fermented foods such as mawe, uji, koko, togwa and busaa, however, have rarely been isolated as the dominating microorganism in spontaneous cereal based food fermentations (see Table 2.1)

***Lactococcus* species**

Lactococci are intimately associated with dairy products, but have been identified in cereal based fermented beverages such as mahewu (Holzapfel, 1989) and bushera (Muyanja, 2003).

***Weissella* species**

Weissella is a relatively new genus within the lactic acid bacteria, heterofermentative and includes both cocci and rods. The G+C content of DNA is about 37-47 mole % (Collin *et al*., 1993). Growth of *Weissella* occurs at 15^{o} C; growth does not occur at 45^{o} C except for the strains of *Weissella confusa* (Collin *et al*., 1993). The species *Weissella confusa* has been identified in African indigenous fermented cereal based foods such as koko (Lei, Jakobsen, 2004), togwa (Mugula, 2003b), and bushera (Muyanja, 2003) see table 2.1.

2.5.1.2 Yeasts

Many studies have reported the involvement of yeasts in the fermentation of indigenous cereal based foods and have shown that yeasts occur in majority of these foods (see table 2.1). *Saccharomyces cerevisiae* is one of the predominant particularly in spontaneous alcoholic fermentations such as cereal opaque beers (Holzapfel, 1997; Jespersen, 2003). In spontaneously fermented foods yeasts coexist with other microorganisms and have been found to stimulate lactic acid bacteria by providing essential metabolite such as pyruvate, amino acids and vitamins (Jespersen, 2003).

Yeasts as a group are difficult to define. Yeasts are fungi which at some stage in their life cycle exist primarily as single cells which reproduce by fission or budding (Beneke and Stevenson, 1987). The possible functions of yeast in cereal fermented foods and beverages are; production of aroma compounds through the conversion of carbohydrates into alcohols, esters, organic acids and carbonyls compounds, inhibition of mycotoxins producing moulds (nutrient completion), degradation of mycotoxins, production of tissue degrading enzymes (cellulases, pectinases) which make substrates available for other microorganisms and Probiotic properties (Jespersen, 2003; Kohajdova and Karovicova, 2007; Osmorio-Cadavid *et al*., 2008)

2.5.1.3 Filamentous fungi (Moulds)

Moulds play a very minor role in fermented foods in Africa, however, moulds have been found during fermentation of cereal based foods such as kenkey (Jespersen, 2004) and ogi (Banigo, 1983). The major species are *Aspergillus, Penicillium* and *Fusarium.*

2.5.2 Microbial Interactions in Fermented cereal foods

Many cereal-based food fermentations rely on spontaneous fermentations by the indigenous microbiota, which are mixed cultures consisting of multiple strains or species, present in the food substrate, on utensils used and environment. Microbial communities (biota) are the inevitable consequence of the flow of energy, carbon and other elements between different organisms at the same time or different trophic levels and thus variations and interactions among these indigenous populations influence the function, stability and flexibility of the fermenting community. This has a direct effect on the product quality (such as flavor, aroma and texture) and the reproducibility of fermentations.

Microbial interactions in mixed cultures occur via multiple mechanisms and can be classified on the basis of effect, as direct or indirect interactions. Indirect interactions refer to competitions, commensalism, mutualism, ammensalism or neutralism and direct interactions refer to predation and parasitism (Bazin, 1981; James *et al*., 1995; Sieuwerts *et al*., 2008). Competition is an indirect interaction which has a negative effect on both populations. If a substance (growth limiting nutrient) required by both species is removed from the environment, the situation is regarded as resource-type competition while secretion of substances inhibitory to each other is regarded as interference-type competition. General microbial interference is an effective non-specific control mechanism common to all populations and environments in foods and was first used to describe the suppression of virulent *staphylococci* by avirulent strains (Bazin, 1981; Caplice *et al*., 1999). In addition, in the microbial communities some microorganisms compete with each other for space. When one population benefits from the presence or activity of the other while the benefactor is unaffected, the phenomenon is termed commensalism. An interaction where both populations benefits is mutualism, which includes obligatory interactions (symbiosis) or interactions that result enhanced production (or consumption) of a certain product (synergism). Ammensalism refers to an interaction where one population has an indirect negative impact on another, such as the production of a bacteriocin by one species that inhibit the growth of another.

Direct negative interactions include predation, where one organism is consumed by another and parasitism where one organism is invaded intracellularly by another. In natural communities interactions can be complex and include mixed interactions where more than one type of interaction occur between two species, as well as interactions involving more than one species. This review will focus on the interactions that occur in fermented cereal based foods such as interactions between lactic acid bacteria and yeast.

2.5.2.1 Lactic acid bacteria and yeasts

Associations between yeast and bacteria are very common in a wide variety of traditional foods and beverage fermentations. Fermentation involving lactic acid bacteria and yeast, and which are carried out in closed vessels will rapidly become anaerobic, acidic, saturated with carbon dioxide and alcoholic. This combination of conditions will certainly be inhibitory to many spoilage microorganisms including filamentous fungi and bacteria associated with various forms of food poisoning (Wood and Hodge, 1985).

Literature indicates that proliferation of yeasts in fermented food is favored by the acidic environment created by lactic acid bacteria. While lactic acid bacteria survival during fermentation process is in association with yeasts (Bazin, 1981; Oyewole, 1997). The death and autolysis of yeast cells release provide growth factors such as vitamins (riboflavin) and soluble nitrogen compounds that stimulate the growth of the bacteria (particularly flavor enhancing bacteria) (Fleet, 2007). The association is mutualistic in which the growth factors produced by yeast are used by the lactic acid bacteria thereby increasing the density of bacteria which in turn produce sufficient lactic acid to reduce the pH of the medium enough to increase the specific growth rate of the yeast. This association of yeast and bacteria also contribute to metabolite which impart taste and flavor to foods whereby individual species (yeast or lactic acid bacteria) would not.

Example, in the study by Zulu *et al*. (1997), preparation of Munkoyo beverage using starter cultures showed that a faint ‘munkoyo’ aroma developed in the beverage when the Lactic acid bacteria *Lactococcus confusus* was use alone, and no ‘Munkoyo’ aroma was perceived when yeast *Saccharomyces cerevisiae* was used alone. Instead, a fruity- alcoholic aroma was apparent. However, the mixed inocula of lactic acid bacteria and yeast gave a typical ‘Munkoyo’ aroma.

2.5.3 **Starter Cultures**

Starter cultures are not usually used in fermentation of many indigenous African foods and there use would be an appropriate approach for the control and optimization of the fermentation processes in order to lessen the problems of variation in organoleptic quality and microbial stability observed in these African indigenous fermented foods. No lactic acid bacteria starter cultures are commercially available yet for small scale processing of traditional Africa foods (Holzapfel, 1997). A large number of reports show that lactic acid bacteria and yeasts are mostly predominant in many African cereal fermented foods (Ali and Mustafa, 2009; Yousif *et al*., 2010). There is need for thorough understanding of the fermentation process particularly the role of these lactic acid bacteria and yeasts in order to identify those functionally most effective for the preparation of the fermented foods (Mugula *et al*., 2003). Fast reduction in pH of fermenting foods is one of the desirable functional properties of lactic acid bacteria, in terms of food safety as it serves to inhibit the growth of especially the gram negative acid sensitive foodborne pathogenic and spoilage bacteria. A study by Yousif *et al*., (2010) showed that use of starter cultures such as *Pediococcus* reduced pH fast due to their high acid producing ability.

2.6 African Non-alcoholic Fermented Cereal Foods and beverages

Gruels and non-alcoholic beverages are fermented cereal products which on processing yield acidic, non-alcoholic and of high water content fluidy gruels (porridge). The best documented of these gruels is *ogi* (Odunfa, and Oyewole, 1998).

2.6.1 Examples of Some African Non-alcoholic Fermented Cereal Beverages

2.6.1.1 Togwa

Togwa is a non-alcoholic beverage consumed in East Africa (Oi and Kitabatake, 2003). In Tanzanian homes, it is widely produced for use directly as weaning food and diluted for use as refreshment. The cereals used in the preparation of the beverage are maize and finger millet malt (Oi and Kitabatake, 2003). Cereal flour is mixed with water and cooked. The gruel is then cooled down to about 35°C, and about 10% of old togwa (back-slopping) is mixed with the gruel, together with 5% malt flour (Farnworth, 2003).

Malt flour is prepared from sorghum or millet. The malt flour is prepared by soaking the cereal grains of choice for 12 hours; then drain- dried and allow the grains to germinate for 3-6 days. After sun- drying and grinding of the germinated grains, the malt flour is ready for use (Farnworth, 2003). After mixing of gruel with malt and old togwa (as a starter culture), the mixture is left to ferment spontaneously. The fermentation can be performed in earthenware pots, aluminum pots or plastic containers. Since fermentation is spontaneous and uncontrolled, resulting togwa products are of variable quality and stability (Mugula *et al*., 2003).

The microorganisms predominant in the fermented product are lactic acid bacteria particularly the genus *Lactobacillus* and yeasts particularly *Issatchenkia orientalis*. However studies have shown that togwa made using *Lactobacillus plantarum* as a single strain starter culture give togwa similar in quality to the spontaneously fermented (Farnworth, 2003).

In spite of the fact that togwa is prepared under poor hygienic conditions, there have been no documented outbreaks of foodborne diseases connected to consumption of togwa. Studies have established that enteropathogens (*Bacillus cereus, Campylobacter jejuni*, enterotoxigenic *Escherichia coli, Salmonella typhimurium* and *Shigella flexineri*) inoculated before fermentation disappear after 24 h in the fermenting gruel, provided that the pH during this 24h period has fallen to less than 4.0, which is the normal pH fall in togwa.

2.6.1.2 Mahewu

Mahewu is a non-alcoholic sour beverage very popular among the Bantu people of southern Africa. Mahewu is also known by the names amahewu, maheu, magou and mageu. The spontaneously fermented mahewu is prepared from either thin or thick maize porridge (Gadaga *et al*., 1999). Sorghum, millet malt or wheat flour is added to the porridge, mixed and left to ferment spontaneously at ambient temperature to pH of about 3.5. It is an adult food type which is commonly used to wean children and is introduced to infants between 4-18 months (Gadaga *et al*., 1999). Little information has been recorded on the general properties, microbiological processes and safety of traditionally prepared Zimbabwean mahewu (Gadaga *et al*., 1999). However, the predominant microorganisms in the South African spontaneously fermented mahewu belong to *Lactococcus lactis* subspp *lactis*. Industrial preparation of Mahewu is carried out in Zimbabwe and South Africa (Holzapfel, 1989).

2.6.1.3 Uji

Uji is widely consumed in Kenya, Uganda and Tanzania. It is made from maize, millet, sorghum or cassava. The cereal is finely ground and slurried with water. The slurry is spontaneously fermented for 2 to 5 days at room temperature. The uji is then diluted and brought to a boil or the slurry is diluted and cooked fresh without fermentation. Preparation methods vary from country to country and tribe to tribe. In Kenya fermented uji is mainly prepared and consumed by the rural housewives (Mbugua, 1977).

Uji is an important component of breakfast and lunch. It is also used as weaning food, thirst-quenching drink, or side dish, and it is believed by some to enhance lactation. In some parts of Kenya the fermented slurry is sun dried and the granulated powder is used thereafter for subsequent uji preparation by hydrating and then cooking (Mbugua, 1977). In a typical spontaneous uji fermentation based on maize and sorghum flour, the coliforms dominate the fermentation for the first 16 to 24 hours, and then the *lactobacilli* become dominant, producing sufficient acid to decrease the number of coliforms. If millet is substituted for sorghum, lactobacilli dominate the fermentation from the start. The dominant lactic acid bacteria during uji fermentation are of the species *Lactobacillus plantarum.* The others include *Lactobacillus cellobiosus, L. buchneri, Pediococcus acidolactici* and *L. fermentum* (Mbugua, 1981).

2.6.1.4 Bushera

Bushera is a traditional sorghum or millet based non- alcoholic beverage of Uganda (Muyanja *et al*., 2003). The flour from germinated sorghum and millet grains is mixed with boiling water and left to cool to ambient temperature. Germinated millet or sorghum flour is then added and the mixture is left to ferment spontaneously at ambient temperature for 1-6 days (Muyanja *et al*., 2003). Low income women at village level produce bushera for home consumption and sale (Muyanja *et al*., 2003).

The product is consumed by both the young and adults. However, for children only a day fermented bushera (Muyanja *et al*., 2003).

In the study by Muyanja *et al*. (2003) initial stages of fermentation of bushera was dominated by lactic acid bacteria and coliforms and in the last stage by lactic acid bacteria and yeasts. Coliforms decreased to undetectable levels during fermentation due to high acidity (low pH). The study indicated that several different species of lactic acid bacteria can be implicated in the fermentation of bushera and they included *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus delbrueckii, Lactobacillus paracasei, Weissella confusa, Leuconostoc citreum, Leuconostoc mesenteroides, Lactococcus raffinolactose, Lactococcus lactis, Enterococcus mundtii* and *Enterococcus faecium.* The lactic acid bacteria identified in bushera have been reported in other African cereal fermented foods (refer to table 2.1).

2.6.1.5 Ogi

Ogi is a fermented cereal gruel or porridge processed from Maize, although sorghum or millet is also employed as the substrate for fermentation (Blandino *et al*., 2003; Ohenhen, and Ikenebomeh, 2007; Osungbaro, 2009). Choice of grain depends on preference and ethnicity and the color of ogi will depend on the cereal grain used; cream for maize, reddish brown for sorghum and dirty grey for millet (Ohenhen and Ikenebomeh, 2007). Ogi is one of the most important staples used as weaning food in West Africa, especially important in Nigeria (Ohenhen and Ikenebomeh, 2007), although it is also consumed by adults (FAO, 1999). Ogi is very smooth in texture and has a sour taste similar to that of yoghurt (Mbata *et al*., 2009). The traditional preparation of ogi involves washing and soaking of maize kernel in water for 1-3 days followed by wet milling and sieving to remove bran, hulls and germ. The sievate is allowed to settle for 1-3 days a process referred to as souring during which time fermentation also proceeds and the solid starchy matter, ogi, sediments (Odunfa and Oyewole, 1998; Osungbaro, 2009). For consumption, the paste is added to some quantity of water and boiled with continuous stirring to make gruel.

Lactic acid bacteria *Lactobacillus plantarum, Lactobacillus plantarum, L. confuses, L. murinus, L. agilis, Leuconostoc mesenteroides*, the aerobic bacteria *Corynebacterium* and *Aerobacter*, the yeasts *Candida mycoderma, Saccharomyces cerevisiae* and *Rhodotorula* and the moulds *Cephalosporium, Fusarium, Aspergillus and Penicillium* are the major organisms responsible for the fermentation and nutritional improvement of ogi (FAO, 1999; Lund *et al*., 2000; Ohenhen and Ikenebomeh, 2007). Moulds are eliminated during the wash and soaking (steeping) period. The major fermentation include lactic, acetic and butyric acids all contributing to the flavor of ogi. Fermentation studies have determined that *Lactobacillus plantarum* was the predominant microorganism in fermentation responsible for lactic acid production. *Corynebacterium* hydrolyzes the maize starch to organic acids while *Saccharomyces cerevisiae* and *Candida mycoderma* contribute to flavor development (FAO, 1999; Osungbaro, 2009). Substantial nutrient losses occur during various steps of cereal processing for ogi preparation and these include fibre, protein, calcium, iron, phosphorus, and vitamins such as thiamin, riboflavin, niacin, folic acid and panthothenic acids (Osungbaro, 2009). Extent of nutrient losses depends on the exact method used in the ogi preparation (Osungbaro, 2009).

2.7 Spoilage and Safety of fermented cereal foods

2.7.1 Spoilage of Cereal based fermented foods

Preservation of food has, since the beginning of mankind been necessary for our survival. The preservation techniques used in the early days relied- without any understanding of the microbiology- on inactivation of the microorganisms through drying, salting, heating and fermentation. All these methods are still being used today (Gram *et al*., 2002).

Spoilage is defined as the deprivation of good effective qualities. Spoiled food undergoes changes that make the food unacceptable for human consumption resulting in losses. The changes, though usually of microbial origin also include; physical damage, drying out, discoloration and staling and rancidity. Microorganisms grow rapidly and foods are such excellent sources of nutrients. Fermented foods are substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins, and lipids to non-toxic products with flavors, aroma and textures pleasant and attractive to the human consumer. If the products of enzyme activities, from microbial invasion, have unpleasant odors, or undesirable, unattractive flavors or the products are toxic or disease producing, the fermented foods are described as spoiled and not safe for consumption (Seinkraus, 2002).

A number of spoilage bacteria, yeasts and food borne pathogenic bacteria have been encountered during solid state fermentation of foods (Katongole, 2008). However, lactic acid fermented foods generally have a very good safety record thus less likely to be vehicles for foodborne infections and intoxication than fresh foods (Roy *et al*., 2007) even in the developing world where the foods are prepared at small scale mainly in homes by people without adequate knowledge of microbiology, often in unhygienic conditions (Seinkraus, 1997).

2.7.2 The role of lactic acid bacteria in food safety

The principle behind the safety of lactic acid fermented foods is the ability of the fermenting microorganisms particularly lactic acid bacteria to produce organic acids which decrease the pH of the food product to values lower than 5, thereby inhibiting growth of pathogenic and spoilage acid intolerant bacteria. The fermentation process can lower the pH to 4 or below (Oyewole, 1997; Mensah, 1997). There is also production of bacteriocins, hydrogen peroxide, carbon dioxide, ethanol and diacetyl and also competition for nutrients and space. Spoilage and pathogenic bacteria are eventually unable to compete with the fermenting microorganisms (antagonized). The antimicrobial effect is believed to result from the action of the acids in the bacterial cytoplasmic membrane, which interferes with the maintenance of the membrane potential and inhibits the active transport (Gram *et al*., 2002; Blandino *et al*., 2003).

Growth inhibition depends on the microorganisms involved, the temperature, amounts of un-dissociated acids and the buffering capacities of the food. Fermented foods are generally weakly buffered and will therefore easily achieve low pH (Lei, 2006). However several bacteria have remarkable ability to survive different environmental stress conditions making it hard to exclude them from food products (Han *et al*., 2001). In addition unhygienic handling, external contamination, contaminated water, inferior quality of raw materials and high temperatures and humidity as well as lack of refrigeration, increase the survival chances of many bacteria in these fermented foods (Roy *et al*., 2007) which is usually the case in most countries where fermentation is practiced (Mensah, 1997).

In foods that are cooked and then fermented using another source of fermenting material such as malt, or in the case of munkoyo beverage the ‘munkoyo’ root, the level of food contamination mainly depend on the quality and safety of the raw fermenting materials, water used and the hygienic and sanitary conditions of the food preparation sites as well as the degree to which the pH of the food falls. The first step in the preparation of Munkoyo beverage is the cooking of the cereal maize meal to a thick or thin porridge. Then while the porridge is still warm, addition of the ‘munkoyo’ root and or extract and allow it to spontaneously ferment at ambient temperatures (25 – 30 ^{o} C) in 24 - 48 hrs before consumption. Fermentation is primarily by lactic acid bacteria and yeasts (Simwamba and Elahi, 1986; Zulu *et al*., 1997). The pH decreases to 3.5 and there is production of ethanol (Zulu *et al*., 1997) presenting conditions in the beverage inhibiting microorganisms sensitive to low pH. However there is not information on the microorganisms (contaminants) associated with the ‘Munkoyo root or whether the root also contribute some anti-microbial activities that improve the quality and safety of the beverage.

2.7.3 Survival of pathogenic bacteria in cereal fermented foods

There are a number of studies that have been carried out on the survival abilities of pathogenic bacteria in cereal based fermented foods (Simango and Rukure, 1992; Svanberg *et al*., 1992; Kingamkono *et al*., 1994; Kunene *et al*., 1999; Tetteh *et al*., 2004). The study by Simango and Rukure, (1992) showed that sour porridge and mahewu had bacteriostatic effects on enteric pathogens *Shigella* and *E. coli*. Other studies have shown that *E. coli* is tolerant to acidic conditions of fermented foods. However the infectious dose of some pathogens can be lower than 10 cells. This would still cause illness particularly for the immune compromised and the infants were the food is used for weaning. There is also little information on the effects of fermentation on parasites such as *Cryptosporidium*, *G. lamblia* and foodborne trematodes. The cysts of these organisms show resistance to adverse conditions, but are believed to be destroyed by adequate cooking (Motarjemi, 2002).

2.7.4 **The role of yeast in food spoilage and safety**

Yeast rarely occurs in foods and beverages as single culture therefore, the impact of yeast on the production, quality and safety of foods and beverages is intimately linked to their ecological and biological activities. Generally most habitats are comprised of a mixture of yeast, bacteria and filamentous fungi (moulds) and product quality is determined by the interactive growth and metabolic activities of the total microbiota (Fleet, 2007; Sieuwerts *et al*., 2008). However, yeasts can generally resist extreme conditions better than bacteria and are found in low pH foods, causing spoilage.

Food spoilage by yeasts consists in the visible or detectable alteration of physical and sensory properties of food. The most known alterations occur in acidic drinks and are characterized by; abundant gas production, sediment or pellicle formation, off-flavors and off tastes. However, in fermented foods and beverages yeast spoilage is not so easily defined because the produced metabolites contribute to the flavor and aroma of these products (Loureiro and Querol, 1999). There is therefore a slight line between what is perceived as spoiled or beneficial activity (Fleet, 1992).

As part of daily life, humans consume large populations of yeast without adverse impact on their health. Unlike bacteria and viruses yeasts are rarely associated with outbreaks of food borne gastroenteritis, intoxications or other infections. Nevertheless, some literature connects the dietary intake of yeasts with a range of gastrointestinal, respiratory, skin and even psychiatric disorders. Overgrowth of yeast in the gastrointestinal tract might contribute to the development of these disorders (Fleet, 2007).

2.7.5 Moulds and mycotoxins in cereal fermented foods

Cereal grains are mainly spoiled by fungi (moulds) of the genus *Penicillium*, *Aspergillus* and *Fusarium.* Tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods lead to fungi proliferation and may produce mycotoxins. (Wagacha and Muthomi, 2008). Poor harvesting practices and improper storage can also contribute to fungal growth and increase the risk of mycotoxins production. These climatic conditions and post harvest practices are characteristic in most parts of Africa, hence the threat of mycotoxins contamination of cereal foods.

Maximum tolerable levels, control mechanisms in developing countries are insufficient and can obviously not be applied at the house hold and small scale level to any significant extent. There is insufficient information on degradation of mycotoxins by typical microorganisms involved in traditional small scale fermentation (Motarjemi *et al*., 1996). Mycotoxins therefore represent one of the important classes of naturally occurring toxicants in food, posing considerable health risk to consumers (Shetty and Jespersen. 2006). The health risk is made even more palpable by the fact that, staple diets in many African households particularly the rural are based on cereal crops such as maize, which are highly susceptible to mycotoxins contamination (Wagacha and Muthomi, 2008). Aflatoxins and fumonisins are particularly a major risk factor in stored cereals and are of the greatest significance in Africa and other tropical developing countries.

2.7.5.1 Aflatoxins

Aflatoxins of which B1 (AFB1) is the most important are produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus* and are known to be potent carcinogens, mutagens, teratogens and suppress the immune system (Davis and

Diener, 1987; Kpodo *et al*., 1996; Mayer *et al*., 2003; Onilude *et al*., 2005; Science in Africa, 2006 and 2010). Positive correlation has been established between the consumption of aflatoxin contaminated foods and the increased incidence of liver cancer, therefore considered a silent killer. People exposed to very high aflatoxin concentrations experience liver failure and rapid death. From 2004 to 2006, nearly 200 unsuspecting people in Kenya died in this manner after eating highly contaminated maize (Wagacha, and Muthomi, 2008; Science in Africa, 2006 and 2010). Therefore large doses (> 6000mg) of aflatoxin may cause acute toxicity with lethal effects whereas small doses for prolonged period are carcinogenic.

A pilot survey conducted in Katete district of Zambia 1981/82, indicated that the prevalence of maize contamination was 18.6 % and mean total aflatoxin content was 17.25 ug/kg (Njapau, 1998). Aflatoxin is a colorless, tasteless chemical that is invisible and only laboratory tests can confirm its presence and contamination levels.

A study by Odhav and Naicker, (2002) indicated that raw ingredients (sorghum, sorghum malt grains, and maize grits) used to commercially produce traditional beers (Utshwala and Utshwala special) and home-brewed beers (Umquombotha, Isiqatha, and Imfulamfula) were contaminated by bacteria and fungi (both yeasts and moulds). The contaminating moulds were found to be *A. flavus*, and other *Aspergillus* species, *Penicillium* species, and *Rhizophus* species. Two of the six commercial beer samples contained aflatoxins (200 and 400 microgram per litre).

In addition, the study by Kpodo *et al*. (1996) indicated that cooking during preparation of Kenkey fermented maize dough did not appear to destroy aflatoxin present and fermentation of the dough did not appear to reduce the levels of aflatoxins.

2.7.5.2 Fumonisins

Fumonisins are produced by different species of the genus *Fusarium* (IARC, 2002; Soriano and Dragacci, 2004; Wagacha and Muthomi, 2008) and are widely distributed in maize in Africa (Bankole and Adebanjo, 2003). Consumption of fumonisins has been associated with elevated human oesophageal cancer incidence in various parts of Africa (Wagacha and Muthomi, 2008) and are classified as possible human carcinogens (IARC, 2002).

In West Africa, fumonisin levels of 65-1830 µg/kg with mean of 390 µg/kg have been detected in maize from Nigeria (Bankole and Adebanjo, 2003) and Doko, *et al*. (1995) reported average fumonisin levels of 3310 ng/g in maize from Benin with 82% incidence of toxin contamination.

The same report by Doko *et al*., (1995) showed fumonisin levels of 1710 ng/g in maize from Zambia with 100% incidence of toxin contamination. All samples of home-made Xhosa maize beer in South Africa were positive for fumonisin B1 with range of 38 to 1066 ng/ml and mean of 281ng/ml and total fumonisins (B1, B2, and B3) ranged from 43 to 1329 ng/ml with a mean of 369 ng/ml (shepherd *et al*., 2005). These levels are well above the provisional maximum tolerable daily intake of 2 µg/kg of body weight/day set by the FAO/WHO expert committee on Food Additives. However, a study by Chelule *et al*. (2010) showed that during *amahewu* (a traditional South African maize-based porridge) fermentation, mycotoxins (AFB_1, FB_1 and ZEA) decreased significantly.

In conclusion therefore, if mycotoxins contaminated raw materials are used for the preparation of fermented foods, chances are that the end product will still have the mycotoxins. However, some lactic acid bacteria *Lactobacillus plantarum*, and *Lactobacillus fermentum* have some antifungal effect on some aflatoxin producing fungi (Onilude *et al*., 2005) and the production of fermented foods usually involves other processing steps, such as cleaning, dehusking, soaking, milling and cooking. These processing steps, even if fermentation cannot be relied upon as a means of detoxifying raw materials contaminated with mycotoxins, can contribute to the reduction in contamination of the final product, thus contributing to the safety of cereal fermented foods (Westby *et al*., 1997).

2.8 Street vended cereal fermented foods and food safety

The country's economic situation, social difficulties and urbanization among other factors promote the growth of informal sector of economy including street food vending (Hanashiro *et al*., 2005). Street vended foods are defined as ready to eat foods and beverages prepared on the street or prepared at home and consumed on the street without further preparation (FAO, 2005).

Familiarity, taste, low-cost and convenience are some of the appealing factors that make street foods popular as food sources. Street foods can also play an important role in the nutritional supply, providing an opportunity for consumers (who include all age groups in Africa) to meet their daily nutritional requirement (Hanashiro *et al*., 2005; FAO, 2005; Omemu and Aderoju, 2008).

However, factors among others such as poor local infrastructure, characteristic of the foods sold and the lack of sanitary surveillance (Umoh and Odoba, 1999) increase concerns about the potential for food poisoning due to microbial contamination (Muleta and Ashenafi, 2001; Gadaga *et al*., 2008) and thus the general perception that street vended foods are unsafe. Study by Gadaga *et al*., (2008) showed serious hygienic problems due to lack of appropriate infrastructure. As mentioned in the above section, safety of food is affected by several factors which include quality of raw materials, handling and storage practices (including poor time-temperature conditions). Street vendors also store water for their hand and utensil washing under vulnerable conditions subject to further contamination, since in most cases there are problems of access to running water posing a safety concern (Tomlin, 2004; Hanashiro *et al*., 2005; FAO, 2005). Generally food safety knowledge is low among street food vendors (Omemu and Aderoju, 2008).

Examples of cereal based fermented foods sold as street foods in Africa include kenkey, banku and koko (Ghana), bushera (Uganda), ogi (West Africa), cereal based fermented porridges (Benin, Togo, Senegal, Burkina Faso, Ivory Coast)(FAO, 2005) and chibwantu and munkoyo beverages (Zambia). Studies have shown presence of food-borne pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella*, *shigella* and high microbial counts in different street foods, an indication of post cooking contamination, posing a serious health hazard to consumers (Umoh and Odoba, 1999; Muleta and Ashenafi, 2001; Hanashiro *et al*., 2005; Gadaga *et al*., 2008). However some street foods are intrinsically safer such as kenkey a fermented cereal food (Tomlin, 2004).

2.9 Antimicrobial potential of Plant Products

Flavonoids belong to a group of polyphenolic compounds widely distributed throughout the plant kingdom and seem to have an important role in the maintenance of human health (therapeutic potential) (Rauha *et al*., 2000; Puupponen-pimiä *et al*., 2001; Narayana *et al*., 2001; Cushnie and Lamb, 2005). It has been suggested that since flavonoids are widely distributed in edible plants and beverages (Puupponen-pimiä *et al*., 2001) and have been previously used in traditional medicine, flavonoids are therefore likely to have minimal toxicity (Cushnie and Lamb, 2005).

Flavonoids and other plant phenolics in addition to their antioxidant activity (free radical scavenging activity) which exhibit several beneficial effects such as anti-inflammatory and anti-allergic activity have multiple biological activities including antimicrobial (anti-bacterial, anti-fungal and anti-viral) and their role as antimicrobial is well recognized (Rice-Evans *et al*., 1996; Rauha *et al*., 2000; Narayana *et al*., 2001; Friedman, 2007). With the growing number of foodborne illness outbreaks caused by some pathogens (Tauxe, 1997; WHO, 2002) and increasing antibiotic resistance of some pathogens associated with foodborne illness (White *et al*., 2001; White *et al*., 2002) and over consumption of medicinal antibiotics (Seppälä *et al*., 1997; Giamarellou and Antoniadou, 2007), plant derived products such as phytochemicals may provide useful intervention to reduce pathogens in foods (Friedman, 2007). Different bacterial species exhibit different sensitivity to phenolics and flavonoids (Puupponen-pimiä *et al*., 2001).

The study by Zulu *et al.* (1994) showed that the Munkoyo roots (*Rhynchosia insignis* and *R. heterophylla*) used in the preparation of the beverages munkoyo and chibwantu have high concentrations of flavonoids and that fermentation had no effect on the flavonoids. There is therefore a potential that munkoyo roots besides giving color and flavor to the beverage, may also be providing the antimicrobial activity, in addition to the probiotic potential due to fermentation, thus improving the microbiological quality and safety the beverages. Regular consumption may also promote good health i.e. reduce the risk of developing conditions such coronary heart disease (Zulu *et al*., 1994), and cancer (Kähkönen *et al*., 1999).

2.10 Future of fermented foods

Fermented foods have been part of the human diet for centuries and the food industry is now directing new product development towards the area of functional foods and functional food ingredients due to consumers demand for healthier foods (Charalampopoulos *et al*., 2002). Food safety and the linkage between diet and health are issues of major concern to the modern consumer.

There is growing scientific evidence indicating that fermented foods are good for health or contain ingredients that are good for health. Foods that improve or change the intestinal microflora are of particular interest because of our increased knowledge of the role the intestinal microflora plays in health and disease resistance. Fermented foods will become even more important in our diet, as we identify different microorganisms that can be used in production of Probiotic foods. Many of the beneficial functions of probiotic bacteria have not yet been defined and a large majority of commercially available probiotic products today are milk based and other foods besides milk will have to be used as basal ingredients in probiotic foods (Farnworth, 2003).

2.11 *Conclusion*

Traditional cereal based fermented foods and beverages of other African countries have been investigated and documented. In Zambia though, limited information is available on indigenous cereal based fermented foods and beverages. To improve the production of these food products for increased consumption and commercialisation as acceptable traditional fermented foods and also if fermentation is to contribute in meeting the challenge of food spoilage and food related diseases which are a challenge, there is need to gather information on consumer beliefs on and perception of indigenous foods including cereal based fermented foods and beverages and acceptance of these food products, as well as information on preparation and utilisation of these foods, nutritional value and health benefits of consuming such foods.

With all the importance and benefits of fermented foods in Africa, spontaneous fermentation is among the predominant production method and the trend of producing cereal based fermented foods by spontaneous fermentation is likely to continue for many years to come. The microorganisms (biota) responsible are lactic acid bacteria, yeasts and fungi and the process of fermentation is very complex. Knowledge of these microorganisms is important to understand the process. The information on dominant microorganisms and their role in the fermentation process of these indigenous fermented foods and beverages is vital for their improvement to acceptable traditional fermented foods, particularly in the development of starter cultures. Starter cultures with predictable characteristics would help in the manufacturing of fermented foods with stable and consistent quality. The available information on the food quality and safety of these foods would thus be essential for commercialisation through formal and informal markets that can make the foods more accessible thereby contributing to food security.

The spoilage and poisoning of foods by microorganisms is a problem particularly in the developing world and there are different microorganisms responsible. In addition, there is a growing trend in street vended foods with high potential for food spoilage and poisoning due to microbial contamination. Fast reduction in pH of fermenting foods is one of the desirable functional properties of lactic acid bacteria, in terms of food quality and safety as it serves to inhibit the growth of especially the gram negative acid sensitive foodborne pathogenic and spoilage bacteria. Starter cultures such as *Pediococcus* reduce pH fast due to their high acid producing ability. Therefore, information on the microbiological safety of these foods is very important in decision making by relevant stakeholders.

Information on the antimicrobial potential of plant products such as munkoyo roots would also be very valuable in preservation of food and development of functional foods containing both probiotic and plant products. Consumers are increasingly demanding food that is more ‘natural’ not prepared with preservatives of chemical origin. Natural alternatives (safe plant compounds such as flavonoids) are required to minimize spoilage and improve safety with respect to food borne pathogenic microorganisms.

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Table 2-1: Fermented Cereal Foods and Beverages from different parts of Africa

Product	Raw Materials	Country	Predominant Microorganisms	Nature and Use of Product
Agidi	Maize	Nigeria, Benin	*Lactobacillus* spp, *Aerobacter*, *Corynebacterium*, Yeast, Moulds	Sourdough
Amqomboti	Maize, Sorghum	South Africa	*Lactobacillus delbueckii*	Beverage
Banku	Maize	Ghana	Lactic acid bacteria, moulds	Dough
Ben-Saalga	Millet	Burkina Faso	Lactic acid bacteria, *L. plantarum, L. fermentum, Pediococcus pentosaceus*	Beverage
Bogobe	Sorghum, Millet, Maize	Botswana	*Lactobacillus* spp, Yeasts	Dough/ Porridge
Bojalwa	Sorghum	Botswana	unknown	Alcoholic beverage
Borde	Maize, Barley, Wheat, Finger millet, Sorghum	Ethiopia	unknown	Alcoholic beverage
Bouza	wheat	Egypt	unknown	Alcoholic beverage
Burukutu	Sorghum	Nigeria, Benin, Ghana	*Leuconostoc mesenteroides, acetobacter, candida, Saccharomyces cerevisiae, Saccharomyces chavelieri*	Alcohol beverage
Busaa	Finger Millet,	Kenya, Nigeria, Ghana	*L. plantarum, L. helviticus, L. salivarius, L. casei, pediococcus damnosus, saccharomyces cerevisiae*	Sour Beer
Bushera	Sorghum, Millet	Uganda	*Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus delbrueckii, Lactobacillus paracasei, Weissella confuses, Leuconostoc citreum, Leuconostoc mesenteroides, Lactococcus raffinolactose, Lactococcus lactis, Enterococcus mundtii, Enterococcus faecium. Streptococcus thermophilus,* yeasts	Beverage
Chibuku	Maize	Zambia	yeasts	Alcoholic beverage
Chikokivana	Maize, Millet	Zimbabwe	*Saccharomyces cerevisiae*	Alcoholic beverage
Dalaki	Millet	Nigeria	Unknown	Thick porridge
Doro	Sorghum,	Zimbabwe	Yeasts and bacteria	Alcoholic

	Millet, Maize				beverage
Enjera	Sorghum	Ethiopia		*Lactobacillus plantarum, Leuconostoc mesenteroides, Candida guillienmandi*	Flat bread
Hussuwa	Sorghum	Sudan		*Lactobacillus saccharolyticum, Gluconobacter oxydans, Acetobacter xylinum, Saccharomyces cerevisiae*	Dough/ Beverage
Kachasu	Maize	Zimbabwe, Zambia		Yeasts	Alcoholic beverage
Kaffir Beer	Maize	South Africa		LAB, *Saccharomyces cerevisiae, Candida krusei*	Alcoholic beverage
Kamu	Millet	Nigeria		*L. plantarum, Pediococcus pentosaceus*	cake
Katata	Millet	Zambia		Unknown	Alcoholic beverage
Katubi	Millet	Zambia		Unknown	Alcoholic beverage
Kenkey	Maize, sorghum, millet	Ghana		*Obligate heterofermentative lactobacilli, Leuconostoc mesenteroides, Leuconostoc fermenti, Candida krusei, Saccharomyces cerevisiae, Pernicillium, Aspergillus, Fusarium*	Porridge dough
Kisra	Sorghum,	Ethiopia, Sudan		*Lactobacillus fermentum, L. reuteri, L. amylovorus, candida krusei*	Flat bread
Kito	Maize	Tanzania		unknown	Porridge
Koko	Maize, sorghum, millet	Ghana		*Lactobacillus fermentum, L. salivarius, Weissella confusa, Pediococcus spp, Leuconostoc mesenteroides, Leuconostoc fermenti Candida krusei, Saccharomyces cerevisiae*	Sour porridge
Kwunu-Zaki	Millet	Nigeria		LAB, yeasts	Paste
Maasa		Ghana, Nigeria		unknown	Sour cake
Mahewu (maheu, mageu, magou)	Maize	South Africa, Zimbabwe		*Lactococcus lactis, L. delbruckii, L. bulgaricus*	Beverage
Mangisi	Millet	Zimbabwe		unknown	beverage
Mawe	Maize	Benin		*Lactobacillus fermentans, L. cellobiosus, L. brevis, L. salivarius, Pediococcus spp, Candida krusei*	Sourdough
Merissa	Sorghum	Sudan, Ethiopia		Lactic acid bacteria	Alcoholic beverage
Munkoyo	Maize	Zambia		Lactic acid bacteria, yeasts	Beverage
Muramba	Sorghum	Uganda		unknown	Alcoholic beverage
Mutwiwa	Maize	Zimbabwe		Lactic acid bacteria, bacteria¸moulds	Porridge
Obiolor	Sorghum, Millet	Nigeria		*L. plantarum, Bacillus* spp, *Streptococcus lactis*	Beverage
Ogi	Maize	Nigeria,		*Lactobacillus plantarum, L. confusus, L. murinus, L. agilis,*	Soft or

		Benin	*Leuconostoc mesenteroides, Candida mycoderma, Saccharomyces cerevisiae,Rhodotorula*	stiff gel
Pito	Maize, Sorghum	Nigeria, Ghana	Lactic acid bacteria, *Saccharomyces*, *Candida* spp.	Sour Beer,
Pombe	Maize, Millet	Tanzania	unknown	Alcoholic Beverage
Seketeh	Maize	Nigeria	*Saccharomyces cerevisiae, Streptococcus chevalieri, Streptococcus elegans, L. plantarum, Lactococcus lactis, Bacillus subtilis, Aspergillus niger, Aspergillus favus, Mucor rouxii.*	Alcoholic beverage
Seven –day beer	Maize, Millet	Zambia	unknown	Alcoholic beverage
Talla	Maize, Millet	Ethiopia	unknown	Alcoholic beverage
Thobwa	Maize, Sorghum	Zambia	Unknown	Beverage
Togwa	Maize	Tanzania	*Lactobacillus brevis, L. plantarum L. fermentum, L. cellobiosus, L. lactis, Weisella confusa, Pediococcus pentosaceus, Issatchenkia oreintalis, Saccharomyces cerevisiae, candida tropicalis, candida pelliculosa, Candida krusei*	Beverage
Uji	Maize	Kenya, Tanzania, Uganda	*Lactobacillus plantarum, L. cellobiosus, L. buchneri, Pediococcus acidilactici, L. pentosaceus, Leuconostoc fermenti*	Beverage/ Porridge
Umbugug	Maize, Millet	Sudan	unknown	Alcoholic beverage

Lactic acid bacteria (LAB) mostly species of *Lactobacillus, Leuconostoc, Lactococcus, Enterococcus* and *Pediococcus*.
Yeasts mostly species of *Saccharomyces* and *Candida*
Moulds mostly species of *Aspergillus, Penicillium* and *Fusarium*
(Sources: Van Der Walt, 1956; Steinkraus, 1983; Salovaara, 1993; Holzapfel, 1997; Mensah, 1997; Odunfa, 1988; FAO, 1999; Lund *et al*., 2000; Abegaz *et al*., 2002; Blandino *et al*., 2003; Muyanja *et al*., 2003; Lei, 2006; Nout, 2009; Osungbaro, 2009)

Chapter 3

Production and utilisation of cereal-based fermented food products of Lusaka and Chongwe areas in Lusaka province, Zambia

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Chapter 3

3 Production and utilisation of cereal-based fermented food products of Lusaka and Chongwe areas in Lusaka province, Zambia

Abstract

Fermented foods and beverages constitute a significant portion of people's diets all over the world and it is known that in Zambian homes people prepare and utilise fermented food products for different purposes. However, there is limited documented data on the production and utilisation of these indigenous food products and the limited information that is available, is scattered and not easy to find. This study was aimed at gathering information on the production and utilisation of indigenous cereal based fermented food products to ensure the information (especially on production) is not lost as it is usually passed on from one generation to another, verbally.

Data acquired from a questionnaire completed by 205 randomly selected people of Lusaka and Chongwe districts, in Lusaka province, showed that indigenous cereal based fermented food products are prepared at home for home consumption, to sell or to use on special occasions. The main reasons for consuming indigenous cereal based fermented food products are that the foods are a source of energy and are nutritious.

More of non-alcoholic than alcoholic beverages is consumed. The non-alcoholic beverages are munkoyo, chibwantu, mahewu and thobwa, and these fermented food products are also used as weaning and complementary foods for young children. The general process for preparation in different households, of munkoyo and chibwantu beverages is similar. The activities though, at each specific step differ thereby contributing to variability in quality of products from one household to another. The munkoyo roots are used by over 93% of the respondents who prepare munkoyo and chibwantu, making it an important ingredient for the fermentation process of these beverages. The munkoyo roots are mainly bought at local markets and different utensils are used for the fermentation process, mainly plastic buckets and containers. Beverages are offered for sale in pre-used bottles (water or soft drinks bottles). The pre-used bottles are washed before use. Measuring cups are also used and the customer brings along a packaging utensil. The main reason for fermenting cereal based foods is the resulting pleasant traditional taste and flavour.

The majority of respondents did not have any kind of cooling facility and results show that these indigenous cereal based fermented food products can only be stored for a short period of time thus varied methods are employed to prolong the shelf life of these foods. Less than 7% of the respondents experienced medical problems or discomfort after consumption of some of these fermented food products. These food products especially non-alcoholic beverages are also used as alternative treatment and/or prevention methods for diarrhea and other ailments. This study has demonstrated the importance of indigenous cereal based fermented food products for the surveyed population and the country at large.

Key Words: Munkoyo roots, Cereal-Based Fermented Food Products, Utilisation

3.1 Introduction

At household level, a number of people in southern Africa, both in urban and rural areas, are recorded to be suffering from malnutrition due to a limited variety of available commercial foods, which are often of poor nutritional value (Albertson, 1995; Mahgoub *et al*., 2012). Indigenous food plants contain exceptional amounts of vital nutrients and essential vitamins (Simwamba and Elahi, 1986; Lyatuu *et al*., 2009; Lugwaila *et al*., 2011) such as vitamin A, iron, zinc, calcium and vitamin B_{12} (Vinceti *et al*., 2013), important for eliminating deficiencies and proper maintenance of human health, especially for children, pregnant women and those affected by HIV/AIDS who are often vulnerable to malnutrition and diseases.

The 2007' Zambia Demographic and Health Survey (ZDHS) reported that 45% of children under-five year old were stunted (low height for age) and 21% severely stunted in 2006, 5% were wasted (low weight for height) and 15% were underweight (low weight for age). The 2009' National Nutrition Surveillance Report conducted in 32 districts by the National Food and Nutrition Commission found stunting in 54% of boys under five years old and in 44.6% of girls under five years old. The 2007' ZDHS also reported malnutrition in 9.6% of women of childbearing age surveyed (Central Statistics Office (CSO) *et al*., 2009). Malnutrition among people living with HIV is much higher than in the general population (Ministry of Health (MoH), 2011).

In Zambia there is a variety of indigenous food plants. These include **cereals** (e.g. maize (*Zea mays*), sorghum (*Sorghum bicolor*), pearl and finger millet (*Pennisetum glaucum* and *Eleusine coracana* respectively), **leafy vegetables** (e.g. amaranthus (ibondwe) (*Amaranthus* spp), spider plant (lubanga) (*Cleome* spp) and cassava (*Manihot esculenta*) leaves (katapa) (Guarino, 1997; Biodiversity Community Network (BCN), 2006; Lyatuu *et al*., 2009)), **wild fruits** (e.g. baobab (mubuyu) (*Adasonia digitata*), masau (*Ziziphus mauritiana*), masuku (*Uapaca kirkiana*) and mupundu (*Parinari curatellifolia*) (Mingochi, 1998; BCN, 2006)), **wild nuts** (e.g mungongo (*Schinziophyton rautanenii*) nut) , **tubers** (e.g. yam (Dioscorea spp) and chikanda (*Satyria siva*, *Disa* spp and *Habenaria* spp) (Nyomora, 2005; Biodiversity Community Network, 2006; Challe and Price, 2009)) and **roots** (e.g. munkoyo (*Rhynchosia* spp and *Eminia* spp) (Mulobwa, 1998; Malaisse, 2010)). Some of these indigenous food plants are widely distributed and naturally occur in different woodland forests e.g the miombo, munga, mopane, kalahari and termitaria (Sekeli and Phiri, 2002) and are largely underutilized (Mulobwa, 1998; BCN, 2006). Yet indigenous knowledge of these food products can help people use these foods available around them to sustain their lives. Indigenous food plants are an important source of food particularly for people in the rural areas. Indigenous food plants are used to supplement diets and are gaining importance as a major household income and food security commodity, since it is found sold in local markets and along main roadsides (Mulobwa, 1998). A variety of these wild (indigenous) food plants have potential for applications in formal agriculture and thus increase their production and utilisation in order to reduce poverty and malnutrition (Mahgoub *et al*., 2012).

Published information in literature indicates that fermented foods and beverages constitute a major portion of people's diets all over the world and provide 20-40% of the total food supply (Campbell-Platt, 1994).

The Zambian diet is mainly composed of cereals that provide almost two third of the dietary energy supply (FAO, 2009). The predominant cereal is maize, consumed by 90% of the population (FAO, 2009). There is limited data on the production and utilisation of indigenous cereal based fermented food products in Zambia. Different types of indigenous cereal based indigenous foods and beverages are prepared by fermentation. Fermentation of indigenous foods is spontaneous (natural) and in some instances, back-slopping is used. The processes in preparation of the same types of food vary from one household to another, hence the variation in quality and safety.

The Zambian indigenous cereal based fermented food products, are prepared and utilised for home consumption, sell, special occasions or both home consumption and sell and are utilised as main meal and breakfast meal in the diet, light meal or snack (for the rural people particularly during field work such as planting, weeding and harvesting), weaning foods and refreshment beverages, as well as beverages for lactating mothers. These food products are prepared and consumed among the rural, peri-urban and urban people and include **nshima** prepared from fermented maize meal, sour porridge also known as **mteteka** from the eastern parts of the country, **chibwantu**, **munkoyo**, **thobwa** and **mahewu** (the non-alcoholic beverages), seven days also known as **gankata** from the southern parts of the country, **katata** and **katubi (**the alcoholic beverages) and **kachasu** (also known as lituku in other parts of the country), a distilled alcoholic beverage. **Chibuku**, **lusaka**, **chat**, **shake-shake** and **nkwazi** beers are commercial opaque alcoholic beverages from different parts of the country.

Chibwantu and munkoyo are prepared from maize meal and maize grit, respectively and munkoyo roots. The munkoyo roots are purchased from the local markets, collected from the forest or requested from family, friends and neighbors (unknown source). Wheat flour is also used or sometimes added in place of the munkoyo roots.

Chibwantu and munkoyo are prepared in the house or outside the house either in the shade or the sun. There are variations from household to household in the preparation of the porridge, e.g. at what stage the munkyo roots or root extract and/or sugar are added to the porridge, thereby giving variation in quality of the final product. In the preparation of chibwantu and munkoyo different types of utensils are used which include calabashes (*insupa*), clay pots (*mbiya*), plastic or metal buckets and drums.

There is limited (particularly published) information on cereal based fermented food products in Zambia. At home these food products are prepared and utilised for different purposes. Therefore, the purpose of this work was to gather information on the use of munkoyo roots and on the production and utilisation of indigenous cereal based fermented food products and to establish the relevance of these foods in households and the country at large with particular emphasis on the preparation of chibwantu and munkoyo beverages.

3.2 Materials and Methods

3.2.1 **Study area**

Zambia (Fig. 3-1) is situated in the tropical belt of south central Africa, 10 to 18 degrees south latitudes and on a plateau 1,300 meters above sea level and covers 752, 629 sq. km. The country has a tropical climate moderated in much of its territories by high altitudes and the land is of high geographic diversity. This diversity extends to its people as well in terms of social and culture thereby influencing the preparation and utilisation of food products by its people.

The study was conducted in Lusaka and Chongwe districts which are located in Lusaka province (Fig. 3-2) of Zambia. The geographic coordinates of the province are 15 degrees south latitude and 29 degrees east longitude. According to the 2010' Zambia population census, the country's population stands at 13 million and is predominantly rural, only 39% of the population lives in urban areas. Lusaka province has a population of 2, 198 996, of which 49.1% are males and 50.9% females and 79.3% of the population lives in Lusaka district and 8.5% in Chongwe district (Fig. 3-2). Lusaka province is the smallest of the ten provinces of Zambia with area of 21, 896 sq. km; however, it has the highest share of the country's population at 17.2% (Zambia Population Census, 2010; FAOSTAT (FAO), 2004). The study was conducted in Lusaka province because it is culturally diverse, people are originally from different parts of the country and ethnic groups and the province has the highest share of the country's population.

3.2.2 **Administration of the Questionnaire**

The structured questionnaires for face to face interviews were administered in Lusaka and Chongwe districts of Lusaka province. Two hundred and five people were randomly selected, 32.7% males and 67.3% females. The respondents completed the questionnaire after the purpose of the exercise was explained to them, which was to gather information on the production and utilisation of cereal based fermented food products, particularly the indigenous processing methods and raw materials used for the preparation of munkoyo and chibwantu beverages. The data was analysed statistically, using *t*- and chi square tests and the software used for data analysis was IBM SPSS statistics 21.

Figure 3-1: Map of Zambia with provinces (http://en.wikipedia.org/wiki/Districts_of_Zambia). Muchinga the tenth province was separated from Northern Province in 2011.

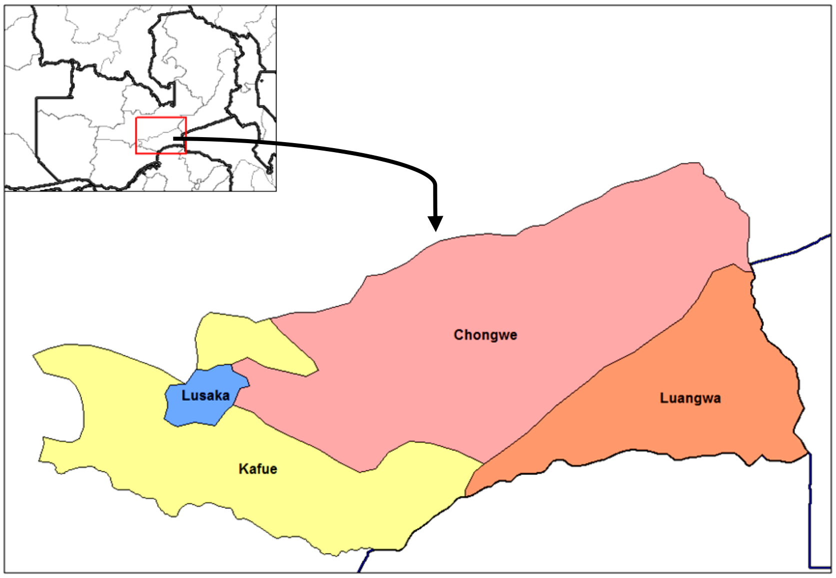

Figure 3-2: Lusaka province of Zambia with districts (Chongwe, Kafue, Luangwa and Lusaka) (http://en.wikipedia.org/wiki/Districts_of_Zambia)

3.3 Results

3.3.1 **Respondent's demographic characteristics**

Majority of the respondents were in the age range 21 to 40 years old, collectively accounting for 62.7%. Ninety-five of the 138 (67.3% of the total female respondents) female respondents were married. The number of households with five people per household was the highest (21.5%). The number of people in the household surveyed ranged from 3-7, these households collectively accounted for 73.7%. Within this range (3-7 persons / household), is where the majority had babies (0-2 years old) accounting for 30.2%. The majority of the respondent's households however, did not have babies (61.5%). A small portion of the respondents (8.3%) had two or three babies per household. A percentage of 58.1% of households had at least one to three members in the age range three to 12 years old, 44.4% had one or two members in the age range of 13-18 years old and 69.2% had adult members above 18 years old.

Almost seven percent (6.8%) of the respondents did not attend any school. Of the respondents, 20% had tertiary training, 24.9% had at least secondary education or vocational training with 48.3% attending primary or up to junior secondary education. Few respondents were government or salary workers (17.6%). The majority were unemployed (41.7%) or self employed (27.5%) (Table 3-1(a) and 3-1 (b)). The unemployed respondents were mainly from rural areas as well as the high density areas of the urban area.

A percentage of 3.7 of the respondents had a monthly income of less than $20, 50.5% (the majority of the respondents) in the range of $21-$200, 36.5% in the range of $220-$1000 and 9.3% above $1000.

Eighty four of the respondent's households (41%) where located in the urban areas of which 46 were in high density (54.8%), 20 in medium density (23.8%) and 18 in low-density areas (21.4%). Fifty one households where located in the peri- urban areas (24.9%) and 70 in the rural areas (34.1%). A percentage of 56.6 of the respondent's households had pipe borne water, were as 41.9% used water from a borehole or well. Only 48.3% had electricity. The main source of fuel for cooking was charcoal and/or firewood (67.2%), the percentage of respondents that used electricity was 32.3% and 0.5% used gas. The majority of respondents had no form of cooling facility (56.4%) while 43.6% had a refrigerator, a freezer or both.

3.3.2 Part A: Preparation of Cereal Based Fermented Food Products

One hundred and seventeen (57.1%) of the total respondents prepared one or more type of the indigenous cereal based fermented foods and beverages and majority of these respondents, 92, were females (78.6%), out numbering the total number of respondents, 88, who did not prepare any of these type of food products (42.9%). The parameters tested for respondents who did not prepare these foods and beverages were:

1. difficult to prepare
2. lack of knowledge on how to prepare them
3. no time to prepare them
4. other reasons

The majority, 36 of the 88 respondents (40.9%), who did not prepare these foods and beverages, knew how but had no time to prepare them, 26 lacked the knowledge on how to prepare them (29.5%) while a small number of five persons (5.7%) found these foods and beverages difficult to prepare.

Twenty-one respondents (23.9%) had other reasons for not preparing these indigenous foods and beverages. Of those 21 respondents, eight persons lacked interest to prepare them or learn how to prepare them (38.1%), five did not like them or lacked appetite for them (23.8%) and two were cautious of food poisoning (9.5%). The others (28.6%) lacked materials to prepare them, felt it was a waste of maize meal and that the number of people preparing these food products had increased thereby making the food products easily accessible. Fig. 3-3 and 3-4 give a summary of respondents that prepare indigenous cereal based fermented foods and beverages or not, as well as the reasons why they do prepare these food products or not.

The types of indigenous cereal based fermented foods and beverages that are prepared include nshima from fermented maize meal, sour porridge (mteteka), chibwantu, munkoyo, thobwa, mahewu and seven days (gankata). The majority of respondents prepare the non-alcoholic beverages with the greater part preparing munkoyo (82.1%), chibwantu (43.6%) and thobwa (26.7%). Forty seven and nine tenth percent (47.9%) prepared more than one type of the food products; munkoyo or chibwantu and other types.

The main reason for preparation of these foods and beverages was home consumption (78.4%). Others prepared them for home consumption and to sell (21.6%) and rarely were these foods and beverages prepared to sell only (1.7%). From the results obtained only the beverages were prepared for selling. The frequency of preparation varied for the different fermented food products. The respondents prepare chibwantu (75% of the respondents) and munkoyo (55.2% of the respondents) more often whereas for thobwa, 45.2% of the respondents prepare the beverage occasionally, 25.8% more often and 29.0% rarely. Table 3-2 shows the description of these food products. Table 3-3 and 3-4 the frequency of respondents who prepare these foods and reason for preparation and the frequency of preparation. Sixty one percent of the respondents rarely prepare sour porridge and 57.1% rarely prepare nshima from fermented maize meal (Table 3-4).

3.3.3 Part B: Preparation of Chibwantu and Munkoyo non-alcoholic beverages

3.3.3.1 Ingredients

All the respondents who prepare the munkoyo beverage use maize meal and 10.4% of them add a bit of maize grit during preparation of the porridge and all those who prepare chibwantu use maize grit and 76% of them, also add maize meal during the preparation of the porridge. A percentage of 93.8 of the respondents who prepare munkoyo and 94.2% of those who prepare chibwantu use the munkoyo roots. Ninety nine percent of the respondents who prepare munkoyo and 96.2% of those who prepare chibwantu add sugar at some stage during the preparation.

The other ingredients used in the preparation of these beverages include wheat flour, sweet potatoes, sorghum and yeast. Only 13.7% of the respondents who prepare these beverages add these other ingredients during preparation, with 13.5% of the respondents who prepare munkoyo and 9.6% of those who prepare chibwantu, using wheat flour (Table 3-5).

3.3.3.2 Munkoyo roots

One hundred and eight (108) of the respondents (92.3%) who prepare indigenous cereal based fermented foods and beverages use the munkoyo roots. Of the respondents who use the munkoyo roots in preparation of munkoyo or chibwantu beverages, the majority, 83 (76.9%), prefer to add the yellow type of roots whereas 13 persons prefer the white (12%) and 12 prefer both the yellow and white roots (11.1%).

Of the respondents that prefer the yellow roots, the main reasons for their preference are that:

- it makes the beverage taste good and gives it a very nice strong flavor (38.6%) and that it is available at the markets (28.9%)
- it is the type that the respondents were familiar with or that they were used to use these types of roots (18.1%)
- once added to the porridge during beverage preparation, it quickly liquefied the porridge and quickly changed the color of the beverage (14.5%)

- it makes the final product sweeter compared to when the white roots were used (8.4%)
- respondents (7.2%) thought these roots are the “original” munkoyo roots or believed they are the “real” munkoyo roots
- it is the traditional “Bemba” munkoyo root (1.2%) (Bemba is one of the ethnic tribes of Zambia),
- it is common (easily accessed) compare to the white root (2.4%)
- it is the “one” (root commonly) found in the area (1.2%)
- it is a body cleanser; “removes” disease (heals malaria) (2.4%)
- with the yellow roots, you are assured of its safety unlike the white munkoyo roots which might be poisonous (1.2%).

Of the respondents that prefer the white roots, the main reasons for their preference are that:

- The roots are easily available at the markets (84.6%)
- Familiar with these types of roots or the ones preferred in the household (46.2%)
- Gives better taste and flavor to the beverage compared to when the yellow roots are used (30.8%)
- For quick fermentation process compared to the yellow roots (7.7%)
- Makes the beverage sweet; no need to add sugar (7.7%)
- Like the white appearance of the beverage imparted by the white roots (7.7%)
- Alternative in case there are no yellow roots (15.4%)

Table 3-6 and 3-7 gives the summary of the reasons for the preferred types of munkoyo roots.

3.3.3.3 The process of preparation for munkoyo and chibwantu beverages

The process of preparation of the beverages is:

1. Preparation of the porridge
2. Cooling down of the porridge
3. Addition of fermenting substrates to the porridge
4. Fermentation

Flow diagram that gives an outline of the steps during munkoyo and chibwantu beverage preparation is presented in Fig. 3-6.

There are a varied ways of preparation of the porridge for making munkoyo or chibwantu beverages. For the preparation of munkoyo, the main way is using maize meal (93.7% of the respondents) and few (5.4% of the respondents) add a bit of maize grit during preparation of the porridge. For the preparation of chibwantu, the respondents either use maize grit only (51%) or use maize grit and adding a bit of maize meal (49%) during preparation of the porridge.

For munkoyo and chibwantu beverage preparation, the majority of the respondents (91.1%, accounting for total respondents who prepare chibwantu and munkoyo beverages) allow the porridge to cool down before addition of fermenting substrates. Few of the respondents add the munkoyo roots or munkoyo roots and sugar to the hot porridge before the fermentation process. Majority of the respondents (41.8%) add the munkoyo roots directly to the warm porridge, 20.5% add the pre-soaked roots and root extract and 11.6% add the root extract. Few of the respondents add other substrates such as sorghum, wheat flour, sweet potatoes or water to the warm porridge before the fermentation process or ferment the porridge without adding anything.

The fermentation process last most often for 18-24 hours while some of the respondents ferment for up to two or three days. Few of the respondents (11.6%) add the sugar with the fermenting substrates; the majority adds the sugar after the fermentation process when the beverage is ready for consumption and/or to sell. The munkoyo roots are left within the beverage or removed before addition of sugar. Table 3-8 and 3-9 give a summary of the activities performed at each stage of the process of munkoyo and chibwantu beverages preparation.

There is also variation in the time the munkoyo roots are soaked (Table 3-10). Of the respondents who soak the munkoyo roots, the majority (54.1%) soak them for about an hour and 29.8% of the respondents for 30 minutes or less. Few soak them for more than of two hours.

3.3.3.4 Source of munkoyo roots, utensils used during fermentation and packaging of beverages for sell

Majority of the respondents (95.4%) buy the roots from the market and others collect them from the forest or ask from family and friends. Plastic buckets (79.6%) are the main utensils used for fermentation, then calabash (insupa) (23.9%). The other utensils used are metal buckets, plastic containers and drums (metal or plastic). Fermentation is done mainly in the house (73.2%) otherwise outside the house; 35.7% in the sun and 0.9% in shady spaces. Few of the respondents do not ferment the beverage. Tables 3-11, 3-12 and 3-13 summarise the respondents' frequencies for their source of munkoyo roots, the utensils used and the conditions for the fermentation process.

Of the respondents who prepare the indigenous cereal based fermented beverages, a total of 17 packages the beverage in a way to sell. The majority of the respondents put the beverage in a bucket or calabash and sells to customers by measuring out the beverage using a measuring cup into the customer's packaging utensil (small containers, water jars, or cups). Thus sometimes when purchasing the beverage, customers carry their own packaging utensils. The main packaging materials used for the beverages are used soft drink plastic bottles (that is used bottles of Havana cola, Tangi, Tango pina, coca cola) and used water bottles; accounting for a total of 64.7% of the producers who package the beverages. Eleven and eight tenth percent (11.8%) of the producers buy new packaging bottles or containers for packaging of the beverages (Table 3-14).

For 95.6% of the respondents who prepare the indigenous cereal based fermented food products, their main reason for fermenting is improved taste and flavor and very few (7.9%) ferment the beverage for longer keeping (Table 3-15).

3.3.3.5 Cooking fuel and cooling facilities

A combined total of 71% of the respondents who prepare indigenous cereal based fermented food products including munkoyo and chibwantu beverages use charcoal and firewood as their main source of fuel for cooking purposes. The majority (55.5%) had no cooling facility such as a refrigerator or freezer in their households (Table 3-16).

3.3.4 Part C: Consumption of Cereal Based Fermented Food Products

3.3.4.1 Consumption

An overwhelming majority of the respondents 188 (91.7%) consume some kind of indigenous fermented food products or beverages (Fig. 3-7). Of those, 117 prepared the food themselves (62.2%)._Seventy one of the respondents (80.7%) who do not prepare indigenous cereal based fermented food products do consume cereal based fermented food products (Fig. 3-8). Thirteen of the respondents that do not consume indigenous cereal based fermented food products had alternatives available to indigenous cereal based fermented food products. Others do not consume these food products for health reasons, as they are scared of food poisoning, or that the fermented foods were bad or that they just had no knowledge of fermented foods.

The majority consume the beverages compared to sour porridge and nshima prepared from fermented maize meal, with higher percentages of respondents consuming the non-alcoholic beverages (Table 3-17). Females made up the majority of the respondents who consume alcoholic beverages (Fig. 3-9). Generally

The majority of the non-alcoholic beverages consumers in this study usually prepare it in their households, buy it or it is prepared by family and friends both at church, during or for functions. Mahewu is mainly bought (96.3%). For sour porridge and nshima from fermented maize meal, the majority of the respondents who consume it, prepare it in their households and to a lesser extent it is prepared by family and friends. Alcoholic indigenous cereal based fermented beverages, such as seven days, katata and kachasu, are also prepared in households for home consumption. Table 3-18 give a summary of the frequency of respondents and the producers of the fermented foods consumed.

The majority of the respondents rarely consume sour porridge and nshima prepared from fermented maize meal. However, for the majority of the non- alcoholic indigenous cereal based fermented beverages, which are predominantly consumed, the frequency of consumption (Table 3-19) is very varied with a significant number of respondents consuming the products on a daily basis; others rarely. Table 3-19 summarise the frequency of consumption of the cereal based fermented food products. The majority of the respondents who consume the commercial alcoholic beverages (e.g. chibuku and others) consume them on a daily basis. These consumers consisted mainly of women (Fig. 3-9 and Table 3-19).

The non- alcoholic indigenous cereal based fermented beverages are used for refreshment purposes according to 98% of respondents for all the different types of non-alcoholic beverages consumed, however, the beverages are also significantly used as light meals or snacks, breakfast meals, as main meal as well as weaning and/or complementary foods. Sour porridge is mainly used as a breakfast meal (98.3%) and to a lesser extent as light meal or weaning food. Nshima from fermented maize meal is only used as a main meal in the diet. The alcoholic beverages are used mainly for refreshment purposes. Table 3-20 gives a summary on the use of fermented food products consumed.

3.3.4.2 Factors that influence selection of indigenous cereal based fermented food products

Price influenced the selection of cereal based fermented food products for consumption. The majority of the respondents (over 70% of respondents for each food category) found the food products to be “cheap” (affordable) and that the food products were easily available. For the food products that are prepared at home for home consumption such as the non-alcoholic beverages munkoyo, chibwantu and thobwa, as well as nshima prepared from fermented maize meal, are the ones that are mainly offered as gifts (Table 3-21). For all the respondents taste and/or flavour influenced their selection of these foods and 98% of respondents for all food categories believe that cereal based fermented food products are nutritious. Few of the respondents use the foods for medical reasons and the cereal based fermented food products used for this purpose are mainly the non-alcoholic beverages, munkoyo, chibwantu, thobwa and mahewu, particularly munkoyo (Table 3-22).

For the majority of the cereal based fermented food products that are prepared at home for home consumption or to sell; the majority of the respondents felt that the food products were easy to prepare except for mahewu. There is a commercial brand of mahewu on the market that most consumers buy. Table 3-18 shows the distribution of respondents on consumption of cereal based fermented food products and the producers of these food products and 96.3% of the respondents, who consume mahewu, purchase their beverage while only 3.7% prepare it at home for personal consumption and 2.4% consume it when they are at their family and friends’ houses. Table 3-23 shows that, from the respondents who prepare mahewu none of them prepare it to sell, implying that majority of the consumers of mahewu purchase the commercial brand. The majority of the respondents (75%) who consume mahewu, probably felt that the beverage was too complex to prepare as most bought the commercial brand. When answering the question on ease of preparation the consumers of mahewu most likely referred the commercial brand. The results on mahewu consumption indicate that once the indigenous beverages are commercialised, consumers are willing to purchase the food products.

A significant number of respondents ($p < 0.01$) who consume thobwa (27.3%) felt that it was difficult to prepare but the majority found it easy. Sour porridge and nshima prepared from fermented maize meal are not usually consumed on festive occasions or after child birth; however, significant numbers of respondents ($p<0.01$) use the indigenous cereal based beverages, both alcoholic and non-alcoholic during festive occasions and after child birth. The cereal based fermented food products are generally used at certain periods of the day (Table 3-24). In households, majority of consumers of indigenous cereal based fermented food products are adults accounting for more than 98% (for each food category) of respondents who consume these foods. All the types of non-alcoholic beverages; munkoyo, mahewu, chibwantu, and thobwa were offered to babies and toddlers (Table 3-25) as well as sour porridge. Children between the age of three and 12 years old and adolescents also prefer these foods. All family members do consume indigenous cereal based fermented food products in households surveyed.

3.3.4.3 Spoilage

For all the indigenous cereal based fermented food products consumed within the area of study, except for kachasu which is a distilled alcoholic beverage (Table 3-1) and the commercial mahewu brand, the respondents indicated that these food products could not be stored for a long period of time. There was a very significant difference ($p<0.01$) in storability within different food categories. However, for the non-alcoholic beverages thobwa, munkoyo, and chibwantu, the majority of respondents felt that the beverages could be stored for longer than two days. Most of the consumers of the commercial alcoholic beverage, chibuku, indicated that it can be stored for less than a day and therefore chibuku purchased has to be consumed the same day (Table 3-26).

Since almost all the respondents who consume indigenous cereal based fermented food products were influenced by sensory properties taste and flavour in their selection of these foods. During spoilage of these foods, a change in flavour was the main characteristic observed (Table 3-22 and 3-27). The food products get affected by moulds as significant numbers of respondents observed development of moulds on the different indigenous cereal based fermented food products, the most affected being nshima prepared from fermented maize meal and sour porridge. Other characteristic observed on non-alcoholic beverages and sour porridge were changes in viscosity; were the beverage or porridge became watery ("light") and slimy. For munkoyo, thobwa and chibwantu, there was also the presence of bubbles or foam on top of the beverages (Table 3-27). Once the food products were spoiled, the majority of the respondents threw them away, however, the respondents tried to prepare or purchase just enough to avoid spoilage and wastage. A percentage of 4.8 of the respondents consume the food even when the food products were spoiled (Table 3-28).

One hundred and thirteen (56.1%) of all the respondents had no cooling facility in their household. Table 3-27 shows that only 28.8% and 14.1% of all the respondents use a refrigerator and freezer respectively; accounting for 42.9% of respondents using some form of refrigeration for controlling spoilage of indigenous cereal based fermented food products. There are varied ways of controlling spoilage of these fermented food products (Table 3-29) and the main ones include:

- preparation or purchase of just enough of the food products which will all be consumed or sold before spoilage
- placing the food products in a container or any utensil with low humidity such as a clay pot then placing it on "cold/ cool" surfaces or in a cool place such as a thatched hut submerging under water.

To a lesser extent, respondents employ one or more of the following methods to avoid or reduce the rate of spoilage

- for non-alcoholic beverages;

- remove it from the fermenting utensil such as a calabash to stop further fermentation
- addition of sugar only during consumption of the fermented food products to slow down the fermentation process and subsequently spoilage
- submerge a container of very cold water in the beverage
- put the beverage in a container with a jute sack around it (keeping the jute sack wet and cool all the time).
- for the beverage ready for sale; place ice blocks in a basin used to carry the beverage bottles or containers (Fig.. 3-10).
- when the beverage spoils(becomes alcoholic) give to beer consumers
- give or share excess fermented food products with neighbors.

3.3.4.4 Medical issues

Majority of the consumers (92.9%) of indigenous cereal based fermented food products within the study area do not experience any medical problems /or discomfort after consumption of these food products; only 14 of the respondents (7.1%) indicated that they experience some form of discomfort after consumption of these fermented food products and the main symptom experienced was diarrhea, experience by six respondents (42.9% of the respondents who experienced the discomfort), stomach cramps, four (28.6%), nausea, four (28.6%), headache; two and coughing; one respondent. None of the respondents experienced fever or poor vision. The symptoms were mainly observed within 30 minutes of consumption of food or the next day and the frequency of experiencing symptoms after consumption of these foods is often. Eleven affected consumers (78.6% of these respondents) mainly abstain from the suspected food product to relieve the symptoms. Four (28.6%) of these respondents administer drugs but two indicated that they allow the symptoms to stop on its own and only one indicated that they employ Oral Rehydration Salt (ORS) and only one seek medical attention. The symptoms experiences are similar to the symptoms associated with microbial food poisoning due to contamination of foods with pathogenic microorganisms. If the assumption, for the respondents who experience discomfort after consumption of indigenous cereal based fermented food products, of the symptoms is microbial food poisoning, then the results in Table 3-28 indicate that majority of the incidents of food poisoning go unrecorded since consumers just abstain from the suspected food and rarely seek medical attention. Table 3-23 shows the percentages of respondents that give these foods to babies and toddler (below the age of three years old). However, none of the babies and toddler from households where indigenous cereal based foods are consumed experienced medical problems or discomforts (according to their mothers) from consumption of these foods. Those who experience symptoms are adults, most likely, as they are the main consumers of these fermented foods (Table 3-25). To a lesser extent, children between three and 12 years old and adolescents did experienced symptoms (Table 3-30).

3.3.4.5 Use of indigenous cereal based fermented food products for treatment and/or prevention of ailments

Only the beverages are used for treatment and/or prevention of ailments and munkoyo is the main one used. Chibwantu, thobwa and mahewu are used for diarrhea, nausea and a lack of appetite, but in addition to these munkoyo is also used for yellow fever, malaria, eye problems, stomach cramps, malnutrition and headaches. Chibuku beer is also use for stomach cramps. Overall the respondents who use indigenous cereal based fermented food products for treatment and/or prevention of ailments are few, less than 12% of the total respondents (Table 3-31).

3.3.5 Importance of cereal based fermented food products as part of the Zambian diet

Table 3-32 gives the summary on the importance of indigenous cereal based fermented food products as part of the Zambian diet. One hundred and eighty three (89.3%) of the respondents think indigenous cereal based fermented food products form an important part of the Zambia diet and their main reasons include the following:

The food products;

- are a source of energy (making the consumer feel full)
- substitute other foods (staples) and are good for people in the rural areas since packaged foods and beverages are not easily found / accessed
- are nutritious, supplement other foods and can be used as starter meals for the whole family
- the beverages are favorable for large gatherings such as marriage ceremonies, arrival of a new baby in a family and funerals as well as for visitors

Other reasons include the fact that:

- indigenous cereal based fermented food products are easy and cheaper to prepare and for the underprivileged (or because of poverty), the foods are affordable and good for their health
- helps patients with a lack of appetite/ and or nausea (even at hospitals it is encouraged to consume these foods)
- likable (God given, local foods, from our ancestors) and very natural hence making them very healthy
- helps with breast milk production in lactating mothers and are used for weaning (children easily forget breast feeding)
- source of national pride

For the respondents that felt that the foods do not form an important part of the Zambian diet their reasons included the following:

- could do without indigenous cereal based fermented food products
- there are a variety of other foods and beverages available making these foods not very important and besides, the foods are only consumed for refreshment/ or fun purposes.
- people just follow the taste/ flavor; the foods are not nutritious (lack vitamins and protein)
- bad because the food products have bacteria and “make one sick”

- rarely consumed in the households

3.3.6 Trends in the consumption patterns of indigenous cereal based fermented food products

There is no information on the consumption patterns of indigenous cereal based fermented foods and the results in Table 33 show the respondents' views in the trends in consumption of these food products. The majority of the respondents, 155 (77%), indicated that the trends in consumption of indigenous cereal based fermented food products were on the increase and the main reasons for that included the following:

- because of the traditional, nice/ or pleasant taste and flavour of the foods
- people have realized that the foods are nutritious (the food products contribute to good health, have a lot of nutrients and vitamins)
- the foods are affordable hence many can afford to buy or offer as gift
- the foods are easily accessed/ highly available (including in the local markets) and availability is on the increase (including resources to prepare them)
- people enjoy the foods (especially the beverages during the hot season) and there is an increase in the number of people consuming these types of food
- give enough energy and supplement other foods
- people no longer consume and/or consider these foods as traditional foods.

Of the 23 respondents (11.3%) who indicated that the trends in consumption of indigenous cereal based fermented food products were declining indicated that their main reasons were:

- too many modern foods and drinks are available on the market
- these are used as supplement foods
- and that there is lack of people with the knowledge to prepare these fermented foods.

For the 25 respondents (12.3%) who were not sure of the trends in consumption of indigenous cereal based fermented food products, their main reasons were that:

- they had no interest (do not pay attention at the food products)
- lack of statistical knowledge / have not been observant/ do not know much about these foods
- rarely prepare them, hence do not know.

3.4 Discussion

A significant number of respondents had some form of education to help them secure a formal job and/or help themselves through self-employment. The results indicated that few respondents were government or salary worker. The majority of the respondents were unemployed or self-employed. Some of the respondents that are self-employed had difficulty keeping track of their total monthly income because they had different sources of income to sustain their households.

A review of literature shows that fermented foods are important part of diets of many and fermentation is an inexpensive way of preserving food, improving its nutritional value and enhancement of its sensory properties (Steinkraus, 1983; FAO, 1998; Abegaz *et al*., 2002; Blandino *et al*., 2003) as well as improving health of people by satisfying their medical needs (Aderiye and Laleye, 2003). The results obtained from this study confirm that a wide range of cereal based fermented food products are produced and utilised. Mainly beverages (alcoholic and non-alcoholic) and the porridges (thick and thin paste) are prepared and utilised. The majority of the respondents from the surveyed area (urban, peri-urban and rural) consume some kind of indigenous cereal based fermented food product and prepare one or more types for their home consumption or to sell to support their households. Generally, the majority of the respondents believed that indigenous cereal based fermented food products form an important part of the Zambian diet mainly because these foods give energy and are a nutritious substitute for other foods. The majority of respondents believed that the trends in consumption of these foods are on the increase mainly because of the traditional pleasant taste and flavour. The majority of the respondents that prepare these food products had no cooling facility making fermentation an important aspect of food preservation.

The munkoyo roots used for preparation of munkoyo and chibwantu beverages are mainly purchased from the local markets and to a lesser extent collected from the forest. At the local markets, the munkoyo roots are usually sold in open spaces (on market tables) with no form of packaging (Fig. 3-11), only packaged for a customer once bought. Therefore there could be contamination from the environment as well as from handling of the roots during selling. In addition, the process and equipment used during preparation of the munkoyo roots from the time they are collected from the forest to time of sell at local markets is not documented to the author’s knowledge. It is most probably that some of the spoilage microorganisms are encountered during that process and since the roots are not washed before use, the microorganisms may find their way into the final product. Fermentation of foods starts generally by inoculation of microorganisms from raw materials, utensils used and the environment or by back slopping.

Plastic containers and buckets usually offer the most diversity when it comes to fermentation containers. Plastic containers and buckets come in different shapes and sizes and are cheaper and easily accessed (Beerlegends, 2011). Plastic containers and buckets can be reused for many other functions in a household such as storage of dry foods and water. The results from Table 3-11 show that more than 80% respondents (who traditionally prepare munkoyo and chibwantu beverages for home consumption or to sell) use plastic buckets and containers for the fermentation process. However, plastic buckets and containers can easily be scratched, especially during cleaning with hard and/or rough scourers. The punctured surfaces can create breeding ground for bacteria, which include those that have been associated with food spoilage such as *Proteus*, *Micrococcus* and *Bacillus* as well as food borne yeasts such as

Saccharomyces, and *Pichia* and food borne moulds such as *Penicillium*, *Aspergillus* and *Fusarium* (Filtenborg, 1996; Jespersen and Jakobsen, 1996; Huis in't Veld, 1996; Sanni *et al*., 1999). Biofilms can also form on the inner surfaces of the buckets and containers thereby compounding contamination of the beverages during preparation, subsequently resulting in varied quality and safety of the products. In addition, some of these spoilage microorganisms maybe capable of producing toxins that could be harmful to the health of the consumers. The fermented food products are normally consumed together with the microorganisms responsible for the fermentation (Sanni *et al*., 1999).

The general preparation process of traditional munkoyo and chibwantu beverages, was found to be similar within the study area, however, there are widely varied activities performed at each of the steps during the preparation process (Fig.. 3-6, Table 3-7 and 3-8) as well as in packaging and general handling between different households, thereby, further contributing to the variation in quality and safety of these food products.

Diarrheal diseases are a major health problem contributing to high rates of malnutrition, particularly in developing countries. It is estimated that up to 70% of these diseases are of foodborne origin and are caused by contaminated foods and drinking water (Motarjemi, 1993; Nout and Motarjemi, 1997; WHO, 2009). There has therefore been a growing interest in the use of indigenous fermented food products because of the microbial inhibitory properties of the organic acids produced (mostly lactic acid) and other inhibitors such as hydrogen peroxide, diacetyl, alcohols, carbon dioxide, and bacteriocins as well as their synergistic inhibitory effects, during the fermentation process (Rodgers, 2001; Caplice, 1999; Helander, 1997).

There are many traditional beliefs about the medicinal properties of indigenous fermented food products (Dirar, 1992) and the inhibitory properties these food products on human pathogens such as *E. coli*, *Staphylococcus aureus*, *Campylobacter jejuni* and some *Shigella* and *Salmonella* species during fermentation, have been reported and the studies reviewed (Mensah, 1997; Quevedo, 1997; Gadaga *et al*., 2004). This present study indicated that 92.9% of the respondents did not experience any form of medical problems or discomfort after consumption of cereal based fermented food products and especially the munkoyo beverage is used by some of the respondents in the treatment and/or prevention of diarrheal diseases and other ailments. The respondents also reported that these indigenous foods are good for nausea and lack of appetite, symptoms or conditions associated with diarrheal diseases and that even at hospitals; the officials encourage consumption of these foods when one is not well.

Ninety three percent of the respondents from the study area that prepare these beverages (munkoyo and chibwantu) use the munkoyo roots (yellow or white) during preparation probably as a source of fermenting substrates, microorganisms and/or enzymes, unlike the majority of other Africa indigenous cereal based fermented foods, such as togwa from Tanzania (Oi and Kitabatake, 2003), bushera from Uganda (Muyanja *et al*., 2003) and mahewu from southern Africa (Gadaga *et al*., 1999) that use malted cereals for the same purpose. However, the munkoyo root itself could also be imparting some therapeutic properties in addition to those of fermenting microorganisms as these beverages are used for medical purposes. Some respondents believe the munkoyo (yellow) root is a body cleanser which removes disease (such as malaria) from the body.

The review of studies by Motarjemi *et al.* (1993) demonstrated that weaning foods prepared under unhygienic conditions are frequently heavily contaminated with pathogens and thus was a major factor in the cause of diarrheal diseases and associated malnutrition. The study has shown that indigenous cereal based foods are used as weaning and/or complementary foods for children; babies and toddler (below the age of three years old). None of the babies and toddler from households where indigenous cereal based foods are consumed experienced medical problems or discomforts from consumption of these foods. Overall, only a small number of the respondents experienced medical problems or discomfort after consumption of the fermented foods, indicating that probably the food products have some prophylactic and therapeutic properties.

3.5 Conclusion

This study has demonstrated the extent to which indigenous cereal based fermented food products are prepared in households of rural, peri- urban and urban areas of Lusaka and Chongwe districts in Zambia using traditional methods. A comprehensive profile of the preparation of cereal fermented food products with emphasis on chibwantu and munkoyo beverages; prepared using munkoyo roots, the supplements added, variation in fermentation techniques and storage was made contributing significantly to annotating indigenous knowledge that was only orally preserved up to date. The results of the survey are of use to indicate the product use differences and consumer-producer preferences of the population surveyed and their concerns in respect of safety, economic, social and medical parameters.

There are a number of factors that contribute to the quality and safety of the food products which include the;

- quality of raw materials used
- handling of raw materials at the local markets, forest (during collection in the case of munkoyo roots) and in households during preparation of the food products
- utensils and methods used during preparation
- environment where the food products are prepared
- packaging materials used especially to sell the food products
- storage conditions both for raw materials and final prepared food products as well as during sale

Information gathered on ingredients and preparation methods of munkoyo and chibwantu beverages is essential for up grading to commercial production particularly in developing products that will be acceptable in terms of sensory properties, quality and safety by the majority of the consumer population. More research is needed to help find ways of improving the nutritional and safety aspects of these food products for increased consumption.

The next chapter (four) will investigate the microorganisms associated with the munkoyo roots and maize grit used during preparation of chibwantu beverage to establish their microbiological quality and safety.

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Table 3-1: (a) Distribution of respondents on Employment status against Location of Household

Employment status	Location of Household			Total
	Urban	Peri-urban	Rural	
Government / Salary worker	27 (32.1)	5 (9.8)	4 (5.8)	36 (17.6)
Self employed	26 (31.0)	18 (35.3)	12 (17.4)	56 (27.5)
Unemployed	20 (23.8)	19 (37.3)	46 (66.7)	85 (41.7)
Others (Allowance (remittance), Piece work, retired or student)	11 (13.1)	9 (17.6)	7 (10.1)	27 (13.2)
Total	84 (100.0)	51 (100.0)	69 (100.0)	204 (100.0)

Pearson chi-square: P< 0.01. Numbers in brackets represent percentages of respondents

Table 3-1: (b) Distribution of respondents on employment status against Location of Household if it is in urban area

Employment status	Location of Household if it is urban area			Total
	High density	Medium density	Low density	
Government / Salary worker	7 (15.2)	12 (60.0)	8 (44.4)	27 (32.1)
Self employed	19 (41.3)	3 (15.0)	4 (22.2)	26 (31.0)
Unemployed	14 (30.4)	2 (10.0)	4 (22.2)	20 (23.8)
Others (Allowance (remittance), Piece work, retired or student)	6 (13.0)	3 (15.0)	2 (11.2)	11 (13.1)
Total	46 (100.0)	20 (100.0)	18 (100.0)	84 (100.0)

Pearson chi-square: P< 0.01. Numbers in brackets represent percentages of respondents

Table 3-2: Indigenous cereal based fermented foods mainly consumed in Lusaka province of Zambia

Food Product	**Raw Materials**	**Nature of the food product based on texture**
Nshima (prepared from fermented maize meal)	Maize	Paste
Sour porridge (Mteteka)	Maize	Semi-solid gruel
Munkoyo	Maize, Munkoyo roots	Non-alcoholic liquid gruel
Chibwantu	Maize, Munkoyo roots	Non-alcoholic liquid gruel
Thobwa	Maize, Sorghum	Non-alcoholic liquid gruel
Mahewu	Maize, Wheat	Non-alcoholic liquid gruel
Seven days (Gankata)	Maize, sorghum	Alcoholic liquid gruel
Katata	Maize, Millet	Alcoholic liquid gruel
Katubi	Maize, Millet	Alcoholic liquid gruel
Kachasu	Maize, Sorghum	Liquid (distilled alcohol)
Commercial opaque beers	The main raw materials are Maize, Sorghum, Millet	Alcoholic liquid gruel
Chibuku		
Shake shake		
Nkwazi		
Lusaka		
Chat		

The predominant microorganisms are not known, limited work has been done on the food products.

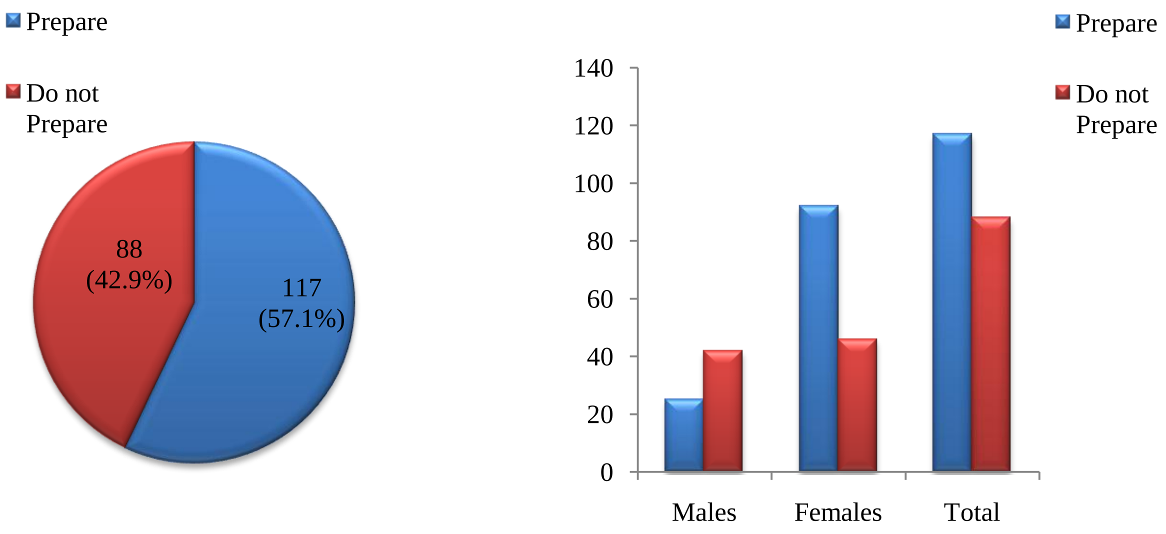

Figure 3-3: Frequency of respondents that prepare indigenous cereal based fermented food products.

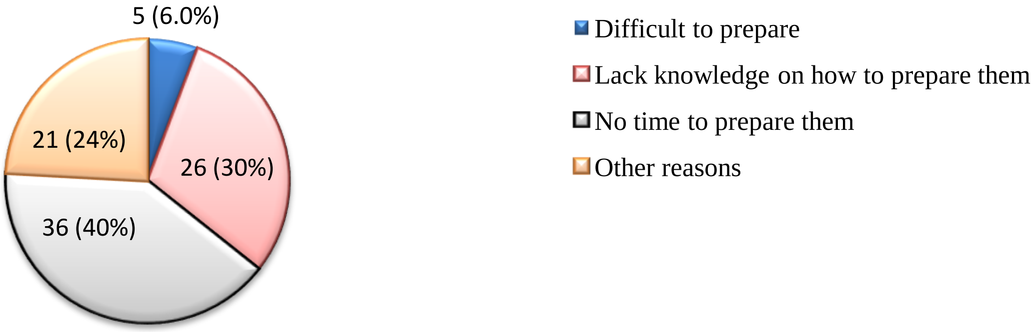

Figure 3-4: (a) The frequency of the respondents who do not prepare indigenous cereal based fermented foods or beverages, the reasons for not preparing them.

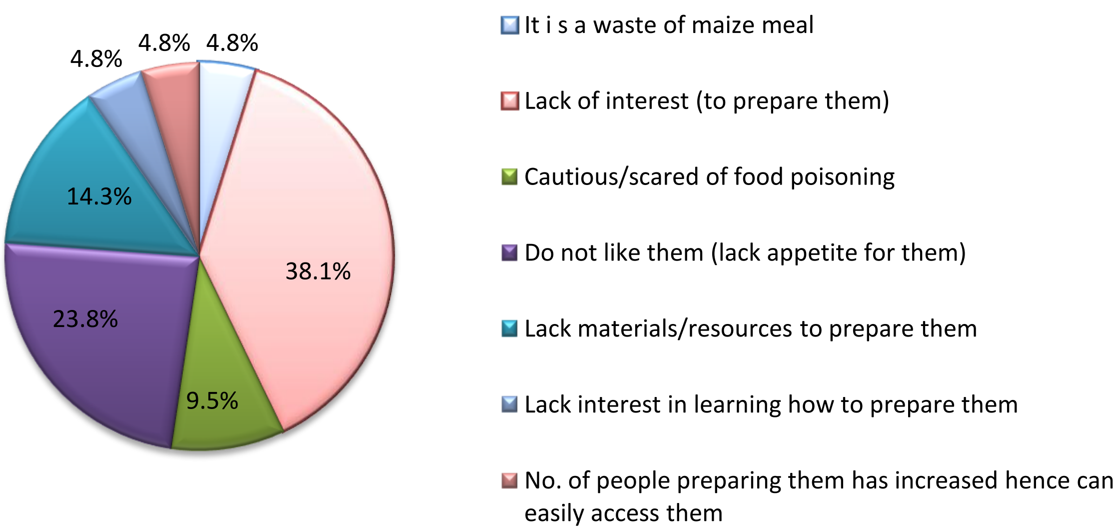

Figure 3-4: (b) Frequencies of respondents for other Reasons (21 respondents)

Table 3-3: The frequency of respondents that prepare indigenous cereal based fermented foods and beverages and the reason for preparation.

Foods and beverages prepared	**Frequency of respondents**	**Reason for preparation**		
		Home consumption	Sell	Both Home consumption and sell
Chibwantu	52 (43.6)	41 (78.8)	-	11 (21.2)
Munkoyo	96 (82.1)	83 (86.5)	1 (1.0)	12 (12.5)
Thobwa	31 (26.7)	30 (96.8)	-	1 (3.2)
Mahewu	2 (1.7)	2 (100)	-	-
Sour porridge	26 (22.2)	26 (100)	-	-
Nshima (from fermented maize meal)	7 (6.0)	7 (100)		
Seven days	1 (0.9)	-	1 (100)	-
Katata	0			
Kachasu	0			
Others	0			

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-4: Frequency of the respondents that prepare indigenous cereal based fermented foods and beverages and the foods and beverages prepared as well as the frequency of preparation.

Foods and beverages prepared	Frequency of respondents	Frequency of preparation		
		Often	Occasionally	Rarely
Chibwantu	52	39 (75)	8 (15.4)	5 (9.6)
Munkoyo	96	53 (55.2)	25 (26.0)	18 (18.8)
Thobwa	31	8 (25.8)	14 (45.2)	9 (29.0)
Mahewu	2	-	-	2 (100)
Sour porridge	26	7 (27)	3 (11.5)	16 (61.5)
Nshima (from fermented maize meal)	7	2 (28.6)	1 (14.3)	4 (57.1)
Seven days	1	-	1 (100)	-
Katata	0	-	-	-
Kachasu	0	-	-	-
Others	0	-	-	-

*Numbers in brackets represent percentages

Table 3-5: Main ingredients used in the preparation of munkoyo and chibwantu beverages

Main ingredients used	Frequency of respondents	
	Munkoyo	Chibwantu
Maize grit	10 (10.4)	51 (98.1)
Maize meal	96 (100)	40 (76.9)
Munkoyo roots	90 (93.8)	49 (94.2)
Water	96 (100)	52 (100)
Sugar	95 (99.0)	50 (96.2)
Other ingredients	16 (13.7)	
Wheat flour	13 (13.5)	5 (9.6)
Yeast	1 (1.0)	1 (1.9)
Sweet potatoes	1 (1.0)	4 (7.7)
Sorghum	1 (1.0)	-

Numbers in brackets represent percentages. 96 respondents prepare munkoyo and 52 prepare chibwantu

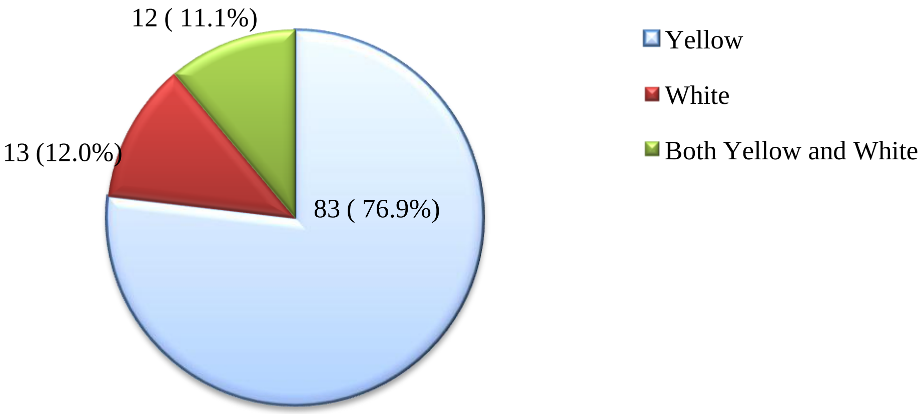

Figure 3-5: For the respondents that prepare munkoyo and chibwantu beverages, the frequency of respondents' preferred type of munkoyo roots.

Table 3-6: Reasons for the preference of the yellow munkoyo roots

Main reasons for the preference of the yellow munkoyo roots	**Frequency of respondents**
Makes the beverage taste good and giving it a very strong nice flavor	32 (38.6)
Available at the markets	24 (28.9)
The type that the respondents are familiar with or the respondents are used to using this type of root	15 (18.1)
When added to the porridge during beverage preparation, the roots quickly makes it light and changes the color of the beverage fast	12 (14.5)
Makes the beverage sweeter compared to when the white root is used	7 (8.4)
Yellow roots are the original munkoyo roots or believe it was the real munkoyo roots	6 (7.2)
Traditional Bemba munkoyo	1 (1.2)
One found in the area	1 (1.2)
Common compared to the white root	2 (2.4)
It is a body cleanser, removes disease from the body (heals malaria)	2 (2.4)
With the yellow roots, you are assured of its safety unlike the white roots which might be poisonous	1 (1.2)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-7: Reasons for the preference of the white munkoyo roots

Main reasons for the preference of the white munkoyo roots	Frequency of respondents
Highly available at the markets	11 (84.6)
Familiar with these types of roots or the ones preferred in the household	6 (46.2)
Gives better taste and flavor to the beverage compared to when the yellow roots are used	4 (30.8)
For quick fermentation process compared to the yellow roots	1 (7.7)
Makes the beverage sweet; no need to add sugar	1 (7.7)
Like the white appearance of the beverage imparted by the white roots	1 (7.7)
Alternative in case there are no yellow roots	2 (15.4)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-8: Process of preparation for munkoyo beverage

Step	Activity	Frequency of respondents
1	Prepare fire and boil water (in a pot, metal bucket or drum)	95 (100)
2	Add maize meal to boiled water to make the porridge	77 (81.1)
	Add maize meal to warm water and let it cook until the porridge turns yellowish or brownish (a bit burnt)	12 (12.6)
	Add maize meal and a bit of washed maize grit to boiled water to make the porridge	2 (2.1)
	Add a bit of washed maize grit to the boiled water, cook a bit and the add maize meal to make the porridge	3 (3.2)
	Add left over nshima and make porridge until it turns yellowish	1 (1.1)
3	Let the porridge cool down (in a pot, calabash, or bucket; in case of a drum, remove it from the fire)	86 (90.5)
	Let the porridge cool down (in a pot, calabash, or bucket) then dilute with water and ferment for 18-24hrs	4 (4.2)

	Add munkoyo roots to the hot porridge and allow to cool down	2 (2.1)
	Add munkoyo roots and sugar to the hot porridge and allow to cool down	2 (2.1)
	Dilute the porridge with water then add wheat flour	1 (1.1)
4	Add munkoyo roots to the warm porridge and ferments (overnight or 2 days)	40 (42.1)
	Add munkoyo roots and a bit of sugar to the warm porridge and ferments (overnight)	4 (4.2)
	Add munkoyo roots to the warm porridge, then water to dilute, remove the roots after a few minutes and ferment (overnight)	1 (1.1)
	Add munkoyo roots to the warm porridge, then a bit of water and ferment (overnight)	3 (3.2)
	Add pre-soaked munkoyo roots and root extract to the warm porridge and ferments (overnight)	23 (24.2)
	Add munkoyo root extract only to the warm porridge and ferments (overnight)	4 (4.2)
	Pour the hot porridge in a bucket (plastic or metal) and let it cool down	2 (2.1)
	Pour the warm porridge in a bucket (plastic or metal) of water to dilute, the next day add munkoyo roots and sugar and ferment for 3 days	2 (2.1)
	Add water to porridge, stir and ferment for less than a day and the beverage is ready	3 (3.2)
	Add wheat flour (or mixture of flour with water) to the warm porridge and ferments (overnight)	2 (2.1)
	Add water mixed with wheat flour and sugar to the warm porridge and allow to ferment	5 (5.3)
	Add sorghum to the warm porridge and stir then allow to ferment	2 (2.1)
5	Add sugar and the beverage is ready for consumption/ sell	54 (56.8)
	Add sugar, remove the munkoyo roots and the beverage is ready	2 (2.1)
	Stir and remove the munkoyo roots and the beverage is ready for consumption/ sell	5 (5.3)
	Stir, remove the munkoyo roots from the beverage	9 (9.5)
	Stir, remove the munkoyo roots and ferment (overnight, 2 days)	2 (2.1)
	Add munkoyo roots to the warm porridge and ferments (overnight)	4 (4.2)
	Add a bit of previously used munkoyo roots to the warm porridge for quick fermentation	1 (1.1)
	Add pre-soaked munkoyo roots, root extract to the warm porridge and ferments (overnight)	3 (3.2)

	Pour warm porridge in a bucket and ferment (overnight)	2 (2.1)
	Add sugar to the warm porridge and ferment (overnight)	1 (1.1)
6	Add sugar and the beverage is ready for consumption	18 (18.9)
	Stir, remove the munkoyo roots, add sugar and the beverage is ready for consumption	4 (4.2)

Numbers in brackets represent percentages of respondents who prepared munkoyo

Table 3-9: Process of preparation for chibwantu beverage

Step	Activity	Frequency of respondents
1	Prepare fire and boil water (in a pot, metal bucket or drum)	51 (100)
2	Add washed grit to make the porridge	25 (49.0)
	Add washed (use sieve) grit to make the porridge and then add a bit of maize meal	25 (49.0)
	Add washed grit, let it cook until it turns brown (burns a bit), then stir	1 (2.0)
3	Let the porridge cool down (in a bucket)	43 (84.3)
	Add maize meal mixed with water to the porridge, stir to avoid sticking at the bottom of the cooking utensil, then cook further (30minutes)	6 (11.8)
	Pour porridge in bucket and add roots directly	2 (3.9)
4	Add munkoyo roots to the warm porridge and ferments (overnight or 2 days)	15 (29.4)
	Add munkoyo roots and sugar to the warm porridge and ferments (overnight)	4 (7.8)
	Add munkoyo roots to the warm porridge, then water to dilute, remove the roots after a few minutes and ferment (overnight)	2 (3.9)
	Add pre-soaked munkoyo roots and root extract to the warm porridge and ferments (overnight)	7 (13.7)
	Add munkoyo root extract only to the warm porridge and ferments (overnight)	9 (17.6)
	Add munkoyo root extract and sugar to the warm porridge and ferments (overnight)	2 (3.9)
	Pour the porridge in a bucket (plastic or metal) and let it cool down	5 (9.8)
	Pour the porridge in a bucket (plastic or metal) and add water the dilute	2 (3.9)
	Add water to porridge, stir and ferment for less than a day and the beverage is ready	1 (2.0)
	Pour the porridge in a bucket/ container and ferment overnight and it is ready	1 (2.0)
	Add sugar to the porridge and ferments (overnight)	1 (2.0)
	Add pounded sweet potatoes to the porridge or pre-soaked roots	1 (2.0)
	Pre-soak roots in water used for cleaning grit	1 (2.0)

5	Add sugar and the beverage is ready for consumption	25 (49.0)
	Stir and remove the munkoyo roots and the beverage is ready for consumption	3 (5.9)
	Stir, remove the munkoyo roots, add sugar and the beverage is ready for consumption	2 (3.9)
	Add munkoyo roots to the warm porridge and ferments (overnight)	3 (5.9)
	Add pre-soaked munkoyo roots and root extract to the porridge and ferments (overnight)	3 (5.9)
	Add pre-soaked munkoyo roots, root extract and sugar to the porridge and ferments (overnight)	1 (2.0)
	Pour warm porridge in a bucket and ferment (overnight)	1 (2.0)
	Add a bit of previously used roots to warm porridge for quick fermentation	1 (2.0)
	Add sugar to the warm porridge and ferment (overnight)	1 (2.0)
6	Add sugar and the beverage is ready for consumption	3 (5.9)
	Stir, remove the munkoyo roots, add sugar and the beverage is ready for consumption	4 (7.8)

* Numbers in brackets represent percentages of respondents who prepared chibwantu

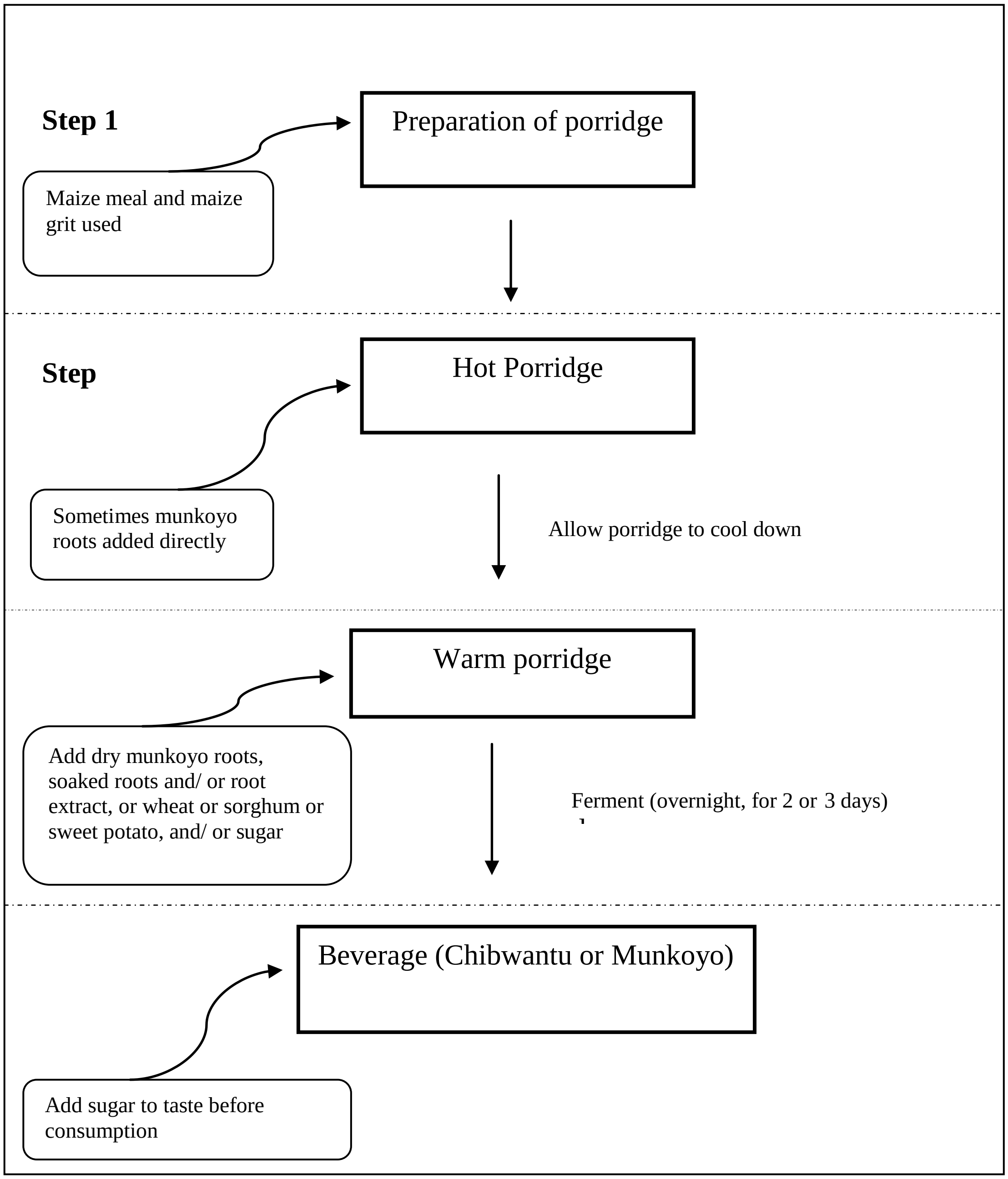

Figure 3-6: Flow diagram: Outline of the steps during munkoyo and chibwantu beverage preparation

Table 3-10: Time of soaking (in water) for the munkoyo roots

Time of soaking		**Frequency of respondents**
Minutes	1-10	8 (10.8)
	11-20	3 (4.1)
	30	11 (14.9)
Hours	1	41 (54.1)
	2	7 (9.5)
	5-10	2 (2.7)
	24	2 (2.7)
	Total	74 (100)

Numbers in brackets represent percentages of respondents

Table 3-11: Source of the munkoyo roots used for preparation of munkoyo or chibwantu beverages (n= 108)

Source	**Frequency of respondents**
Buy from local market	103 (95.4)
Ask from family/friends	18 (16.7)
Collect from forest	15 (13.9)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-12: Utensils used during the fermentation process of munkoyo and chibwantu beverages (n=113)

Utensils used	**Frequency of respondents**
Plastic bucket	90 (79.6)
Calabash (Insupa)	27 (23.9)
Plastic container	14 (12.4)
Metal bucket	6 (5.3)
Drum (plastic/metal)	2 (1.8)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-13: Fermentation conditions of munkoyo and chibwantu beverages (n=112)

Fermentation conditions	Frequency of respondents
In the house	82 (73.2)
Outside the house in the sun	40 (35.7)
Outside the house in open shade spaces	1 (0.9)
Do not ferment	2 (1.8)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-14: Packaging of the beverages for sale (n=17)

Packaging	Frequency of respondents
In a bucket using a measuring cup	13 (76.5)
Buy packaging bottle/ container	2 (11.8)
In used water bottle	4 (23.5)
Used soft drink bottle (of Havana cola, Tangi, Tango pina, Coca cola (plastic)	7 (41.2)
Customers come with their own types of utensils	3 (17.6)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-15: Reasons for fermenting the beverages (n=114)

Reason for fermenting	Frequency of respondents
Flavor/taste	109 (95.6)
Longer keeping	9 (7.9)
Improved nutritional value	6 (5.3)
Not sure	2 (1.8)
Customers come with their own types of utensils	2 (1.8)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-16: Distribution of source of cooking fuel and cooling facilities for respondent that prepare indigenous cereal based fermented food products (n=117)

Source of cooking fuel	**Frequency of respondents**
Electricity	33 (28.2)
Firewood	23 (19.7)
Charcoal	60 (51.3)
Gas	1 (0.9)
Cooling facility	
Refrigerator	20 (17.1)
Freezer	13 (11.1)
Both refrigerator and freezer	19 (16.2)
None	65 (55.5)

Numbers in brackets represent percentages of respondents

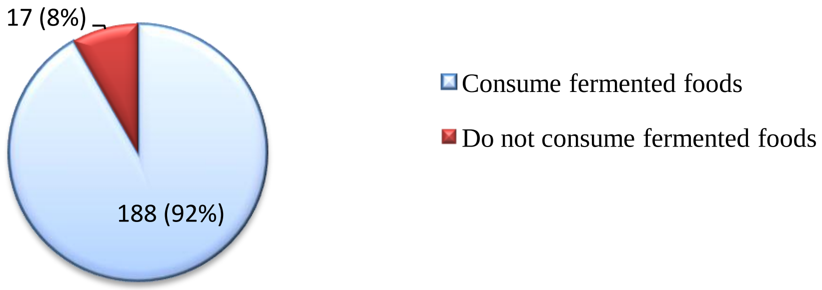

Figure 3-7: Frequency of respondents that consume indigenous fermented cereal based food products

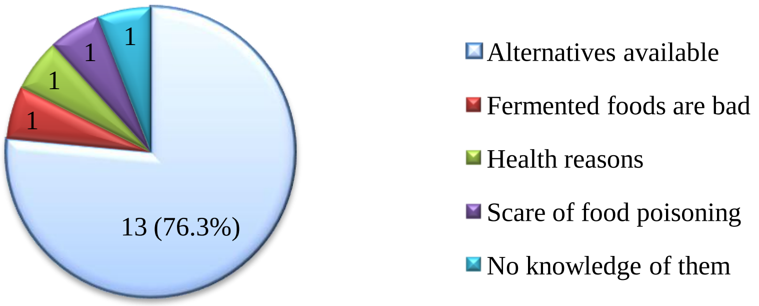

Figure 3-8: Frequency of the respondents who do not consume indigenous cereal based fermented foods or beverages and the reasons for not consuming them

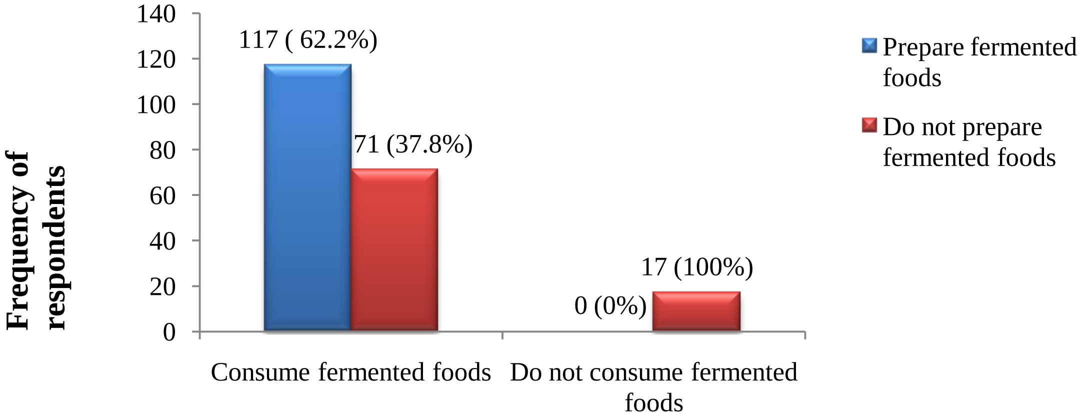

Figure 3-9: Distribution of respondents that consume and prepare indigenous cereal fermented food products

Table 3-17: Frequency of respondent consumption of indigenous cereal based fermented food products

Foods and beverages Consumed	**Frequency of respondents**
Chibwantu	140 (74.5)
Munkoyo	172 (91.5)
Thobwa	142 (75.5)
Mahewu	163 (86.7)
Sour porridge	58 (30.9)
Nshima (from fermented maize meal)	28 (14.9)
Chibuku	48 (25.5)
Seven days (gankata)	4 (2.1)
Katata	4 (2.1)
Kachasu	8 (4.3)
Other opaque commercial beers (Nkwazi, Shake shake, Lusaka and Chat)	6 (3.2)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

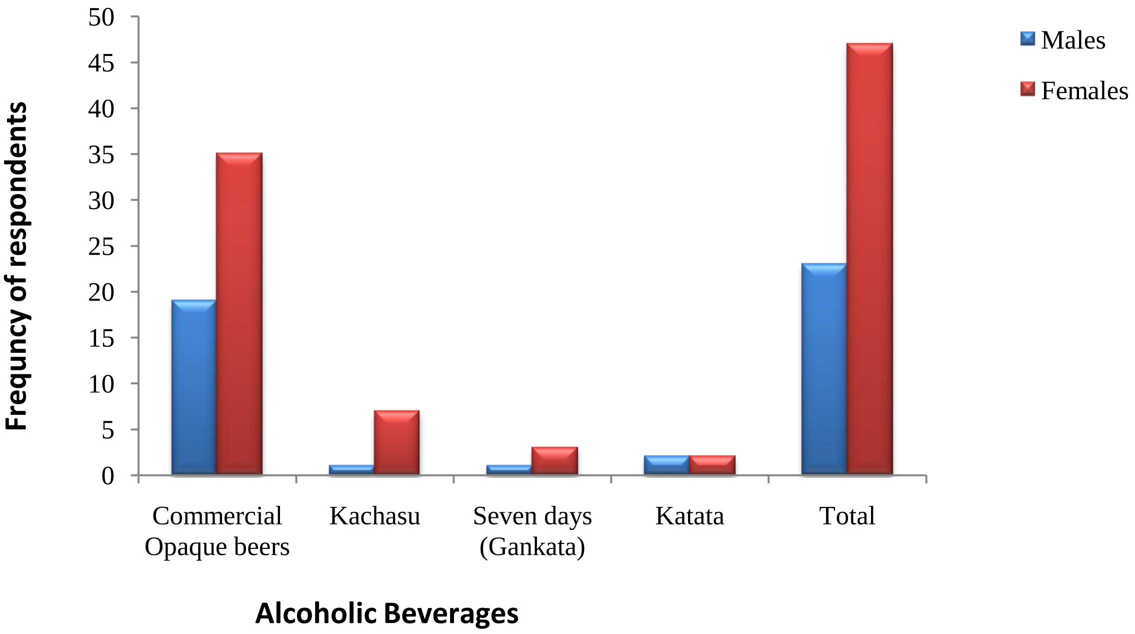

Figure 3-10: Distribution of respondents that consume alcoholic beverages

Table 3-18: Distribution of respondents on the consumption of cereal based fermented food products and the producers of the foods.

Food consumed	**Frequency of respondents**	**Producer**						
		Personal	**Commercial**	**Others**				
				Friends	**Family**	**Church**	**Neighbors**	**Unknown**#
Chibwantu	140	55 (39.3)	45 (32.1)	30 (21.4)	23 (16.4)	2 (1.4)	3 (2.1)	1 (0.7)
Munkoyo	172	99 (57.6)	43 (25)	25 (14.5)	25 (14.5)	2 (1.2)	-	
Thobwa	142	36 (25.4)	50 (35.2)	38 (26.8)	33 (23.2)	6 (4.2)	-	1 (0.7)
Mahewu	163	6 (3.7)	157 (96.3)	3 (1.8)	1 (0.6)	-	-	-
Sour porridge	58	44 (75.9)	2 (3.4)	8 (13.8)	6 (10.3)	-	-	-
Nshima (from fermented maize meal)	27	20 (74.1)	-	2 (7.4)	6 (22.2)	-	-	1 (3.7)
Chibuku	48	-	47 (97.9)	2 (4.2)	-	-	-	-
Seven days (gankata)	4	-	3 (75)	-	1 (25)	-	-	-
Katata	4	2 (50)	1 (25)	1 (25)	1 (25)	-	-	-
Kachasu	8	1 (12.5)	4 (50)	1 (12.5)	-	-	-	-
Other opaque commercial beers (Nkwazi, Shake shake, Lusaka and Chat)	7	-	7 (100)	-	-	-	-	-

Respondents had multiple choices. Numbers in brackets represent percentages of respondents
Unknown producer: beverages consumed at functions such as weddings or whenever the beverage is available

Table 3-19: Frequency of consumption of indigenous cereal based fermented food products

Food product	Frequency of respondents	Frequency of consumption					
		Daily	Twice weekly	Once weekly	Once monthly	Occasionally	Rarely
Chibwantu	139	23 (16.5)	16 (11.5)	14 (10.1)	16 (11.5)	25 (18)	45 (32.4)
Munkoyo	171	39 (22.8)	20 (11.7)	14 (8.2)	32 (18.7)	29 (17)	37 (21.6)
Thobwa	142	4 (2.8)	7 (4.9)	8 (5.6)	14 (9.9)	42 (29.6)	67 (47.2)
Mahewu	158	17 (10.8)	21 (13.3)	20 (12.7)	30 (19)	26 (16.5)	44 27.8)
Sour porridge	58	4 (6.9)	2 (3.4)	2 (3.4)	3 (5.2)	7 (12.1)	40 (69)
Nshima (from fermented maize meal)	28	2 (7.1)	-	-	-	2 (7.1)	24 (85.7)
Chibuku	45	23 (51.1)	6 (13.3)	5 (11.1)	1 (2.2)	5 (11.1)	5 (11.1)
Seven days (gankata)	4	2 (50)	-	-	-	1	1
Katata	3	-	-	-	1	1	1
Kachasu	5	3 (60)	1	-	-	-	1
Other opaque commercial beers (Nkwazi, Shake shake, Lusaka and Chat)	7	2 (28.6)	1 (14.3)	1 (14.3)	-	2 (28.6)	1 (14.3)

Numbers in brackets represent percentages of respondents

Table 3-20: Frequency of respondents on what the fermented foods are mainly consumed for

Food product	**Frequency of respondents**	**Main use**						
		Main meal	**Breakfast meal**	**Snack /and or light meal**	**Weaning food**	**Refreshment**	**Others**	
							To get drunk	**Impress others**
Chibwantu	139	4 (2.9)	14 (10.1)	14 (10.1)	13 (9.4)	139 (100)	-	-
Munkoyo	170	8 (4.7)	22 (12.9)	15 (8.8)	33 (19.4)	167 (98.2)	-	-
Thobwa	142	3 (2.1)	18 (12.8)	14 (9.9)	11 (7.8)	139 (98.6)	-	1 (0.7)
Mahewu	158	2 (1.3)	5 (3.1)	11 (6.9)	27 (17.0)	156 (98.1)	-	-
Sour porridge	59	-	58 (98.3)	5 (8.5)	5 (8.5)	-	-	-
Nshima (from fermented maize meal)	27	27 (100)	-	-	-	-	-	-
Chibuku	46	-	1 (2.2)	2 (4.4)	1 (2.2)	44 (95.7)	2 (4.4)	-
Seven days (gankata)	4	-	-	-	-	4 (100)	-	-
Katata	3	-	-	-	-	2 (66.7)	1 (33.3)	-
Kachasu	5	-	-	-	-	6 (85.7)		
Other opaque commercial beers (Nkwazi, Shake shake, Lusaka and Chat)	7	-	-	-	-	7 (100)	-	-

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Factors that influence selection of indigenous cereal based fermented food products (Table 21-25)

Table 3-21: Influence of price in the selection of the fermented food products

Foods Products	Frequency of respondents	Influence of selection		
		Cheap	Availability	Offered as gift
Chibwantu	138	116 (84.1)	57 (41.3)	39 (28.3)
Munkoyo	170	146 (85.9)	69 (40.6)	49 (28.8)
Thobwa	137	101 (73.7)	51 (37.2)	52 (38)
Mahewu	157	151 (96.2)	51 (32.5)	12 (7.6)
Sour porridge	58	52 (89.7)	28 (48.3)	5 (8.6)
Nshima (from fermented maize meal)	27	22 (81.5)	14 (51.9)	6 (22.2)
Chibuku	44	44 (100)	14 (31.8)	6 (13.6)
Seven days	4	3 (75)	1 (25)	-
Katata	2	2 (100)	-	-
Kachasu	6	6 (100)	3 (50)	-
Others	7	6 (85.7)	5 (71.4)	4 (57.1)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-22: Influence of Sensory properties, health and nutritive value in the selection of the fermented food products

Foods Products	**Frequency of respondents for each food category**	**Influence of selection**		
		Taste/Flavour	**Nutrition**	**Medical reasons**
Chibwantu	136	136 (100)	135 (99.3)	3 (2.2)
Munkoyo	168	168 (100)	166 (98.8)	6 (3.6)
Thobwa	137	137 (100)	136 (99.3)	1 (0.7)
Mahewu	158	158 (100)	157 (99.4)	1 (0.6)
Sour porridge	57	57 (100)	57 (100)	-
Nshima (from fermented maize meal)	26	26 (100)	26 (100)	-
Chibuku	46	46 (100)	46 (100)	-
Seven days	4	4 (100)	4 (100)	-
Katata	2	2 (100)	2 (100)	-
Kachasu	6	6 (100)	5 (83.3)	2(33.3)
Others	5	5 (100)	5 (100)	-

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-23: Influence of ease of preparation in the selection of the fermented food products

Foods Products	**Frequency of respondents**	**Influence of selection**		
		Easy	**Difficulty**	**Complex**
Chibwantu	137	116 (84.7)	18 (13.1)	3 (2.2)
Munkoyo	171	152 (88.9)	15 (8.8)	4 (2.3)
Thobwa	139	84 (60.4)	38 (27.3)	17 (12.2)
Mahewu	156	20 (12.8)	19 (12.2)	117 (75)
Sour porridge	58	53 (91.4)	2 (3.4)	3 (5.2)
Nshima (from fermented maize meal)	27	24 (88.9)	2 (7.4)	1 (3.7)
Chibuku	46	4 (8.7)	12 (26.1)	30 (65.2)
Seven days	3	2	-	1
Katata	3	3 (100)	-	-
Kachasu	6	5 (83.3)	-	1 (16.7)
Others	5	1 (20)	-	4 (80)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-24: Influence of family food habits/ traditions in the selection of the fermented food products

Foods Products	Frequency of respondents	Influence of selection		
		Festive occasions	After childbirth	At certain periods of the day
Chibwantu	136	31 (22.8)	17 (12.5)	120 (88.2)
Munkoyo	167	36 (21.6)	26 (15.6)	147 (88)
Thobwa	136	22 (16.2)	15 (11)	120 (88.2)
Mahewu	157	13 (8.3)	20 (12.7)	145 (92.4)
Sour porridge	54	1 (1.9)	3 (5.6)	52 (96.3)
Nshima (from fermented maize meal)	24	1 (4.2)	1 (4.2)	22 (91.7)
Chibuku	46	19 (41.3)	3 (6.5)	38 (82.6)
Seven days	4	2 (50)	1 (25)	4 (100)
Katata	3	1 (33.3)	-	2 (66.7)
Kachasu	7	5(71.4)	2 (28.6)	2 (28.6)
Others	6	4 (66.7)	1 (16.7)	3 (50)

*Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-25: Category of members consuming each food product

Foods Products	Frequency of respondents	Category of member			
		Babies and Toddlers	Children between 3 and 12 years	Adolescents	Adults
Chibwantu	138	50 (36.2)	105 (76.1)	103 (74.6)	137 (99.3)
Munkoyo	171	62 (36.3)	119 (69.6)	125 (73.1)	170 (99.4)
Thobwa	140	46 (32.9)	102 (72.9)	105 (75)	138 (98.6)
Mahewu	160	61 (38.1)	116 (72.5)	115 (71.9)	159 (99.4)
Sour porridge	59	23 (39)	43 (72.9)	45 (76.3)	58 (98.3)
Nshima (from fermented maize meal)	27	7 (25.9)	23 (85.2)	25 (92.6)	27 (100)
Chibuku	45	-	-	1 (2.2)	45 (100)
Seven days	4	-	-	-	4 (100)
Katata	3	-	-	-	3 (100)
Kachasu	7	-	-	-	7 (100)
Others	7	-	-	-	7 (100)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-26: Storability of the fermented food products consumed

Foods Products	Frequency of respondents	Storability			
		Less than 1 day	1 or 2 days	Few days	Long period
Chibwantu	138	41 (29.7)	32 (23.2)	65 (47.1)	-
Munkoyo	170	45 (26.5)	43 (25.3)	82 (48.2)	-
Thobwa	140	34 (24.3)	36 (25.7)	70 (50)	-
Mahewu	157	43 (27.4)	18 (11.5)	12 (7.6)	84 (53.5)
Sour porridge	58	53 (91.4)	4 (6.9)	1 (1.7)	-
Nshima (from fermented maize meal)	27	25 (92.6)	2 (7.4)	-	-
Chibuku	46	31 (67.4)	5 (10.9)	10 (21.7)	-
Seven days	4	3 (75)	1 (25)	-	-
Katata	3	1	-	2	-
Kachasu	6	2 (33.3)	-	-	4 (66.7)
Others	6	4 (66.7)	-	2 (33.3)	-

Numbers in brackets represent percentages of respondents

Table 3-27: Spoilage characteristics of the fermented food products

		Spoilage characteristics								
					Others					
Food consumed	**Frequency of respondents**	**Change in colour**	**Change in flavour**	**Develop** moulds	**Change in viscosity (watery/light)**	**Slimy**	**Presence of bubbles on top/ Foam**	**See organisms**	**Stops fermenting / becomes bitter**	**No idea/ never seen before/ not sure**
Chibwantu	137	26 (19)	130 (94.9)	41 (29.9)	3 (2.2)	1 (0.7)	2 (1.5)	2 (1.5)*	-	-
Munkoyo	170	29 (17.1)	164 (96.5)	50 (29.4)	1 (0.6)	1 (0.6)	5 (2.9)	1 (0.6)	-	-
Thobwa	138	23 (16.7)	132 (95.7)	39 (28.3)	2 (1.4)	1 (0.7)	3 (2.2)	-	-	1 (0.7)
Mahewu	157	22 (14)	144 (91.7)	37 (23.6)	1 (0.6)	1 (0.6)	-	-	-	7 (4.5)
Sour porridge	59	14 (23.7)	55 (93.2)	25 (42.4)	4 (6.8)	1 (1.7)	-	1 (1.7)**	-	1 (1.7)
Nshima (from fermented maize meal)	26	7 (26.9)	25 (96.2)	19 (73.1)	-	2 (7.7)	-	1 (3.8)***	-	-
Chibuku	42	11 (26.2)	40 (95.2)	9 (21.4)	-	-	-	-	-	-
Seven days (gankata)	4	-	4 (100)	1	-	-	-	-	-	-
Katata	2	-	2 (100)	1	-	-	-	-	1#	-
Kachasu	5	1 (20)	4 (80)	-	-	-	-	-	-	-
Other opaque commercial beers (Nkwazi, Shake shake, Lusaka and Chat)	7	-	3 (42.9)	6 (85.7)	-	-	-	-	1##	-

Respondents had multiple choices. Numbers in brackets represent percentages of respondents
*Maggots, ** Insects, ***Presence of flies, # Stops fermenting, ## Becomes bitter.

Table 3-28: Frequency of respondent on what they do with the spoiled fermented food products (n= 186)

what is done with spoiled fermented food products	**Frequency of respondents**
Consume it	9 (4.8)
Throw it away	180 (96.8)
For livestock feeding	2 (1.1)
Only prepare or purchase adequate amounts to avoid spoilage	63 (33.9)
Others	
Use as manure	1 (0.5)
Add fresh porridge to the spoiled fermented beverage and consume	1 (0.5)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-29: Frequency of respondent on methods of spoilage control of fermented food products (n= 205)

Spoilage control method applied	**Frequency of respondents**
Refrigerator	59 (28.8)
Freezer	29 (14.1)
Submerge under water	18 (8.8)
Place in dry container with low humidity then place the container on cold surfaces or in a cool/ cold place (thatched hut, clay pot)	53 (25.9)
Others	
Give/ share with neighbors the excess	5 (2.4)
Prepare or purchase just enough which will all be consumed or sold (Drink to avoid spoilage)	35 (17.1)
For beverages; remove from calabash to stop further fermentation	5 (2.5)
Submerge a container of cold water into the beverage	1 (0.5)
Put beverages in containers with a jute sack around (keep the jute sack wet and cool)	1 (0.5)
Place ice blocks in the basin used to carry the beverage bottles/ containers (for sell)	2 (1.0)
Only add sugar to the food during consumption	5 (2.4)
Re-cook	1 (0.5)
When the non-alcoholic beverage gets spoiled give to alcoholic beverage (beer) consumer	1 (0.5)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Figure 3-11: Ice blocks placed in a basin with bottles of munkoyo beverage for sell.

Figure 3-12: Munkoyo roots on display at a local market

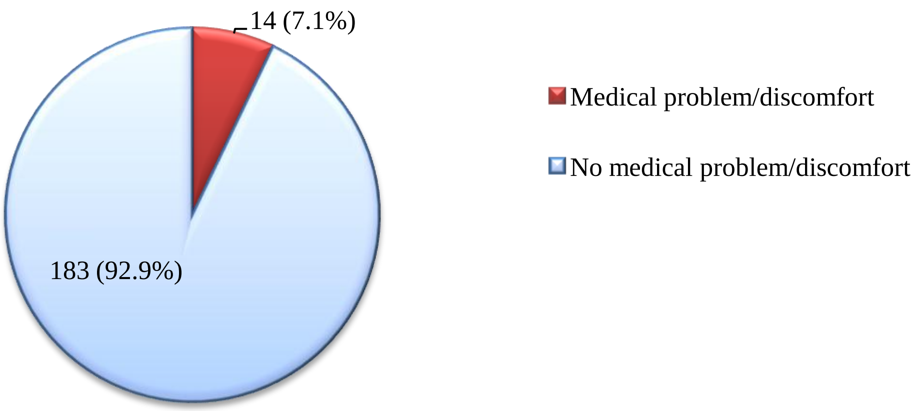

Figure 3-13: Frequency of respondents who develop medical problems / or discomfort after consumption of indigenous fermented cereal based food products

Table 3-30: Frequency of respondents who experience medical problem/ symptoms (n=14)

Medical problem/ symptoms*	**Frequency of respondents**
Diarrhea	6 (42.9)
Stomach cramps	4 (28.6)
Nausea	4 (28.6)
Headache	2 (14.3)
Coughing	1 (7.1)
Fever	-
Poor visibility	-
Time to development of symptoms	
Within 30 minutes of consumption	5 (35.7)
1 hour after consumption	2 (14.3)
3-6 hours after consumption	1 (7.1)
Next day	5 (35.7)
Do not know/ cannot remember	1 (7.1)
Frequency of symptoms	
Often	10 (71.4)
Occasionally	3 (21.4)
Rarely	1 (7.1)
Type of treatment employed*	
Administration of drugs	4 (28.6)
Employ Oral Rehydration Salts (ORS)	1 (7.1)
Others	
Abstain from suspected foods	11 (78.6)
Symptom stops on its own	2 (14.3)
Seek medical attention	1 (7.1)
Category of members involved each time symptoms were observed	
Babies and Toddlers	-
Children between 3 and 12 years old	2 (14.3)
Adolescents	2 (14.3)
Adults	10 (76.9)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

Table 3-31: Frequency of respondents that use fermented food products to treat or prevent ailments (n=205)

Food Products	Ailment treated or prevented	Frequency of respondents
Chibwantu	Diarrhea	1 (0.5)
	Nausea	1 (0.5)
	Lack of appetite	1 (0.5)
Munkoyo	Diarrhea	4 (2.0)
	Yellow fever	3 (1.5)
	Malaria	2 (1.0)
	Nausea	1 (0.5)
	Eye problems	1 (0.5)
	Stomach ache/ cramp	1 (0.5)
	Malnutrition	1 (0.5)
	Lack of appetite	1 (0.5)
	Headache	1 (0.5)
Thobwa	Diarrhea	1 (0.5)
	Lack of appetite	1 (0.5)
Mahewu	Diarrhea	1 (0.5)
	Lack of appetite	1 (0.5)
Opaque beers (chibuku)	Stomach ache/ cramp	1 (0.5)

Numbers in brackets represent percentages of respondents

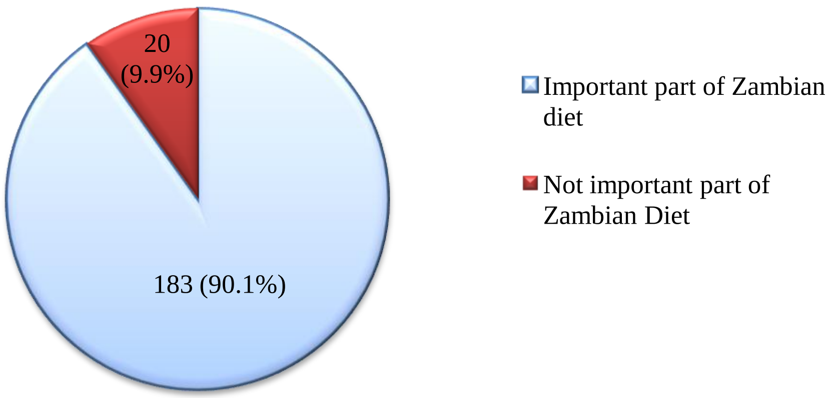

Figure 3-14: Frequency of respondents who think cereal based fermented foods form an important part of the Zambian diet (n=203).

Table 3-32: Indigenous cereal based fermented food products an important part of the Zambia diet

a. The respondents' reasons for thinking indigenous cereal based fermented food products form an important part of the Zambia diet (n=182).

Reasons	**Frequency of respondents**
The foods are nutritious	55 (30.2)
Gives/ source of energy (makes you feel full)	74 (40.7)
Cheaper/ easily sourced source of energy (than foods such as soft drinks)	22 (12.1)
Substitute for other foods (staple foods like nshima, in rural areas packaged foods are not easily seen or found)	57 (31.3)
Supplement other foods	13 (7.1)
Starter meals	9 (4.9)
Refreshment beverages for large gathering, visitors (at weddings, funerals)	10 (5.5)
Easy and cheaper to prepare	4 (2.2)
For the underprivileged (or because of poverty), the foods are affordable and good for their health	7 (3.8)
Helps patients with lack of appetite/ and or nausea (even at hospitals it is encouraged	

to consume these foods)	5 (2.7)
Likable (God given, local foods, from our ancestors)	8 (4.4)
Very natural hence making them very healthy	1 (0.5)
Used for weaning (children easily forget breast feeding)	3 (1.6)
Helps with breast milk production in lactating mothers	1 (0.5)
Beers (such as chibuku) help relieve stress and reduce tempers	2 (1.1)
Non-alcoholic beverages are better than beer	2 (1.1)
Source of national pride	1 (0.5)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

b. The respondents' reasons for thinking indigenous cereal based fermented food products do not form an important part of the Zambia diet (n=18).

Reasons	**Frequency of respondents**
Can do without fermented foods	7 (38.9)
The foods are only taken for refreshment purposes for the fun of it	3 (16.7)
There are a variety of other foods and beverages available making these foods not very important	(3 (16.7)
People just follow the taste/ flavor; the foods are not nutritious (lack vitamins and protein)	2 (11.1)
Rarely consumed in the household	1 (5.6)
Bad because the food products have bacteria	1 (5.6)
Makes you sick	1 (5.6)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

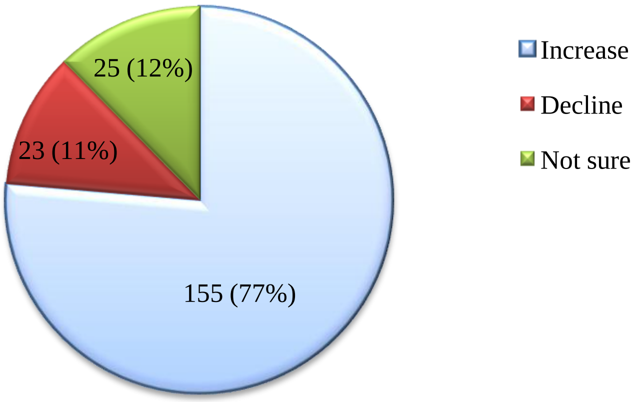

Figure 3-15: Frequency of respondents on trends in the consumption of cereal based fermented food products in Zambian (n=203).

Table 3-33: Trends in the consumption of indigenous cereal based fermented food products in Zambia

a. The respondents' reasons for thinking the trends are on the increase (n=155).

Reasons	**Frequency of respondents**
Traditional/ nice/ pleasant taste and flavour of the foods	47 (30.3)
People have realized how nutritious the foods are (contribute to good health, have lots of nutrients and vitamins)	22 (14.2)
Cheap/ affordable hence many can afford to buy or offer as gift	16 (10.3)
Cheaper and easy to prepare (especially for large gatherings like marriage ceremonies, funeral)	4 (2.6)
Easily accessed/ highly available (including in the local markets) and availability is on the increase (including resources to prepare them)	16 (10.3)
People enjoy the foods (especially the beverages during the hot season)	18 (11.6)
There is an increase in the number of people consuming these types of food	7 (4.5)
People are learning about these foods and are liking them #	5 (3.2)
Give enough energy and are refreshing (more filling especially when beverages are	

compared with soft drinks on the market)	19 (12.3)
People no longer take these foods as traditional foods	2 (1.3)
Supplement other foods (reduce cost of living especially during hunger periods	5 (3.2)
The non-alcoholic beverages are cheaper and replace the need for beer	5 (3.2)
Natural unlike other foods on the market that have side effects	2 (1.3)
Helps when one is not feeling well (lack of appetite/ and or nausea)	3 (1.9)
Convenient meals when people are busy (with field work such as planting, weeding, harvesting)	1 (0.6)
Part of the diets of people	1 (0.6)
Information about their preparation is being passed on from parents to children and the children will continue to prepare these foods	1 (0.6)
The young are joining in drinking the alcoholic beverages (opaque beers) due to lack of recreation, jobs, peer pressure thereby increasing the numbers of consumers	3 (1.9)
Not sure	1 (0.6)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

[#] People from one culture are requesting foods from other cultures and once they taste the different foods, they continue consuming them (especially the non-alcoholic beverages)

b. The respondents' reasons for thinking that the trends are declining (n=23).

Reasons	**Frequency of respondents**
Too many modern foods and drinks on the market and these are used as supplements	10 (43.5)
Lack of people to prepare these fermented foods	9 (39.1)
Less people are consuming these foods	1 (4.3)
People are forgetting their roots	1 (4.3)
A waste of resources for preparing staple food (nshima)	1 (4.3)
People have realized that the food products have bacteria	1 (4.3)
Scared of food poisoning	1 (4.3)

Respondents had multiple choices. Numbers in brackets represent percentages of respondents

c. The respondents' reasons for not being sure of the trends (n=25).

Reasons	**Frequency of respondents**
No interest (do not pay attention at the food products)	4 (16)
Lack of statistical knowledge / have not been observant/ do not know much about these foods	5 (20)
Rarely prepare them hence do not know	3 (12)
Depends on ones traditional background	1 (4)
No idea	1 (4)

Numbers in brackets represent percentages of respondents

Appendix

Appendix 1: Questionnaire for Production and Utilisation of Cereal based Fermented Food Products.

Questionnaire serial number: | | | |

Questionnaire- Production and utilisation of cereal based fermented food products

The purpose of this exercise is to gather information on the production and utilisation of cereal based fermented food products and to establish the relevance of these foods in households and the country at large.

You have been asked to participate in this exercise and please note that by completing this questionnaire you are voluntarily agreeing to participate in this study. You will remain anonymous and all information given will be treated as confidential.

Please mark the appropriate block with an X or write your answer on the space provided.

Section A: Demographic Characteristics

1.0 Name of interviewer

2.0 Date of interview dd/mm/yy | / | / | / |

Respondent

3.0 Gender
(a) Male ☐ (b) Female ☐

4.0 Age in years
(a) 15-20 ☐ (b) 21-30 ☐ (c) 31-40 ☐
(d) 41-50 ☐ (e) 51-60 ☐ (f) Above 60 ☐

5.0 Marital status
(a) Married ☐ (b) Single ☐ (c) Divorced/ separated ☐

(d) Cohabiting ☐ (e) Widowed ☐

6.0 Number of people living in the household: specify……………
(a) 0-2 year | | |
(b) 3-12 year | | |

(c) 13-18 years ☐☐
(d) Above 18 years ☐☐

7.0 Highest level of education
(a) None (did not attend school) ☐ (b) Primary (grade 1-7)
☐
(c) Junior secondary (grade 8-9) ☐ (d) Senior secondary (grade10-12) ☐
(e) College ☐ (f) University ☐

8.0 Employment status
(a) Government / salary worker ☐ (b) Self employed ☐
(c) Allowance (remittance) ☐ (d) unemployed ☐
(e) Others specify………………………

9.0 Monthly Income (Zambian Kwacha)
Specify………………

10.0 Location of Household
(a) Urban ☐ (b) Peri-urban ☐ (c) Rural ☐

11.0 If urban, is it
(a) High density ☐ (b) Medium density ☐ (c) Low density ☐

12.0 Does the household have electricity?
(a) Yes ☐ (b) No ☐

13.0 Household's main source of drinking water
(a) Pipe borne ☐ (b) Borehole ☐ (c) River/ pond/ Dam ☐
(d) Sachet/ bottled/ filtered ☐ (e) Others specify………..

14.0 Household's main source of fuel for cooking
(a) Electricity ☐ (b) Firewood ☐ (c) Charcoal ☐
(d) Gas ☐ (e) Others specify………………..

15.0 Household's cooling facilities
(a) Refrigerator ☐ (b) Freezer ☐ (c) Both Refrigerator and Freezer ☐
(d) Others specify…………….

Section B: Production of cereal fermented foods

16.0 Do you process or prepare cereal based fermented foods or beverages
(a) Yes ☐ (b) No ☐

17.0 If not, indicate the reasons
(a) Difficult to prepare ☐

(b) Lack knowledge on how to prepare them ☐
(c) No time to prepare them ☐
(d) Others specify…………………………………….

18.0 If yes , indicate the types of foods or beverages produced
(a) Chibwantu ☐ (b) Munkoyo ☐ (c) Thobwa ☐
(d) Mahewu ☐ (e) Nshima from fermented maize meal ☐
(f) Sour porridge ☐ (g) Chibuku ☐ (h) Seven days/ Gankata ☐
(i) Katata ☐ (j) Kachasu ☐ (k) Others
specify…………………………………………….

19.0 For the cereal based foods and beverages, indicate the reason for production

	Foods or beverages	Reasons for production			
		Home consumption	Sell	Both home consumption and sell	Others specify

20.0 For the cereal based foods or beverages produced, indicate the frequency of production

	Foods or beverages	Frequency of production		
		often	Occasionally	Rarely

Preparation or production of Chibwantu or Munkoyo

21.0 If you produce or prepare chibwantu or munkoyo, what are the main ingredients used

	Ingredients used in production	
	Munkoyo	Chibwantu
1		
2		
3		
4		

22.0 If you use the munkoyo root, which is the preferred type

(a) Yellow ☐ (b) White ☐

Give reasons for your choice

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………………………………………………………………………..

23.0 Briefly describe the process for preparation of the beverage(s)

……………………………………………………………………………………………

……………………………………………………………………………………………

…………………………………………………………………………………………….

24.0 How do you use the roots

(a) Add the roots directly to the warm porridge ☐
(b) Soak the roots; indicate how long (hours or days)………… ☐
(c) Add only the soaked roots ☐
(d) Add only the root extract ☐
(e) Add both the soaked roots and the root extract ☐

25.0 What is the source of the munkoyo root

(a) Buy from market ☐ (b) collect from the forest ☐
(c) Ask from family/ friends ☐ (d) Others
specify……………………………………..

26.0 What type of utensils do you use for the fermentation process of the beverage(s)

(a) Calabash ☐ (b) Plastic bucket ☐ (c) Metal bucket ☐
(d) Others specify…………………………………………….

27.0 How do you ferment the beverages

(a) In the cooling facility; specify……………… ☐
(b) In the house in open space ☐
(c) Outside the house in the sun ☐
(d) Outside the house in shade ☐
(e) Others specify……………………………………….

28.0 If you produce chibwantu or munkoyo for sell, how do you package the beverage before sell?

(a) In bucket using a measuring cup ☐
(b) In used water bottles ☐
(c) Others specify ☐

29.0 What are your main reasons for fermenting the foods or beverages before consumption

(a) Longer keeping ☐ (b) Flavour/ taste ☐
(c) Improved nutritional value ☐ (d) Detoxifying ☐
(e) Others specify…………………………………………………….

Section C: Consumption of cereal fermented foods

30.0 Do you consume cereal based fermented foods and beverages
(a) Yes ☐ (b) No ☐

31.0 If no, indicated reasons (and proceed to question 41)
(a) Alternatives available such as tea/ soft drinks ☐
(b) Fermented foods are bad ☐
(c) For health reasons ☐
(d) Others specify……………………………………

32.0 If yes , indicate the types consumed
(a) Chibwantu ☐ (b) Munkoyo ☐ (c) Thobwa ☐
(d) Mahewu ☐ (e) Nshima from fermented maize meal ☐
(f) Sour porridge ☐ (g) Chibuku ☐ (h) Seven days/ Gankata ☐
(i) Katata ☐ (j) Kachasu ☐ (k) Others
specify……………………..

33.0 Of the food products selected above indicate the producer

		Producer		
	Food product	Personal	Commercial	Others specify

34.0 Of the food products selected above indicate the frequency of consumption

		Frequency of consumption					
	Food product	Daily	Twice weekly	Once weekly	Once monthly	Occasionall y	Rarel y

35.0 Of the food products selected indicate what they are mainly consumed for

		Main use					
	Food product	Main meal	Breakfast meal	Snack /and or light meal	Weaning food	Refreshment beverage	Others specify

36.0 What factors do you think influence the selection of the fermented foods and/or beverages you consume

Factors influencing selection of food		Food product							
Price	Availability in nature/ season								
	Cheap								
	Offered as gift								
Health and nutritive value	Nutritious								
	Medical reasons								
Sensory properties	Taste/ flavor								
Ease of preparation	easy								
	complex								
	difficult								
Family food habits/ tradition	On festive occasions								
	After childbirth								
	At certain periods of the day								
Storability	Less than a day								
	1 or 2 days								
	Few days								
	Long period								

37.0 Of the food products selected above indicate the category of members consuming each food product or beverage

		Category of member			
	Food product	Babies and Toddlers	Children between 3 and 12 years	Adolescents	Adults

Spoilage characteristics

38.0 How do you know when the Cereal based fermented food or beverage is spoiled

	Food product	Spoilage characteristics			
		Changes in color	Changes in Flavor: taste and/ or aroma	Develop mold	Others Specify

39.0 What do you do with the spoiled food or beverage
(a) Consume it ☐ (b) Throw it away ☐ (c) Livestock feeding ☐
(d) Only prepare or purchase adequate amounts to avoid spoilage ☐
(e) Others specify……………………

40.0 What methods of prevention or control of spoilage of cereal based fermented foods and beverages do you apply?
(a) Refrigerator ☐ (b) Freezer ☐ (c) Submerge under water ☐
(d) In dry container with low humidity ☐ (e) Salting ☐
(f) Sun drying ☐ (g) Others specify ☐

Medical issues

41.0 Do you or any other member of your household experience any medical problem or discomfort after consumption of cereal based fermented foods or beverages?
(a) Yes ☐ (b) No ☐

42.0 If yes, indicate the problem or symptoms
(a) Fever ☐ (b) Diarrhea ☐ (c) Stomach cramps ☐
(d) Nausea ☐ (e) General body malaise☐ (f) Poor visibility ☐
(g) Others specify……………………………….

43.0 From the time of consumption, how long does it take before developing symptoms
(a) Within 30 min of consumption ☐ (b) 1hr after consumption ☐
(c) 3-6 hrs after consumption ☐ (d) 12 hrs after consumption ☐
(e) Next day ☐

44.0 What is the frequency of occurrence of discomfort or symptoms

(a) Often ☐ (b) Occasionally ☐ (c) Rarely ☐

45.0 What type of treatment is employed to take care of the discomfort or symptoms

(a) Administration of drugs ☐ (b) Employ the Oral rehydration therapy (ORS) ☐

(b) Use other foods, vegetables ☐ (d) abstain from suspected food products ☐

46.0 Which category of members in the household were involved each time discomfort or symptoms were observed

Number of members affected at any time	Category of member			
	Babies and Toddlers	Children between 3 and 12 years	Adolescents	Adults
1				
2				
3				
4				
All				

47.0 Of the cereal based fermented foods or beverages consumed, are there any used to treat or prevent any ailments? If so indicate food and the ailment treated or prevented

Food	Ailment treated or prevented

Importance of Cereal based fermented foods and beverages

48.0 Do you think cereal based foods form an important part of the Zambian diet?

(a) Yes ☐ (b) No ☐

Explain your reasoning

……………………………………………………………………………………………

……………………………………………………………………………………………

49.0 What do you think is the trend in consumption of cereal based fermented foods and beverages in the country

(a) It is on the increase ☐ (b) It is declining ☐ (c) Not sure ☐

………

Thank you for your patience and valuable contribution

Chapter 4

Microorganisms associated with the munkoyo roots and maize grit used in the preparation of a Zambian beverage- Chibwantu

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Chapter 4

4 Microorganisms associated with the Munkoyo Roots and Maize grit used in the preparation of a Zambian beverage- Chibwantu

Abstract

Microorganisms associated with plant roots are found in the rhizosphere where there is high and complex microbiological activity. The microbial population associated with roots is diverse qualitatively and quantitatively. The rhizophylic bacteria associated with the roots produce various hydrolytic enzymes that are involved in the conversion of complex carbon sources such as starch, cellulose, casein, urea and gelatin.

There is no published work available on microorganisms associated with *Rhynchosia* spp (or possibly *Eminia* spp) roots that are used during preparation of chibwantu beverage. Several studies showed that medicinal plant roots are associated with bacteria, actinomycetes and fungi and similar microorganisms are expected to be associated with *Rhynchosia* spp roots. These microorganisms could play a role in the fermentation and spoilage of the beverage. Chibwantu is a non-alcoholic beverage produced from maize grit and the root (*Rhynchosia insignis* and/or *Rhynchosia heterophylla* or *Rhynchosia venulosa*).

Samples of the munkoyo roots and maize grit were purchased from different market sites in Zambia. The microbiological quality of all the samples was investigated and Lactic Acid Bacteria (LAB) and yeast and moulds associated with the maize grit and munkoyo roots (yellow and white types) were isolated and identified to species level. The total aerobic count for the maize grit ranged from 1.98 to 3.43 log cfu/g with mean count of 2.91 ± 0.44 log cfu/g, yellow root ranged from 2.70 to 8.93 log cfu/g, with mean count of 6.12 ±1.82 log cfu/g and white root from 4.48 to 8.46 log cfu/g with mean count of 7.02 ± 1.28 log cfu/g.

The isolated LAB were identified as *Enterococcus gallinarum* (89%) and *Enterococcus casseliflavus* (11%) from the maize grit, *Enterococcus faecalis* (71%) and *Enterococcus casseliflavus* (29%) from the yellow munkoyo roots and *Enterococcus casseliflavus* (100%) from the white munkoyo roots. Yeasts and moulds were identified as *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, *Aspergillus oryzae*, *Fusarium* species, *Lichtheimia ramosa*, and *Acremonium* cf. *zeae* from maize grit. *Wickerhamomyces anomalus*, *Cryptococcus albidus*, *Cryptococcus laurentii*, *Cryptococcus liquefaciens*, *Clavispora lusitaniae*, *Yarrowia lypolitica*, *Aspergillus oryzae*, *Fusarium annatatum* and *Fusarium* species from the yellow root and *Wickerhamomyces anomalus, Cryptococcus liquefaciens, Cryptococcus laurentii*, *Rhodotolula mucolaginosa*, *Aspergillus oryzae*, *Aspergillus* species, *Fusarium* species, *Penicillium citrinum*, *Penicillium* species, *Lichtheimia ramosa*, *Scopulariopsis brevicaulis* from the white root. *Wickerhamomyces anomalus* was the dominant yeast species for both yellow and white munkoyo roots.

The high aerobic microbial counts indicates that the root contribute to the fermentation process as well as final microbial quality of the cereal beverage. The different species of yeast indicate that they contribute to the beverage becoming alcoholic after 48 hours of fermentation.

Key Words: Microorganisms, Munkoyo roots, Chibwantu, Maize grit, Zambia

4.1 Introduction

Microorganisms associated with the plant roots are found in the rhizosphere. The rhizosphere is the region of contact between root and soil where the soil is affected by roots (Damle and Kulkarni, 2012). The rhizosphere is where the microorganisms are closely associated with the roots and is the area of intense microbiological activity (Damle and Kulkarni, 2012). When the root is uprooted from the soil, the microorganisms remain adhering to the root.

The microbial (bacteria, actinomycetes and fungi) population associated with roots is diverse qualitatively and quantitatively (Tsavkelova *et al*., 2007; Tamilarasi *et al*., 2008; Damle and Kulkarni, 2012; Ramesh *et al*., 2012). Studies by Tamilarasi *et al*. (2008) and Ramesh *et al*. (2012) found that bacterial population was higher in the entire root zone of medicinal plants followed by fungal populations. *Pseudomonas* was highest (57%) in comparison to other genera (*Klebsiella*, *Xanthomonas*, *Bacillus*, *Erwinia* and *Arthrobacter*) in the rhizosphere of the plant velarian (*Velariana officinalis*) a perennial herb used as medicinal plant (Ghodsalavi *et al*., 2013). Tamilarasi *et al*. (2008) isolated various bacteria from rhizosphere of 50 medicinal plants which among the isolated bacteria the dominant species was *Bacillus*, followed by *Pseudomonas*, *Enterobacter, Corynebacterium*, *Micrococcus* and *Serratia*. Among the fungal isolates, from the study by Tamilarasi *et al*. (2008). *Rhizopus* was found to be higher in number followed by *Aspergillus*, *Penicillium*, *Mucor* and *Fusarium*. The population of microorganisms in the rhizosphere differs from one plant to another (Tsavkelova *et al*., 2007; Damle and Kulkarni, 2012).

The difference in microbial population in the rhizosphere is influenced by the root exudates (Rovira, 1956; Damle and Kulkarni, 2012; Kumar *et al*., 2012; Nallanchakravarthula, S. 2013). Root exudates are defined as those substances which are released into the surrounding medium by healthy and intact plant roots (Rovira, 1969). The substances include sugars, amino acids, peptides, vitamins, organic acids, nucleotides, fatty acids, sterols, enzymes, fungal stimulators and inhibitors and other unknown compounds (Rovira, 1956; Rovira, 1969; Oburger *et al*., 2013). Studies have also shown that rhizophylic bacteria produce various hydrolytic enzymes that are involved in the conversion of complex carbon sources such as starch, cellulose, casein, urea and gelatin (Tamilarasi *et al*., 2008; Damle and Kulkarni, 2012; Kumar *et al*., 2012; Ghodsalavi *et al*., 2013).

Chibwantu is a Zambian fermented beverage produced at household level with traditional techniques and consumed as major breakfast for the whole family, in between meals, during ceremonies and during field works such as planting, weeding and harvesting. It is prepared from maize grit and the root *Rhynchosia insignis* and/or *Rhynchosia heterophylla* (Zulu *et al*., 1997) or *Rhynchosia venulosa* (Simwamba and Elahi, 1986).

The *Rhynchosia* spp plant is a subshrub (Fig. 4-1) found as an under storey plant in the miombo woodland forests of Zambia. The root is tuber-like and fibrous (FAO, 1993) (Fig. 4-2), yellow or white in colour and is generally called the yellow or white munkoyo by the local people. The munkoyo root, after digging it from the ground, is debarked, beaten into fibrous strands, dried and stored uncovered prior to use. There is inadequate information on the species of the munkoyo roots frequently used in different parts of the country. This study showed that majority (76.9%) in Lusaka and Chongwe districts of Lusaka province prefer to use the yellow type of munkoyo.

The first step in preparation of the chibwantu beverage is to prepare the thin or thick maize grit porridge. Munkoyo roots and/or extract are added to the warm porridge and left to ferment due to naturally occurring microorganisms. The microorganisms are inherent in the raw materials (roots and maize grit) and/or are present on utensils (gourds, earthenware pots, plastic and metal or plastic buckets) used in the preparation of the beverage and/or originate from the surrounding environment. Maize grit is a coarse maize meal from dried ground maize (Fig. 4-3). The consequence of cooking the maize grit first is that indigenous enzymes (e.g. amylases) and many of the microorganisms present in the maize grit are inactivated before the fermentation process. The munkoyo root is most probably added as a source of the right type of the microbiota, fermentable carbohydrate or amylolytic enzymes. Fermentation occurs at ambient temperatures (25-30^{o} C) for 18 – 48 hours.

Munkoyo roots are sold in open air markets and without any form of packaging. This way of selling the roots exposed to unhygienic selling tables and dust in the environment (Fig. 4-2B) could be a source of spoilage and pathogenic microorganisms (microbial hazards) for the food products (beverages). Since the beverages produced are a product of spontaneous fermentation, the origin and types of associated microorganisms are diverse and consequently, establishment of undesirable microorganisms that may be potentially pathogenic cannot be ruled out, through microbial succession (Sanni *et al*., 1999).

Spoilage of foods and beverages is the result of microbial activity of a variety of microorganisms (including bacteria, yeast and moulds) (Filtenborg *et al*., 1996; Jos, 1996). These microorganisms originate from varied environments such as plants, animal products, soil, water and insects. Yeasts and moulds are relatively tolerant to low pH, low water activity, low temperature and other compounds (e.g. preservatives) considered to have an inhibitory effect on the growth of many microorganisms (Jos, 1996). Growth of spoilage microorganisms may result in several kinds of food spoilage: off-flavours, toxins, discoloration and rotting. The deterioration of sensory properties is due to the production of exoenzymes during growth. The most important aspect of mould spoilage is however the formation of mycotoxins that could be harmful to the consumers (Filtenborg *et al*., 1996). If the mycotoxins are produced on the munkoyo roots before use, the mycotoxins could possibly be found in the food products. The shelf life of chibwantu is short and the beverage undergoes deterioration within 48 hours of production (Zulu *et al*., 1997). The short shelf life of chibwantu, therefore, could possibly be due to the spoilage microorganisms associated with the munkoyo roots.

There is a commercial brand of cereal based fermented beverage on the market called mahewu which is similar to chibwantu. Due to economic pressures, those that know how to prepare chibwantu do it at home and sell it at local markets in used soft drink plastic bottles (that is used bottles of Havana cola, Tangi, Tango pina, coca cola) and used water bottles implying that there is still a demand for the traditionally prepared beverage (Fig. 4-4).

There is no published work available on microorganisms associated with *Rhynchosia* spp (or possibly *Eminia* spp) roots that are used during preparation of chibwantu beverage. Association of bacteria, actinomycetes and fungi with *Rhynchosia* spp roots is likely and these microorganisms could play a role in the fermentation or spoilage of beverages. This study was conducted to isolate, characterise and identify LAB, coliforms, yeasts and moulds associated with the munkoyo root used during preparation of chibwantu beverage.

4.2 Materials and Methods

4.2.1 **Samples**

The samples, maize grit (300 - 600 g) and munkoyo root bunches (100 - 150 g), were purchased and collected at market sites (Soweto and City) in Lusaka, Livingstone and Chingola. Samples of the maize grit and munkoyo roots (white and yellow types) were collected in the bags offered at the market, by the traders, after purchase and the bags placed in sterile sampling bags.

4.2.2 **Microbiological analyses**

Representative 25 g (BAM, 1995) of the 12 maize grit samples as well as 20 of the munkoyo root samples (10 yellow and 10 white types) were aseptically weighed and mixed (blended) with 225 ml of buffered peptone water to have a 10^{-1} dilution. Serial decimal dilutions were prepared with buffered peptone water and duplicate counting plates were prepared of all appropriate dilutions, by surface plating.

4.2.2.1 Enumeration and isolation of microorganisms

Plate Count Agar (Merck, Gauteng, South Africa) was used for the enumeration of total aerobic mesophilic bacteria. The plates were incubated at 30^{o}C for 48 hours. De Man, Rogosa and Sharpe (MRS) agar (Merck, Gauteng, South Africa) was incubated anaerobically in anaerobic jars with gas generating kits (Microbiology Anaerocult. Merck, Darmstadt, Germany) at 30^{o}C for 48 hours and M17 agar (Oxoid, England) incubated aerobically at 30^{o}C for 48 hours for the enumeration and isolation of LAB. Violet Red Bile Agar (VRBA) (Merck, Gauteng, South Africa) incubated aerobically at 30^{o}C for 24 hours for enumeration of total coliforms and Violet Red Bile Agar - 4 - methylumbelliferyl-ß-D-glucuronide (VRBA-MUG) (Merck, Gauteng, South Africa) incubated aerobically at 37^{o}C for 24 hours for enumeration of *E. coli.* For confirmation of coliforms, representative colonies were inoculated into Brilliant Green Lactose Bile Broth (BGLB) and incubated at 35^{o}C. Examination of tubes was done at 24 hours and 48 hours. Colonies producing gas in this broth were confirmed as coliforms. Confirmation of *E. coli* was done by observing for fluorescent colonies under longwave UV light (BAM, 1995), and suspected colonies were inoculated into tryptone water and incubated at 37^{o}C for 48 hours. Kovacs' reagent was used to detect the production of indole from tryptophan. Baird-Parker Agar (Merck, Gauteng, South Africa), supplemented with egg-york tellurite emulsion (5% v/v) incubated at 37^{o}C for 48 hours for the enumeration of *Staphylococcus aureus*. Rose Bengal Cloramphenicol Agar (RBCA) (Merck) incubated at 30^{o}C for 72 hours for enumeration and isolation of yeasts and moulds.

Isolates of microorganisms were obtained randomly from selected plates of MRS, M17 and RBCA and cultivated in MRS broth, M17 broth and Yeast Malt (YM) broth respectively. Purity was checked by successive streaking on the same media and stored on slants (of the same media) at 4^{o}C for further investigation.

4.2.2.2 Isolation and Identification of Lactic Acid Bacteria (LAB) to Genus Level

For the identification of LAB, 18 – 24 hours culture of the isolates from MRS and M17 agar slants were characterized by microscopic examination, conventional biochemical and physiological tests. The isolates were examined for colony and cell morphology and cell arrangement. All isolates were tested for gram reaction and the catalase enzyme production according to the methods described by Harrigan and MacCance, 1976. Gram positive and catalase negative isolates were then examined for production of gas (carbon dioxide) from glucose using MRS broth containing 5% glucose with inverted Durham tubes, growth at different temperatures 10^{o}C, 15^{o}C, 40^{o}C and 45^{o}C, tolerance of different salt levels (2%, 4% and 6.5%), production of ammonia from arginine and reduction of litmus before clotting in litmus milk (Harrigan and MacCance, 1976; Axellssion, 1993; Savadogo *et al*., 2004). After identification all gram positive and catalase negative isolates were cultivated in MRS broth with 10% glycerol for 18 hours at 30^{o}C and stored at -21^{o}C for further use.

4.2.2.3 Identification of the lactic acid bacteria to species level

Pure isolates that were gram positive and catalase negative; 9 isolates from maize grit, 7 from the yellow munkoyo roots and 5 from the white munkoyo roots were sub-cultured on the same media from which they were isolated MRS and M17 for identification to species level.

4.2.2.4 Identification of coliforms from munkoyo roots

For the isolates that were confirmed as coliforms from Violet Red Bile Agar (VRBA) (Merck, Gauteng, South Africa) and Brilliant Green Lactose Bile Broth (BGLB), they were sub-cultured onto Xylose Lysine Deoxycholate (XLD) agar and Brilliant Green Agar and incubated at 37^{o}C for 24 hours for differential identification. Pure cultures were prepared.

4.2.2.5 Isolation and Identification of Yeast and Moulds

For identification of yeast and moulds, colonies were examined for their colony color, shape and texture. Colonies with different colony morphology were randomly picked from selected RBCA plates. For purification a small portion of the suspected colony type was suspended into a tube of sterile distilled water and a loop full of the suspension was streaked onto the RBCA plate. The plates were incubated at 30^{o}C for 48 hours. The pure cultures were sub-cultured onto YM agar slants and stored at 4^{o}C.

4.2.3 Identification of Microorganisms to species Level

For the identification of the microorganisms (LAB, coliforms, yeasts and moulds) the pure isolates were sequenced by Ingaba Biotech. The results on the sequences obtained from Finch TV (http://www.geospiza.com/ftvdlinfo.html) were aligned against those for the NCBI (National Center for Biotechnology Information) databases using BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify homologous (very similar) sequences.

4.2.4 Statistical Analysis

The data obtained were analyzed using one way analysis of variance (IBM SPSS Statistics 21)

4.3 Results and Discussion

4.3.1 Enumeration of microorganisms

4.3.1.1 Maize Grit

The mean count on M17 agar were 2.98 ± 0.51 log cfu/g higher than on MRS agar (2.71 ± 0.40 log cfu/g) and PC agar (2.91 ± 0.44 log cfu/g) indicating the predominance of presumptive *Lactococci*. Mean count on VRB agar was 1.41 ± 1.30 log cfu/g. Mean count on RBC agar was 1.76 ± 0.99log cfu/g. The mean counts were higher than on VRB agar (for coliforms) but lower than on PCA, MRS and M17 agar.

Table 4-1 summarizes the mean counts obtained from the maize grit purchased at local markets in Lusaka, Zambia.

E. coli, *Salmonella* species and *Staphylococcus aureus* were not detected in all the samples examined. The presence of coliforms is an indication that there is likely contamination of the grit during processing and handling at the markets. However, the maize grit is cooked before use during the preparation of chibwantu indicating that very few microorganisms would find their way into the beverage. The concern is cross contamination during preparation of the beverage.

4.3.1.2 Munkoyo Roots

For all the parameters examined; Total aerobic mesophylic bacteria, LAB, coliforms and yeast and moulds, mean counts from the white munkoyo root were higher than from the yellow munkoyo root except for the coliforms as shown in Table 4-2. However, the differences were not significant at $p < 0.001$. The ranges of counts (log cfu/g) for the yellow munkoyo roots were higher than the ranges for the white munkoyo roots. Mean counts on M17 agar for both types of roots were (yellow 5.91± 1.59 log cfu/g and white 6.73 ± 0.97 log cfu/g) higher than the mean counts on MRS agar (yellow 5.56 ± 1.47 log cfu/g and white 6.47 ± 0.90 log cfu/g). The presence of coliforms (with a range of 2.48 log cfu/g to 8.50 log cfu/g) for both types of the munkoyo root, higher than 2.00 log cfu/g is an indication that there is likely contamination of the products with enteric pathogens. In addition, coliforms may influence other characteristics such as sensory attributes of the fermented food. Mbugua (1982) reported that coliforms resulted in the production of off-flavors and instability in Kenyan uji, a fermented maize based product. Since the munkoyo root undergoes minimal processing after its dug from the ground, it signifies that it could still harbor significant amounts of soil microorganisms with characteristics similar to those of coliforms and thereby responsible for the rapid spoilage of the beverage. Despite the high counts of coliforms from both types of the munkoyo root, no *E. coli*, *Staphylococcus aureus* or *Salmonella* species were detected from all the samples.

The mean count on RBC agar for both types of roots was 4.12log cfu/g lower than the counts on PC, MRS, M17 and VRB agar. The munkoyo roots are soaked in a small amount of water for up to approximately one hour and the munkoyo roots and/or extract are then added to the warm porridge and left to ferment spontaneously (Lovelace, 1977; Simwamba, 1986; Zulu *et al*., 1997). This suggests that all the microorganisms (LAB, coliforms, yeasts and moulds and other soil bacteria) on the roots play a role in the spontaneous fermentation of the beverage. The fermentation conditions for production such as temperature, humidity and aeration are often not optimized (Odunfa, 1988) and the ranges of the microorganisms on the munkoyo roots vary significantly, thus implying that the products prepared from these munkoyo roots are expected to be of varying quality.

4.3.2 **Identification of Lactic Acid Bacteria**

The phenotypic characteristics of the 21 LAB isolates (from both maize grit and munkoyo roots) suggest their resemblance to *Enterococcus* species. All the isolates were similar in their physiologic characteristics with the exception of their cellular arrangement (Table 4-3). The isolates were gram positive, catalase negative cocci which produced no gas from glucose. The microorganisms were all able to grow at different temperatures and levels of salt concentration, produced ammonia from arginine and there was no clotting or color change in litmus milk.

LAB isolates were identified as *Enterococcus gallinarum* (89%) being the dominant species and *Enterococcus casseliflavus* (11%) from the maize grit. *Enterococcus faecalis* (71%) being the dominant species and *Enterococcus casseliflavus* (29%) from the yellow munkoyo root. *Enterococcus casseliflavus* (100%) was the only LAB species identified from the white munkoyo root.

4.3.3 Identification of coliforms

The coliforms associated with the munkoyo roots are either *Enterobacter* species and/or *Klebsiella* species or with other unidentified coliforms.

4.3.4 Yeasts and moulds

The yeasts were identified as *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* from maize grit, *Wickerhamomyces anomalus*, *Cryptococcus liquifaciens*, *Cryptococcus laurentii*, *Cryptococcus albidus*, *Yarrowia lypolytica*, *Clavispora lusitaniae* and *Sporobolomyces nylandii* from the yellow munkoyo roots and *Wickerhamomyces anomalus*, *Cryptococcus liquifaciens*, *Cryptococcus laurentii*, *Pichia fabianii*, and *Rhodotolula mucilaginosa* from the white munkoyo roots (Table 4-4).

The moulds were identified as *Aspergillus oryzae*, *Fusarium* species, *Lichtheimia ramosa* and *Acremonium* cf. *zeae* from the maize grit. *Aspergillus oryzae*, *Fusarium annatatum* and *Fusarium* species from the yellow roots. *Aspergillus oryzae*, *Aspergillus* species, *Fusarium* species, *Penicillium citrinum*, *Penicillium* species, *Lichtheimia ramosa*, *Scopulariopsis brevicaulis* from the white roots. The summary is shown in Table 4-5. Among the yeast and moulds from the maize grit, *Saccharomyces cerevisiae* was the dominant accounting for >80% occurrence and the smaller percentage occurrence accounted for *Wickerhamomyces anamalus* and small amount of moulds. From the yellow munkoyo roots *Wickerhamomyces anamalus* was dominant accounting for > 80% occurrence. The yellow root had much diverse yeast occurrence compared to the white root. Only *Aspergillus oryzae* and few *Fusarium* species are found on the yellow root. From the white munkoyo roots *Wickerhamomyces anamalus*, *Cryptococcus liquefaciens* and *Cryptococcus laurentii* together accounted for >85% occurrence of all the yeast and moulds. The white root had much diverse mold occurrence compared to the yellow root.

The diverse species of yeast associated with munkoyo roots indicate that they contribute to chibwantu beverage becoming alcoholic after a longer time (approximately more than 24 hours) of fermentation, thus contributing to the short shelf life (about 48 hours) of the beverage.

4.4 Conclusions

The raw materials maize grit and munkoyo root, used in the preparation of the beverage- chibwantu consist of coliforms, lactic acid bacteria- genus *Enterococcus*, and yeast and moulds. Coliforms, LAB and yeast and mould mean counts were significantly lower for maize grit compared to munkoyo roots at $p < 0.001$. No pathogenic bacteria tested for were found on the raw materials.

The information obtained from the study demonstrates that there is a diversity of microorganisms on the raw materials used in the preparation of chibwantu. Since the maize grit is cooked prior to fermentation, thus inactivating much of the microorganisms, it can be concluded that munkoyo root contributes a diverse type of microorganisms responsible for the fermentation and resulting quality of the beverage-chibwantu. The aim of the next chapter is to examine the microbial changes and to isolate, characterise and identify the essential microorganisms involved during the fermentation process of chibwantu.

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Table 4-1: Microbiology of maize grit used in the preparation of chibwantu

	Maize Grit	
Medium	Ranges of counts for all samples	Mean counts for all samples with standard deviation
PC agar (Total aerobic mesophilic bacteria	1.98 - 3.43	2.91 ± 0.44
MRS agar (*Lactobacilli*)	2.0 – 3.28	2.71 ± 0.40
M17 agar (*Lactococci*)	2.3 – 3.73	2.98 ± 0.51
VRB agar (coliforms)	0.0 – 3.23	1.41 ± 1.30
RBC agar (yeast and moulds)	0.0 – 3.56	1.76 ± 0.99
XLD (*Salmonella*)	ND	
VRB – MUG agar (*E.coli*)	0.0 – 0.0	0.0
Baird-Parker agar (*Staphyloccocus aureus*)	0.0 – 0.0	0.0

Counts for all samples were in Log cfu/g, ND: Not Detected

Table 4-2: Microbiology of Munkoyo roots (yellow and white) used in the preparation of chibwantu

	Both Roots (counts for all samples)		**Yellow Munkoyo Root** (counts for all samples)		**White Munkoyo Root** (counts for all samples)	
Medium	Ranges	Mean with standard deviation	Ranges	Mean with standard deviation	Ranges	Mean with standard deviation
PC agar (Total aerobic mesophilic bacteria	2.70-8.93	6.57 ± 1.76	2.70-8.93	6.12 ± 1.82^{a}	4.48-8.46	7.02 ± 1.28^{a}
MRS agar (*Lactobacilli*)	2.70-8.66	5.98 ± 1.46	2.70-8.66	5.56 ± 1.47^{a}	4.70-7.57	6.47 ± 0.90^{a}
M17 agar (*Lactococci*)	2.60-8.49	6.32 ± 1.55	2.60-8.49	5.91 ± 1.59^{a}	4.78-8.0	6.73 ± 0.97^{a}
VRB agar (coliforms)	2.48-8.50	5.83 ± 1.58	2.48-8.50	5.83 ±1.84^{a}	3.48-6.83	5.83 ± 0.95^{a}
RBC agar (yeast and moulds)	2.00-5.99	4.12 ± 1.10	2.00-5.99	3.99 ± 1.22^{a}	2.95-5.08	4.24 ± 0.75^{a}
XLD (*Salmonella*)	ND					
VRB – MUG agar (*E.coli*)	0.0 – 0.0	0.0	0.0 – 0.0	0.0	0.0 – 0.0	0.0
Baird-Parker agar (*Staphyloccocus aureus*)	0.0 – 0.0	0.0	0.0 – 0.0	0.0	0.0 – 0.0	0.0

Counts for all samples were in Log cfu/g, ND: Not Detected. Means with different superscripts in the same row differ significantly. Significance level $p < 0.001$

Table 4-3: Characteristics of the LAB isolated from Maize grit and Munkoyo root (yellow and white)

a. Genus Level

Gram		+	+
Catalase		-	-
Cell form		spherical (cocci)	spherical (cocci)
Cellular arrangement		Singles, pairs and chains	Bunches, few pairs forming chains
Growth (°C) at	10	+	+
	15	+	+
	37	+	+
	40	+	+
	45	+	+
Ammonia from arginine		+	+
Gas from glucose		-	-
Reaction in Litmus milk		No clotting or color change	No clotting or color change
Growth at different NaCl levels (%)	2	+	+
	4	+	+
	6.5	+	+
		n=8 *Enterococcus* sp	n=13 *Enterococcus* sp
Source of Isolates		n=1 from maize grit n=2 from yellow and n=5 from white root type	n=8 from maize grit n=5 from yellow root type

b. Species Level

Maize Grit (Nine Isolates)	**Munkoyo root**	
	Yellow type (Seven Isolates)	**White type** (Five Isolates)
Enterococcus gallinarum (Dominant 89%) *Enterococcus casseliflavus* (11%)	*Enterococcus faecalis* (Dominant 71%) *Enterococcus casseliflavus* (29%)	*Enterococcus casseliflavus* (100%)

Table 4-4: Yeasts associated with Maize grit and Munkoyo root (yellow and white)

Maize grit	**Munkoyo Roots**	
	Yellow	**White**
Wickerhamomyces anomalus	*Wickerhamomyces anomalus*	*Wickerhamomyces anomalus*
Saccharomyces cerevisiae	*Cryptococcus liquifaciens*	*Cryptococcus liquifaciens*
	Cryptococcus laurentii	*Cryptococcus laurentii*
	Cryptococcus albidus	*Pichia fabianii*
	Yarrowia lypolytica	*Rhodotolula mucilaginosa*
	Clavispora lusitaniae	
	Sporobolomyces nylandii	

Table 4-5: Moulds associated with Maize grit and Munkoyo root (yellow and white)

Maize grit	**Munkoyo Roots**	
	Yellow	**White**
Aspergillus oryzae	*Aspergillus oryzae*	*Aspergillus oryzae*
Fusarium species	*Fusarium annatatum*	*Aspergillus* species
Lichtheimia ramosa	*Fusarium* species	*Fusarium* species
Acremonium .cf. *zeae*		*Penicillium citrinum*
		Penicillium species
		Lichtheimia ramosa
		Scopulariopsis brevicaulis

Figure 4-1: Munkoyo (*Rhynchosia* species) shrub

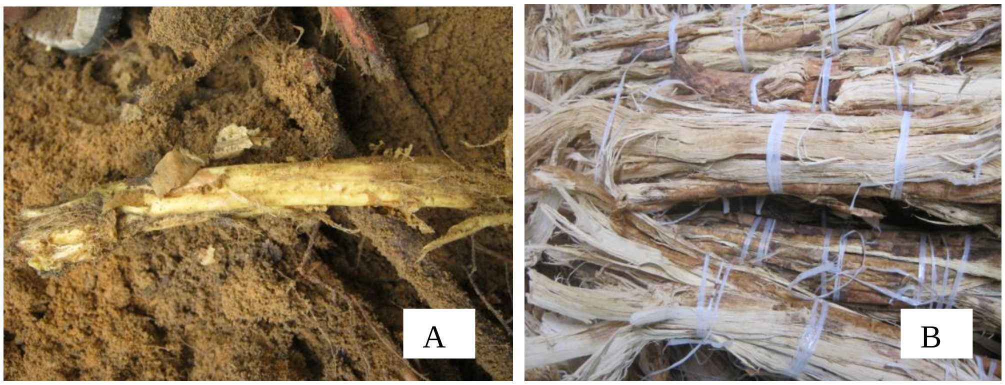

Figure 4-2: The Munkoyo (*Rhynchosia* species) Root

Figure 4-3: Maize grit sold in the local markets

Figure 4-4: Munkoyo beverage; sold in the local markets

Chapter 5

Isolation, Characterization and Identification of the Essential Microorganisms involved during Fermentation a Zambian beverage - Chibwantu

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Chapter 5

5 Isolation, Characterization and Identification of the Essential Microorganisms involved during Fermentation of a Zambian beverage - Chibwantu

Abstract

Fermentation is used as an effective and inexpensive means to preserve the quality and safety of foods. In Zambia, cereal grains such as maize, sorghum and millet are among the common substrates used for preparing cereal based fermented foods and beverages. For cereal based beverages such as chibwantu and munkoyo, the roots *Rhynchosia insignis, Rynchosia heterophylla* or *Rhynchosia venulosa* are used during preparation. The munkoyo roots are either white or yellow in color. Microorganisms involved during fermentation of chibwantu using the white and yellow munkoyo roots as well as during the fermentation of the white and yellow munkoyo root extracts were investigated during natural fermentation for 72 hours.

The microorganisms involved in fermentation of chibwantu using munkoyo root extracts as well as of munkoyo root extracts include aerobic mesophiles, Lactic Acid Bacteria (LAB), coliforms and yeast and moulds. The mesophiles, presumptive LAB (presumptive *Lactobacilli* - *Leuconostoc* on MRS and presumptive *Lactococci*) and coliforms increased during the fermentation process for chibwantu beverage prepared with either yellow or white munkoyo root extract and for the munkoyo root (white and yellow) extracts. There were no significant differences ($p < 0.05$) in microbial counts for the mesophiles, LAB and coliforms from the different parameters tested; Yellow munkoyo root extract (S2), White munkoyo root extract (S3), control; porridge with water instead of munkoyo root extract (S4), chibwantu using the yellow munkoyo root extract (S5), chibwantu using the white munkoyo root extract (S6) at different intervals during the fermentation process. Total microbial counts for yeasts and

moulds did not change significantly during the fermentation process for both chibwantu gruel using the white and yellow munkoyo root extracts or during fermentation with only root extracts. However, moulds decreased significantly with fermentation and yeasts dominated towards the end of the fermentation process.

Parameters associated with the white munkoyo roots (S3 and S6) had higher initial pH compared to the parameters associated with the yellow roots (S2 and S5). The pH at the initial stages of the fermentation process was generally in the range of pH 6.27 to 7.40 (for S2, S3, S5 and S6). S2 pH decreased from an initial pH 6.96 to 6.09 at 24 hours and increased to pH 7.48 at 72 hours, S3 pH also decreased from pH 7.69 to 6.11 at 24 hours and increased to pH 7.83 at 72 hours. For S5, S6 and S4 (control with no munkoyo root extract), the pH decreased during the fermentation process. S5 decreased from pH 6.68 to pH 4.24, S6 from pH 7.17 to pH 4.01 and S4 from pH 7.45 to 4.71 at 72 hours of fermentation.

Preliminary results

LAB from S2 were identified as *Enterococcus casseliflavus*, *Enterococcus thailandicus*, *Weissella confusa* and some unidentified LAB. The yeasts were identified as *Wickerhamomyces anomalus*, *Cryptococcus liquifaciens*, *Cryptococcus albidus*, *Pichia fabianii*, *Rhodotolula mucilaginosa*, *saccharomyces cerevisiae* and *Sporobolomyces nylandii* and the moulds as *Penicillium* species and *Chaetothyriales* species. From S3 LAB were tentatively identified as *Enterococcus gallinarum*, *Enterococcus casseliflavus*, *Lactococcus lactis* and some unknown LAB. The yeasts were identified as *Wickerhamomyces anomalus*, *Cryptococcus albidus*, *Pichia fabianii*, and *Sporobolomyces nylandii* and the moulds as *Verticillium* species, *Fusarium* species, *Aspergillus oryzae*, *Penicillium citrinum* and *Penicillium* species. From S5 LAB were identified as *Enterococcus thailandicus*, *Enterococcus casseliflavus*, *Enterococcus gallinarum*, *Leuconostoc lactis*, *Weissella confusa*, *Lactococcus lactis* and some unknown LAB. The yeasts were identified as *Wickerhamomyces anomalus*, *Cryptococcus liquifaciens*, *Cryptococcus albidus*, *Pichia fabianii*, *Pichia kudrriavzevii*, *Rhodotorula mucilaginosa*, *Saccharomyces* *cerevisiae*, *Meyerozyma guilliermondii, Clavispora lusitaniae* and *Sporobolomyces nylandii*. *Penicillium* species was the only mold identified during the fermentation process. From S6 LAB were tentatively identified as *Enterococcus gallinarum, Enterococcus casseliflavus, Lactobacillus casei, Lactococcus lactis* and *Weissella confusa.* The yeasts were identified as *Wickerhamomyces anomalus, Pichia fabianii, Pichia ciferrii, Rhodotorula mucilaginosa, Meyerozyma guilliermondii* and *Sporobolomyces nylandii* and the only mold identified was *Aspergillus oryzae*. From S4 (control) all the isolates were identified as *Lactococcus lactis*. *Wickerhamomyces anomalus* was the only yeast identified and no moulds were identified during the fermentation process.

From S2, S3, S5 and S6 the dominant coliforms were also identified as *Enterobacter cloacae, Enterobacter* species and *Klebsiella* species. Other microorganisms such as *Micrococci* species and *Bacillus* species were also encountered during the fermentation process.

A diversity of microorganisms associated with the munkoyo roots in particular are responsible for the fermentation of the chibwantu beverage. Due to the wide range of coliforms, LAB and yeast and moulds, the quality of the beverage is expected to vary from one fermentation process to another.

Key Words: Fermentation, chibwantu, munkoyo roots, lactic acid bacteria, yeasts and moulds

5.1 Introduction

Many indigenous cereal based fermented beverages are prepared in different parts of the world and these possess peculiar nutritional and sensory properties, such as taste and flavor, derived from the fermentation of specific raw materials. Depending on country of origin or even local region, various names may be given to the same or similar products produced with slight variations. For example Togwa a non-alcoholic beverage consumed in East Africa and Mahewu (amahewu) a non-alcoholic beverage very popular among the people of southern Africa are both prepared from maize porridge and millet or sorghum malt flour (Gadaga *et al*., 1999; Farnworth, 2003; Oi and Kitabatake, 2003).

Depending on country or even local region, various names may be given to the same product or to products that are basically similar but are produced with slight variation.

Natural cereal based fermentations are carried out by yeast, lactic acid bacteria and filamentous fungi, sometimes forming complex microbiota acting in cooperation (Blandino *et al*., 2003; Nout, 2009). The microorganisms are often naturally occurring on raw materials, utensils used in preparation and in the environment of the production site (Zulu *et al*., 1997; Tamang, 1998; Gassem, 2002). The fermentation conditions for production such as temperature, humidity and aeration are often not optimized (Odunfa, 1988) and this may result in proliferation of undesirable microorganisms, resulting in fermented products of varying quality and safety because the preparation of many of these cereal fermented foods and beverages is still a traditional art in homes, villages and small-scale industries (Jespersen, 2003) and the food products are little known outside their native regions (Abegaz, 2007).

Chibwantu a Zambian cereal based non-alcoholic beverage is prepared from maize grit and the root *Rhynchosia insignis* and/or *Rhynchosia heterophylla* (Zulu *et al*., 1997) or *Rhynchosia venulosa* (Simwamba and Elahi, 1986). The root is generally known as yellow or white munkoyo by the local people. The beverage has to be consumed while actively fermenting within 18 to 48 hours for the reason that once the fermentation process is left for a longer time (more than 48 hours), the beverage becomes alcoholic and loses its acceptable sensory attributes. Chibwantu is among the favorite indigenous beverages produced in Zambia and is consumed as major breakfast for the whole family, in between meals, during ceremonies and field works such as planting, weeding and harvesting.

Considerable work has been done on the microbiology of naturally fermented cooked cereal based beverages (cereals are cooked first then fermented prior to consumption) such as bushera, togwa and mahewu. During the preparation process, malted cereals are added as a source of fermentable carbohydrates, right type of functional microbiota or source of amylolytic enzymes (Simango, 1997; Gadaga *et al*., 1999; Lund *et al*., 2000; Muyanja, 2003; Mugula *et al*., 2003). Lactic acid bacteria and yeasts have been found to be the predominant microbiota (Gadaga *et al*., 1999; Muyanja, 2003; Mugula *et al*., 2003). There is generally inadequate information on the microbiology of naturally fermented cooked cereal based beverages such as Chibwantu were the roots are added as a source of fermentable carbohydrates, functional microbiota or source of amylolytic enzymes.

Knowledge of the fermentation process of this kind of beverages as well as the microorganisms involved is essential for the development of improved products for increased consumption. The aim of this study was to isolate, characterise and identify essential microorganisms involved and to examine the microbial changes during the fermentation of chibwantu.

5.2 Materials and Methods

5.2.1 **Samples**

The samples, maize grit and munkoyo root bunches (yellow and white), of approximately 100 -150 g per bunch, were purchased. The maize grit was purchased and collected at market sites (Soweto and City) in Lusaka. The munkoyo roots were purchased and collected at market sites (Soweto and City) in Lusaka, as well as markets in Livingstone and Chingola.

5.2.2 **Preparation of chibwantu**

Chibwantu was prepared following traditional methods. Thick or thin maize grit porridge is prepared and the roots (munkoyo) and/or extract added to the warm porridge and left to ferment naturally. Fermentation occurs at ambient temperatures (25 – 30 ^{o} C) in 24 - 48 hours (Lovelace, 1977; Simwamba and Elahi, 1986; Zulu *et al.*, 1997).

5.2.2.1 Maize grit porridge

To prepare the maize grit porridge, 1700 ml of tap water was measured into a cooking pot and allowed to boil on a hot plate. Maize grit (300 g) washed with tap water was mixed with the boiling water. The porridge was allowed to cook; first 30 minutes on high heat setting and the last 30 minutes on low/medium heat setting, while stirring periodically to allow for proper mixing and prevent the grit from burning at the bottom of the pot and then allowed to cool down.

To prepare the munkoyo root extract, 50 g of munkoyo root (yellow or white type) was weighed and soaked in one litre of water (ratio 1:20) (tap water which had been boiled and allowed to cool) for two hours and 30 minutes.

5.2.2.2 Chibwantu

To prepare for fermentation of the gruel, three 500 g portions of the cooked maize grit porridge was weighted into plastic fermentation jars. To the first (1) portion 500ml of the yellow munkoyo root extract was added (designated as S5), to the second (2) portion, 500ml of the white munkoyo root extract was added (designated as S6), and to the third (3) portion, 500ml of the boiled and cooled tap water was added (designated as S4). The different mixtures were mixed vigorously and allowed to ferment at room temperature for 72 hours while appropriate samples were taken at 8 hour intervals for determination of pH, microbial counts and isolation of LAB and yeast and moulds. The fermentation process was repeated four times (n=4).

5.2.3 Munkoyo root extract

To check if fermentation occurred in the munkoyo root extract without addition of the maize grit porridge, 500ml of the boiled and cooled tap water was added to 500ml of the munkoyo root extract (yellow or white type) in the plastic jars (designated as S2 for yellow root extract and S3 for white root extract) and allowed to ferment while appropriate samples were taken at 8 hour intervals for determination of pH, microbial counts and isolation of LAB and yeast and moulds.

5.2.4 Microbiological analyses

Representative 25 ml (BAM, 1995) of samples were aseptically taken at 8 hour intervals during the traditional fermentation of chibwantu and mixed with 225 ml of buffered peptone to have a 10^{-1} dilution. Serial decimal dilutions were then prepared with buffered peptone water and duplicate plates were prepared of all appropriate dilutions, by surface plating.

5.2.4.1 Enumeration and isolation of microorganisms

Plate Count Agar (Merck, Gauteng, South Africa) was used for the enumeration of total aerobic mesophilic bacteria. The plates were incubated at 30^{o}C for 48 hours. De Man, Rogosa and Sharpe (MRS) agar (Merck, Gauteng, South Africa) was incubated anaerobically in anaerobic jars with gas generating kits (Microbiology Anaerocult. Merck, Darmstadt, Germany) at 30^{o}C for 48 hours and M17 agar (Oxoid, England) incubated aerobically at 30^{o}C for 48 hours for the enumeration and isolation of LAB. Violet Red Bile Agar (VRBA) (Merck, Gauteng, South Africa) incubated aerobically at 30^{o}C for 24 hours for enumeration of total coliforms. For confirmation of coliforms, representative colonies were inoculated into Brilliant Green Lactose Bile Broth

(BGLB) and incubated at 35^{o}C. Examination of tubes was done at 24 hours and 48 hours. Colonies producing gas in this broth were confirmed as coliforms. Rose Bengal Cloramphenicol Agar (RBCA) (Merck) was incubated at 30^{o}C for 72 hours for enumeration and isolation of yeasts and moulds.

Isolates of microorganisms were obtained randomly from selected plates of MRS, M17 and RBCA and cultivated in MRS broth, M17 broth and Yeast Malt (YM) broth respectively. Purity was checked by successive streaking on the same media and stored on slants (of the same media) at 4^{o}C for further investigation.

5.2.4.2 Characterization and Identification of lactic acid bacteria

For the identification of LAB, 18 - 24 hours culture from MRS and M17 agar slants were characterized by microscopic examination, conventional biochemical and physiological tests. The isolates were examined for colony and cell morphology as well as cell arrangement. All isolates were tested for gram reaction and the catalase enzyme production according to the methods described by Harrigan and MacCance, (1976). Gram positive and catalase negative isolates were examined for production of gas (carbon dioxide) from glucose using MRS broth containing 5% glucose with inverted Durham tubes, growth at various temperatures 10^{o}C, 15^{o}C, 40^{o}C and 45^{o}C, tolerance of different salt levels (2%, 4% and 6.5%), production of ammonia from arginine and reduction of litmus before clotting in litmus milk (Harrigan and MacCance, 1976; Axellssion, 1993; Savadogo *et al*., 2004).

After identification all gram positive and catalase negative pure isolates were cultivated in MRS broth with 10% (v/v) glycerol for 18 hours at 30^{o}C and stores at -21^{o}C for further use.

5.2.4.3 Characterization and Identification of yeasts and moulds

For characterization of yeast and moulds, colonies were examined for their colony color, shape and texture. Colonies with different colony morphology were randomly picked from selected RBCA plates. Pure isolates were prepared by taking suspending a small portion of the suspected colony type into a tube of sterile distilled water and then a loop-full of the suspension was streaked onto the RBCA plate. The plates were incubated at 30^{o}C for 48 hours. The pure cultures were then sub-cultured onto YM (yeast malt) agar slants and stored at 4^{o}C for further use.

5.2.4.4 Identification of Microorganisms to species Level

For the identification of the microorganisms (LAB, coliforms, yeasts and moulds) the pure isolates were sequenced by Ingaba Biotech. The sequences obtained from Finch TV (http://www.geospiza.com/ftvdlinfo.html) were aligned against those for the NCBI (National Center for Biotechnology Information) databases using BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify homologous (very similar) sequences.

5.2.5 pH

The pH was determined using a pH meter (Cyberscan pH 510, Eutech Instruments). The pH meter was calibrated using standard buffer solutions (Merck) at pH 4.0 and pH 7.0.

5.2.6 Statistical Analysis

Student test was done on the data using SPSS (IBM SPSS Statistics 21) to compare mean of samples.

5.3 Results and Discussion

5.3.1 Enumeration of microorganisms

Microbial counts and growth patterns during fermentation of chibwantu prepared with munkoyo roots (yellow and white type) and fermentation of munkoyo root extracts

During the fermentation period of chibwantu, aerobic mesophylic bacteria were the predominant microorganisms. For S2, the aerobic mesophylic bacteria, presumptive *Lactobacilli-Leuconostoc, Lactococci* and coliforms increased from initial counts of 5.75, 5.33, 5.60 and 5.58 log cfu/ml to 8.14, 7.70, 8.11 and 8.22 log cfu/ml at 48 hours and 8.41, 8.15, 8.51 and 8.34 log cfu/ml respectively at the end of 72 hour fermentation. Yeasts and moulds increased slightly in the initial stages of fermentation from 3.49 to 3.66 log cfu/ml at 24 hours and decreased to 3.37 log cfu/ml at 72 hours of fermentation.

For S3, the aerobic mesophylic bacteria, presumptive *Lactobacilli-Leuconostoc*, *Lactococci* and coliforms increased from initial counts of 5.55, 4.76, 5.12 and 5.07 log cfu/ml to 7.70, 7.68, 7.90 and 7.90 log cfu/ml at 48 hours and 8.54, 8.07, 8.51 and 8.36 log cfu/ml respectively at the end of 72 hour fermentation. Yeasts and moulds increased in the initial stages of fermentation from 3.31 to 4.77 log cfu/ml at 40 hours and decreased to 4.41 log cfu/ml at 72 hours of fermentation.

For S5, the aerobic mesophylic bacteria, presumptive *Lactobacilli-Leuconostoc*, *Lactococci*, coliforms and yeasts and moulds increased from initial counts of 5.93, 5.54, 6.00, 5.80 and 2.83 log cfu/ml to 7.70, 7.89, 7.79, 7.78 and 3.39 log cfu/ml respectively at the end of at 48 hours and to 8.36, 8.26, 7.89 and 4.67 log cfu/ml for aerobic mesophylic bacteria, presumptive *Lactobacilli-Leuconostoc*, *Lactococci* and yeasts and moulds respectively at the end of 72 hour fermentation. Coliforms decreased to 7. 35 log cfu/ml at the end of 72 hour fermentation.

For S6, the aerobic mesophylic bacteria, presumptive *Lactobacilli-Leuconostoc*, *Lactococci*, coliforms and yeasts and moulds increased from initial counts of 5.52, 5.25, 5.35, 5.37 and 3.55 log cfu/ml to 7.84, 8.21, 7.78, 7.33 and 3.86 log cfu/ml respectively at the end of at 48 hours and to 8.03, 8.33, 7.37 and 4.70 log cfu/ml for aerobic mesophylic bacteria, presumptive *Lactobacilli-Leuconostoc*, coliforms and yeasts and moulds respectively at the end of 72 hour fermentation. Presumptive *Lactococci* decreased slightly to 7. 65 log cfu/ml at the end of 72 hour fermentation.

There was no significant difference ($p < 0.05$) in counts of aerobic mesophylic bacteria on PCA, presumptive *Lactobacilli-Leuconostoc* on MRS, presumptive *Lactococci* on M17 and presumptive test of coliforms on VRBA, at the initial stage of the fermentation process (start to 24 hours) for all the parameters tested (S2, S3, S5, and S6). As fermentation progressed, S2 and S5 associated with the yellow munkoyo roots had higher counts compared to S3 and S6 associated with the white munkoyo roots, however, the differences were not significant ($p < 0.05$) for aerobic mesophylic bacteria, presumptive *Lactobacilli-Leuconostoc*, *Lactococci* and test of coliforms. The initial microbial counts for all the tested parameters (except control) were in the range of 4.76 - 6.00 log cfu/ml and final counts after 72 hours of fermentation were in the range of 7.35 - 8.54 log cfu/ml.

For coliform counts, after 40 hours of fermentation, S2 and S3 (water with munkoyo root extracts) had higher counts compared to S5 and S6 (maize grit porridge with munkoyo root extract) respectively. Fig. 5-1 to 5-10 summarised the microbial growth counts and patterns during the fermentation of chibwantu and also munkoyo root extracts (yellow and white type) for a period of 72 hours.

The colonies on VRBA during fermentation of S2 were > 98% similar and for S5 were 100% similar. For S3 and S6 two types of colonies dominated and accounted for > 96 % of the colonies.

One assumption for the growth pattern could be that, the microorganisms growing on the different media types were of the same type (or genus), since from 8 hours of fermentation there were few bacterial colony types growing (with one or two colony type of bacteria dominating) on any given plate from the different media used PCA, MRS, M17 and VRBA throughout the fermentation process. Furthermore studies on other cereal based fermented food products indicate that coliforms decrease in number of counts as fermentation progresses to undetectable levels after 24 hours (Mugula *et al*., 2001; Mugula *et al*., 2003; Kunyanga *et al*., 2009). However, for fermentation of chibwantu using the yellow or white root extract, coliforms increased with fermentation time. The coliforms were among the microorganisms associated with the munkoyo roots, since the colonies observed from S2 were also observed from S5 and those from S3 were also observed from S6.

For yeast and moulds; the yellow root extract (S2, and S5) had diverse yeasts compared to the white root extract (S3 and S6). The white root extract had higher mould colony types compared to the yellow root extract. The counts of yeast and moulds on RBCA were lower, fluctuated and did not change much during the fermentation period for all the parameters tested especially the yellow munkoyo root extract (S2). However, there were differences in counts of yeast and moulds between S2 and S3 indicating that the yellow and white munkoyo root extracts have different effects on the growth of yeast and moulds.

The pH at the initial stage of the fermentation process was in the range of pH 6.68 - 7.69 for all the tested parameters. Parameters S2 and S5 associated with the yellow munkoyo roots had lower initial pH values of pH 6.96 and 6.68 respectively, with less variability in measurements compared to parameters S3 and S6 associated with white munkoyo roots with initial pH values of 7.69 and 7.17 respectively. S3 and S6 had higher variability in measurements.

For S5 and S6 (parameters with the maize grit porridge) the pH decreased during the fermentation process. There was higher drop in pH for S6 (white munkoyo root) compared to S5 (yellow munkoyo root) during the fermentation process (Fig 5-11). At 24 hours of fermentation the pH of S6 was already lower than pH of S5, even though the initial pH for S6 was higher than that of S5.

For S2, pH increased at the initial stage from pH 6.96 to 7.45 at 8 hours, dropped to pH 6.09 at 24 hours and then increased to pH 7.48 at 72 hours of fermentation. S3 pH decreased at the initial stage from pH 7.69 to 6.60 at 24 hours and then increased to pH 7.83 at 72 hours of fermentation. S3 had higher pH values compared to S2.

In general, the white root had higher influence on pH compared to the yellow root. However the differences between S2 and S3 and also between S5 and S6 were not significant ($p < 0.05$).

In addition, the microorganisms associated with munkoyo roots did not seem to get affected by changes in pH. There were insignificant differences in counts of microorganisms from S2, S3, S5 and S6. Implying that if the munkoyo roots were associated with spoilage microorganisms particularly bacteria, the compounds produced during fermentation such as lactic acid had less effect and consequently the microorganisms would still be capable of spoiling the beverages. The results further indicate that munkoyo roots (both yellow and white) contain compounds that sustain the growth of different types of microorganisms (Fig 5-1 and 5-2).

5.3.2 Characterisation and Identification of lactic acid bacteria

Some of the catalase negative and gram positive (presumptive LAB) isolated from different parameters tested (S2, S3, S4, S5 and S6) were difficult to sub-culture on the same media from which they were isolated, for further characterisation and hence could not be identified.

S2 (yellow root extract)

Throughout the fermentation process (from initial stage to 72 hours of fermentation), catalase negative and gram positive isolates from MRS and M17, were in the minority. Some of the isolates that could be sub-cultured for further identification were similar in their physiologic characteristics. The isolates were gram positive, catalase negative and majority were spherical in shape. Some of the isolates produced gas from glucose while others did not. Growth characteristics at different temperatures, levels of salt concentration and in litmus milk varied among the isolates as well as production of ammonia from arginine (Harrigan, 1998; Ali, 2011). The summary of characteristics for the isolates is shown in Table 5-1. The sequences of the LAB isolates obtained were identified as *Enterococcus casseliflavus, Enterococcus thailandicus, Weissella confusa* and some unidentified microorganisms.

S3 (white root extract)

Compared to S2 (yellow root extract), S3 had more catalase negative and gram positive (presumptive LAB) isolates and the sequences of the isolates obtained were identified as *Enterococcus gallinarum, Enterococcus casseliflavus, Lactococcus lactis* and some unknown microorganisms. The LAB were isolated throughout the fermentation process except for *Lactococcus lactis* which was isolated after 16 hours of fermentation. Table 5-2 summarised the growth characteristics.

S5 (chibwantu using yellow munkoyo root extract)

The isolated gram positive and catalase negative (presumptive LAB) from fermentation of chibwantu using yellow munkoyo root extract were identified as *Enterococcus thailandicus, Enterococcus casseliflavus, Enterococcus gallinarum, Leuconostoc lactis, Weissella confusa, Lactococcus lactis* and some unknown microorganisms. Table 5-3 summarised the growth characteristics.

S6 (chibwantu using white munkoyo root extract)

The isolated gram positive and catalase negative (presumptive LAB) from fermentation of chibwantu using white munkoyo root extract were identified as *Enterococcus gallinarum, Enterococcus casseliflavus, Lactobacillus casei, Lactococcus lactis* and *Weissella confusa.* Table 5-4 summarised the growth characteristics.

From the parameters associated with munkoyo roots (S2, S3, S5 and S6) the *Enterococcus casseliflavus* isolates had higher variability in growth characteristics at different temperatures, levels of salt concentration, in production of ammonia from arginine and in litmus milk compared to the other LAB isolates. The presumptive LAB that dominated on MRS and M17 plates in some instances were difficult to subculture on the same media from which they were isolated. The assumption could be that they grow in association with other microorganisms.

S4 (no munkoyo root extract)

Presumptive LAB isolates from the control (fermentation of maize grit porridge without munkoyo root extract) were mainly identified as *Lactococcus* lactis, however, there were variations in physiologic characteristics as shown in Table 5-5.

5.3.3 **Identification of yeasts and moulds**

Table 5-6 shows the types of yeasts and moulds isolated during the fermentation process from the different parameters tested (S2, S3, S4, S5 and S6).

S2 (yellow root extract)

S2 was associated with higher counts of yeasts than moulds during the fermentation process and after 48 hours of fermentation some experimental runs only had yeasts. The yeasts isolated during the whole fermentation process were identified as *Wickerhamomyces anomalus*, *Cryptococcus liquifaciens*, *Cryptococcus albidus*, *Pichia fabianii, Rhodotolula mucilaginosa, saccharomyces cerevisiae* and *Sporobolomyces nylandii*. The dominant yeasts during the fermentation process of the munkoyo root extract were *Wickerhamomyces anomalus*, *Cryptococcus liquifaciens* and *Cryptococcus albidus*. The moulds were identified as *Penicillium* species and *Chaetothyriales* species.

S3 (white root extract)

The yeasts from S3 were identified as *Wickerhamomyces anomalus*, *Cryptococcus albidus*, *Pichia fabianii,* and *Sporobolomyces nylandii*. The dominant yeasts during the fermentation process of the munkoyo root extract were *Wickerhamomyces anomalus* and *Pichia fabianii*. The moulds were identified as *Verticillium* species, *Fusarium* species, *Aspergillus oryzae, Penicillium citrinum* and *Penicillium* species. There were less diverse yeasts and more diverse moulds from S3 as compared to S2.

S5- Chibwantu (Maize grit porridge with yellow munkoyo root extract)

The yeasts associated with the fermentation process of chibwantu with the yellow munkoyo roots, were identified as *Wickerhamomyces anomalus*, *Cryptococcus liquifaciens*, *Cryptococcus albidus, Pichia fabianii*, *Pichia kudrriavzevii, Rhodotorula mucilaginosa, Saccharomyces cerevisiae, Meyerozyma guilliermondii*, *Clavispora lusitaniae* and *Sporobolomyces nylandii*. More yeasts occurred during the chibwantu fermentation as compared to the fermentation of only with the yellow munkoyo root extract (S2) and *Penicillium* species was the only mould identified during the fermentation of chibwantu with the yellow munkoyo roots. Mainly it was only the yeasts that were identified during the fermentation process.

S6- Chibwantu (Maize grit porridge with white munkoyo root extract)

The yeasts associated with the fermentation process of chibwantu with the white munkoyo roots, were identified as *Wickerhamomyces anomalus*, *Pichia fabianii, Pichia ciferrii, Rhodotorula mucilaginosa, Meyerozyma guilliermondii* and *Sporobolomyces nylandii*. More types of yeasts also occurred during chibwantu fermentation as compared to the fermentation of only the white munkoyo root extract (S3) and the only mold identified during the fermentation of chibwantu with the white munkoyo roots was *Aspergillus oryzae*. Like S5, it was mainly the yeasts that were found during the fermentation process. Moulds were rarely encountered.

S4- Chibwantu control (Maize grit porridge with no munkoyo root extract)

Wickerhamomyces anomalus was the only yeast identified during the fermentation process of chibwantu without munkoyo root extract and no moulds were identified.

5.3.4 **Coliforms**

Coliforms were present throughout the fermentation process of chibwantu, both with the yellow and white munkoyo roots and the dominant species were identified as *Enterobacter* and *Klebsiella* species. *Enterobacter* species dominated on parameters associated with yellow munkoyo roots and both *Enterobacter* and *Klebsiella* species were dominant on parameters associated with white munkoyo roots.

5.3.5 **Other microorganisms**

Other microorganisms such as *Micrococci* and *Bacillus* species were also encountered during the fermentation process both with yellow and white munkoyo root extracts.

5.4 Conclusions

There is a diversity of microorganisms on the raw materials used during the preparation of chibwantu beverage particularly the munkoyo roots (yellow and white types) which are added for different reasons including quick liquefaction (fermentation) of the porridge during preparation of the beverage, taste and flavour as well as sweetener. The microorganisms associated with the munkoyo roots (yellow and white) consist of coliforms, LAB, yeasts and moulds and these microorganisms were also responsible for the fermentation of chibwantu.

Since there was no significant difference ($p < 0.05$) in microbial counts from the munkoyo root extracts (S2 and S3) and the chibwantu gruel (S5 and S6) even with decreased pH, it can be assumed that the microorganisms associated with the munkoyo roots are not affected by the acids produced during the fermentation process or that the munkoyo roots contain properties that support microbial growth even with decreased pH. In addition, due the diversity in microorganisms associated with the munkoyo root extract used for preparation of the gruel, the quality is expected to vary from one fermentation process to another.

The next chapter aims at examining the effect of munkoyo root extracts (*Rhynchosia* spp) on growth of selected microorganisms.

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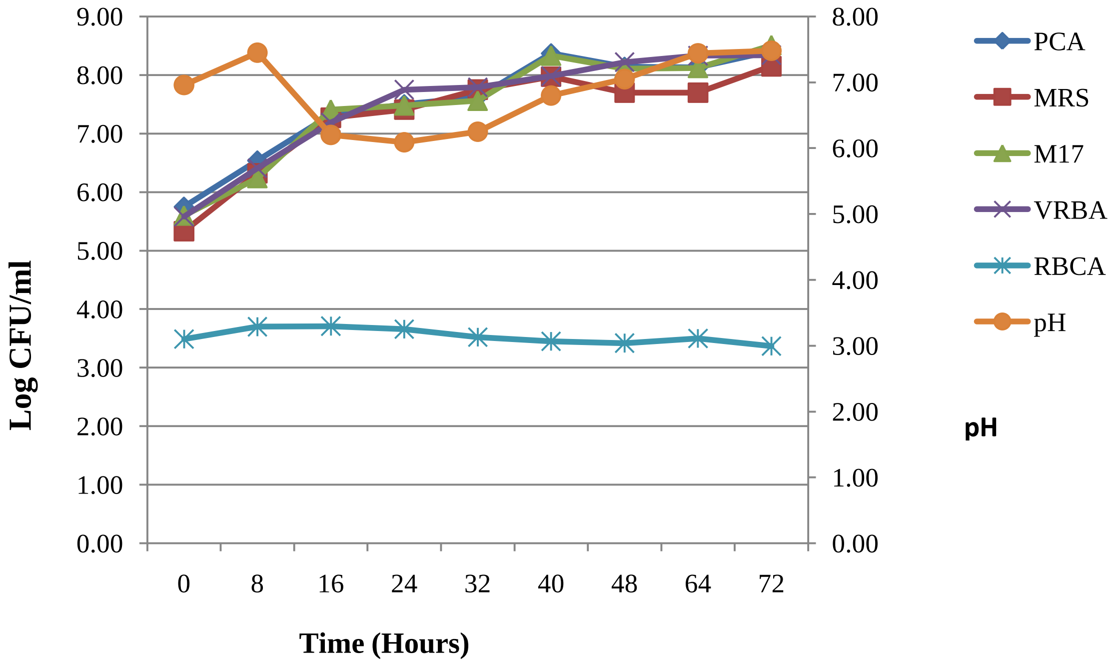

Figure 5-1: Microbial growth patterns during fermentation of yellow munkoyo root extract (S2)

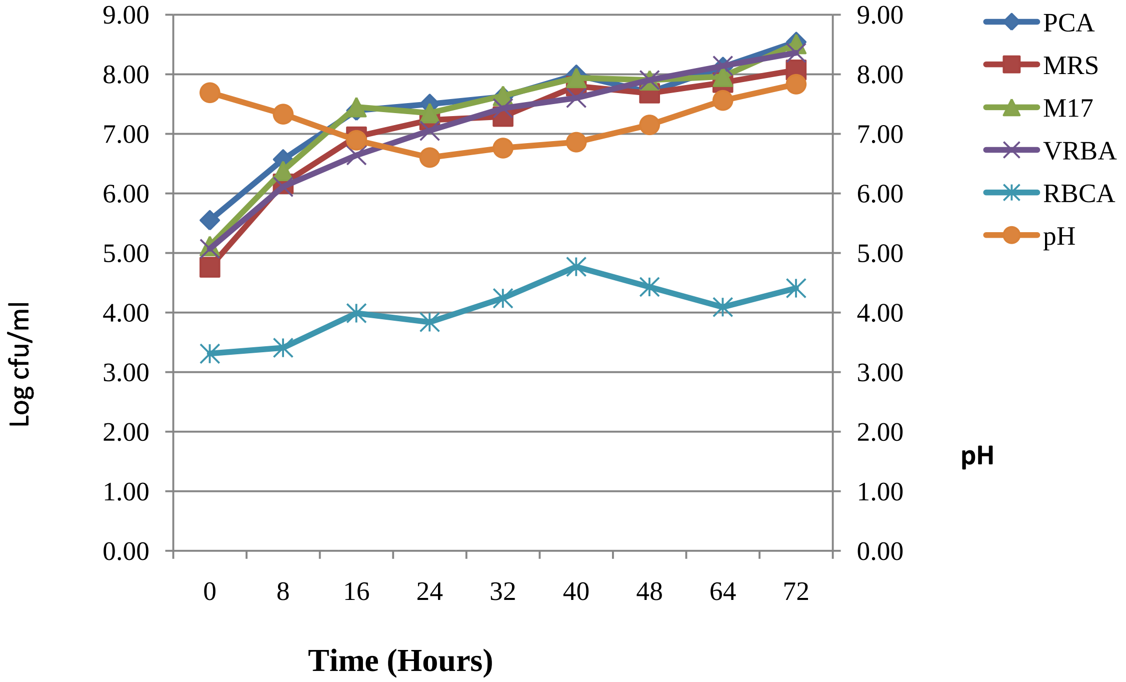

Figure 5-2: Microbial growth patterns during fermentation of white munkoyo root extract (S3)

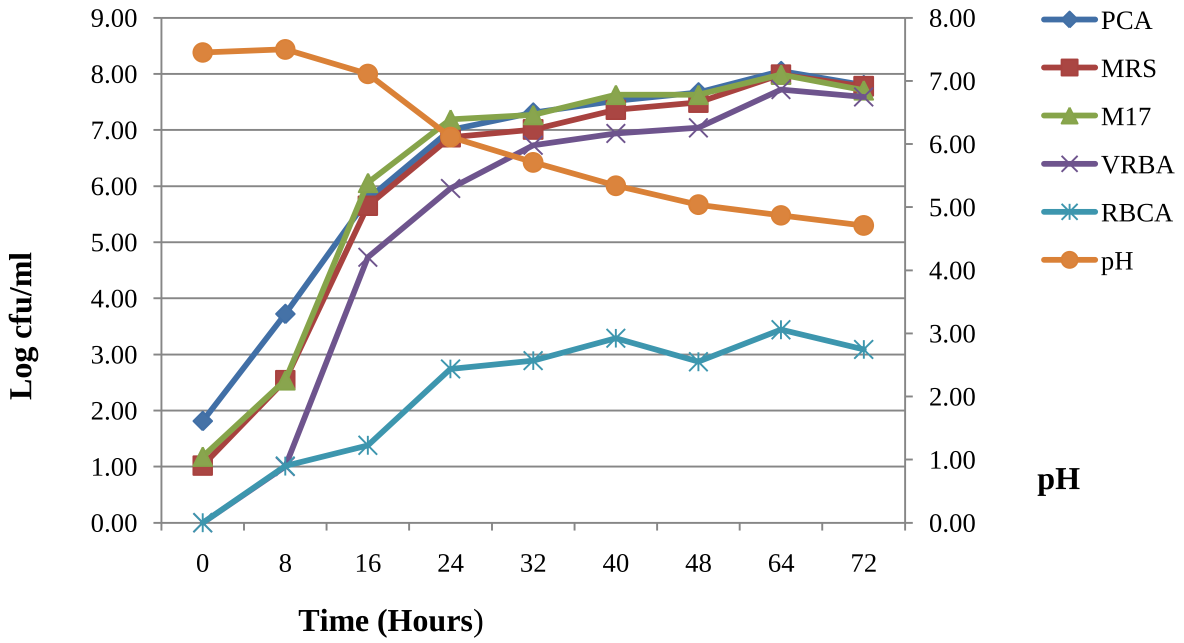

Figure 5-3: Microbial growth patterns during fermentation of grit porridge without munkoyo roots (S4-control)

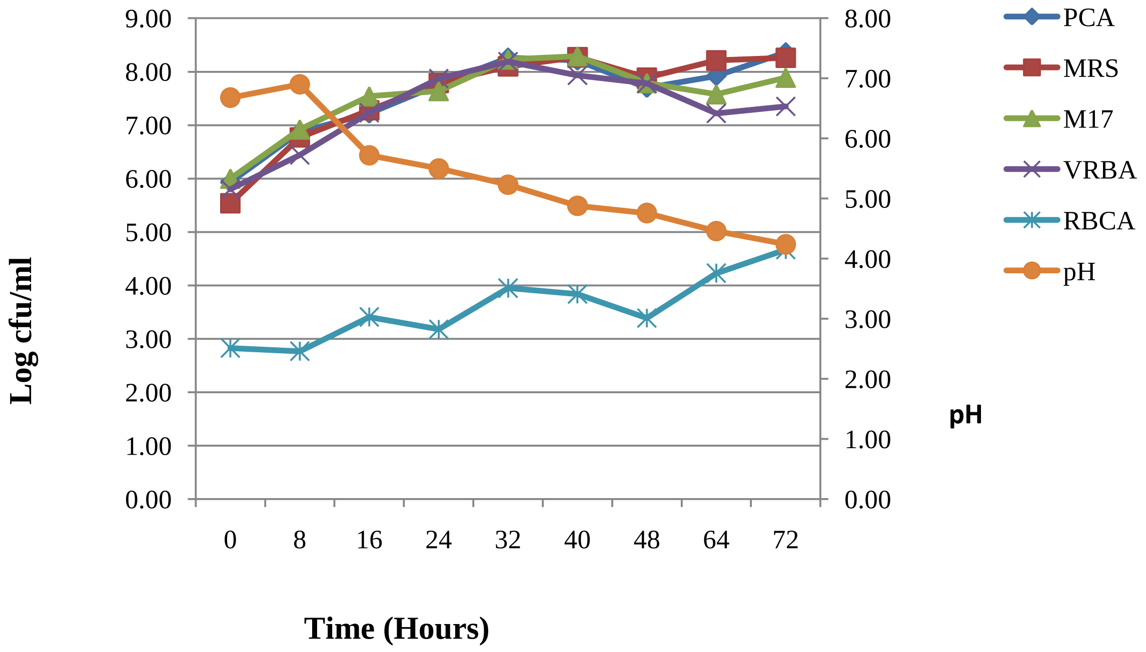

Figure 5-4: Microbial growth patterns during fermentation of chibwantu gruel with yellow munkoyo roots (S5)

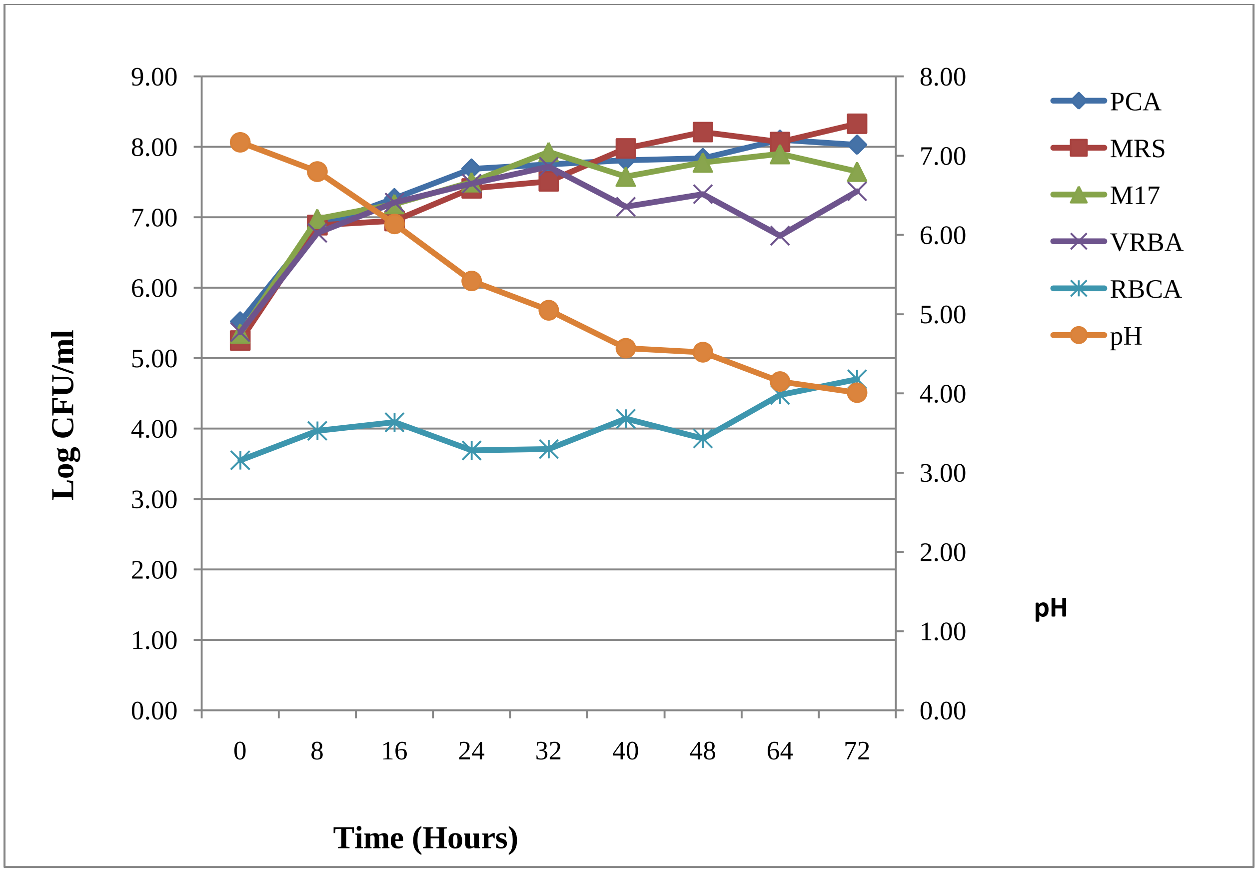

Figure 5-5: Microbial growth patterns during fermentation of chibwantu gruel with white munkoyo roots (S6)

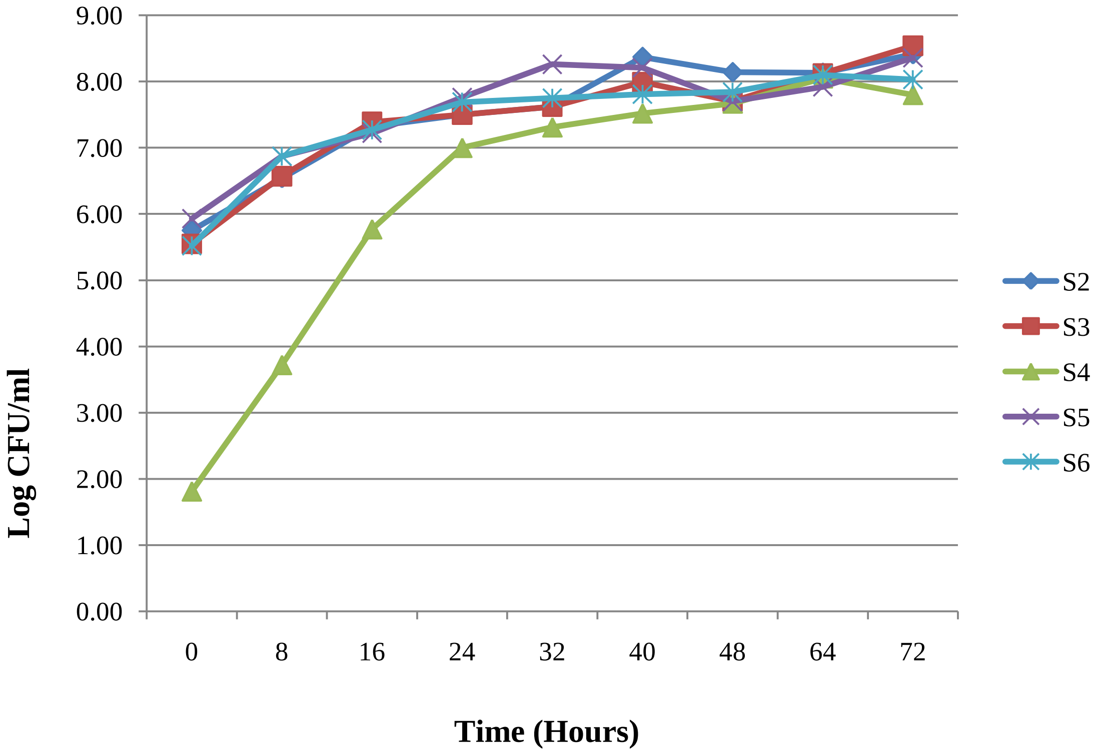

Figure 5-6: Microbial growth patterns on PCA

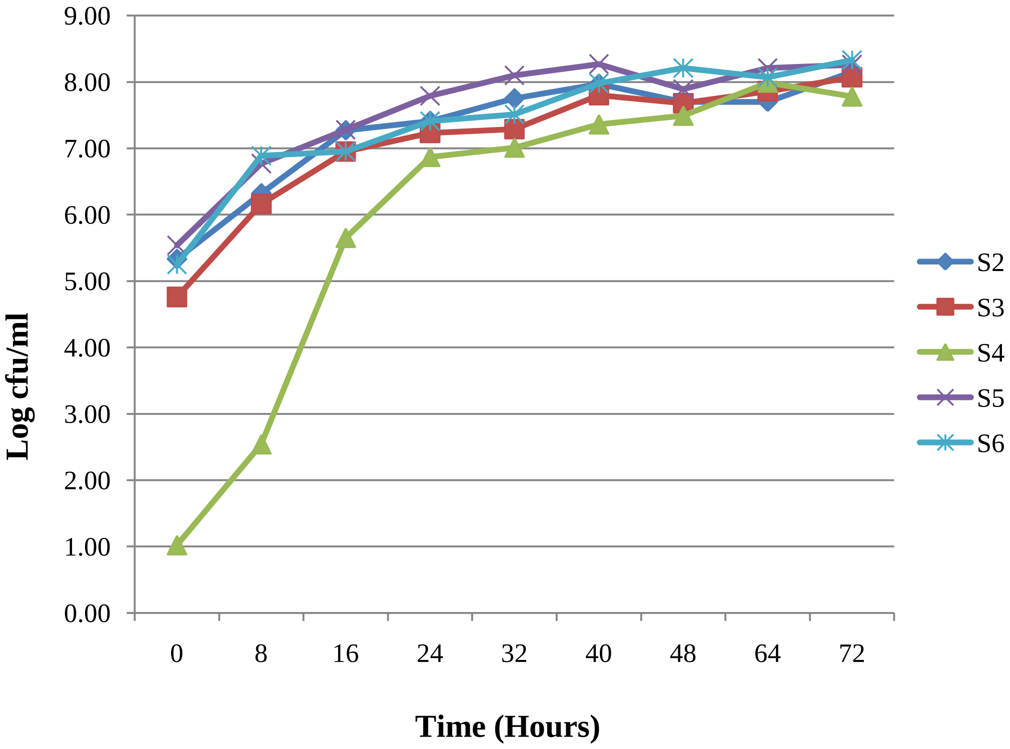

Figure 5-7: Microbial growth patterns on MRS agar

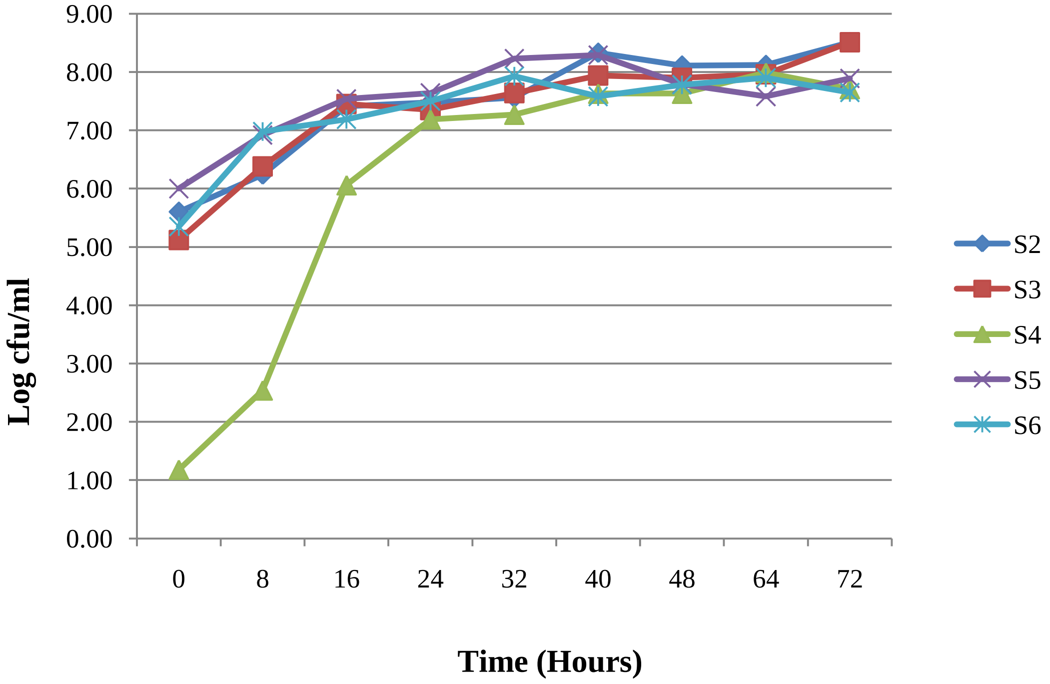

Figure 5-8: Microbial growth patterns on M17

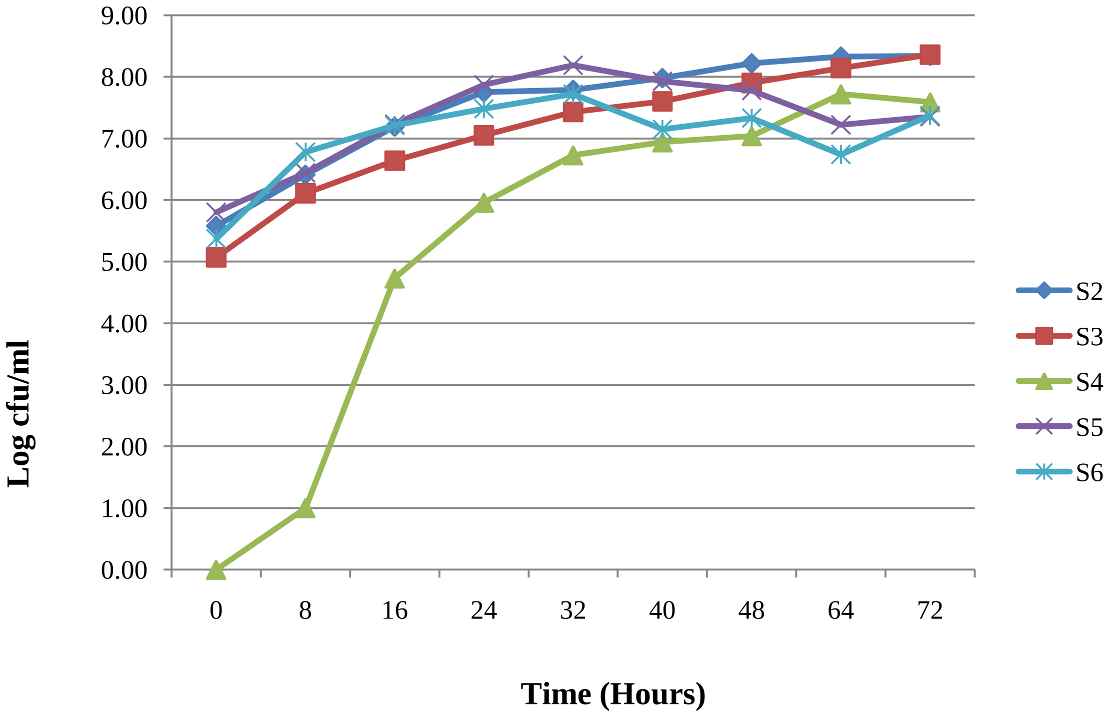

Figure 5-9: Microbial growth patterns on VRBA

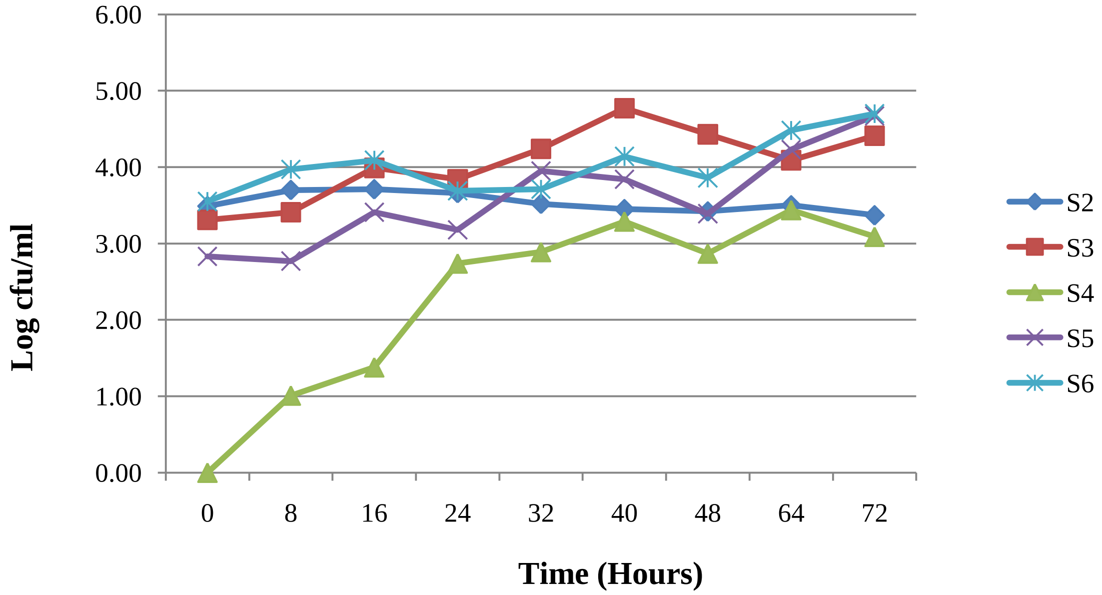

Figure 5-10: Microbial growth patterns on RBCA

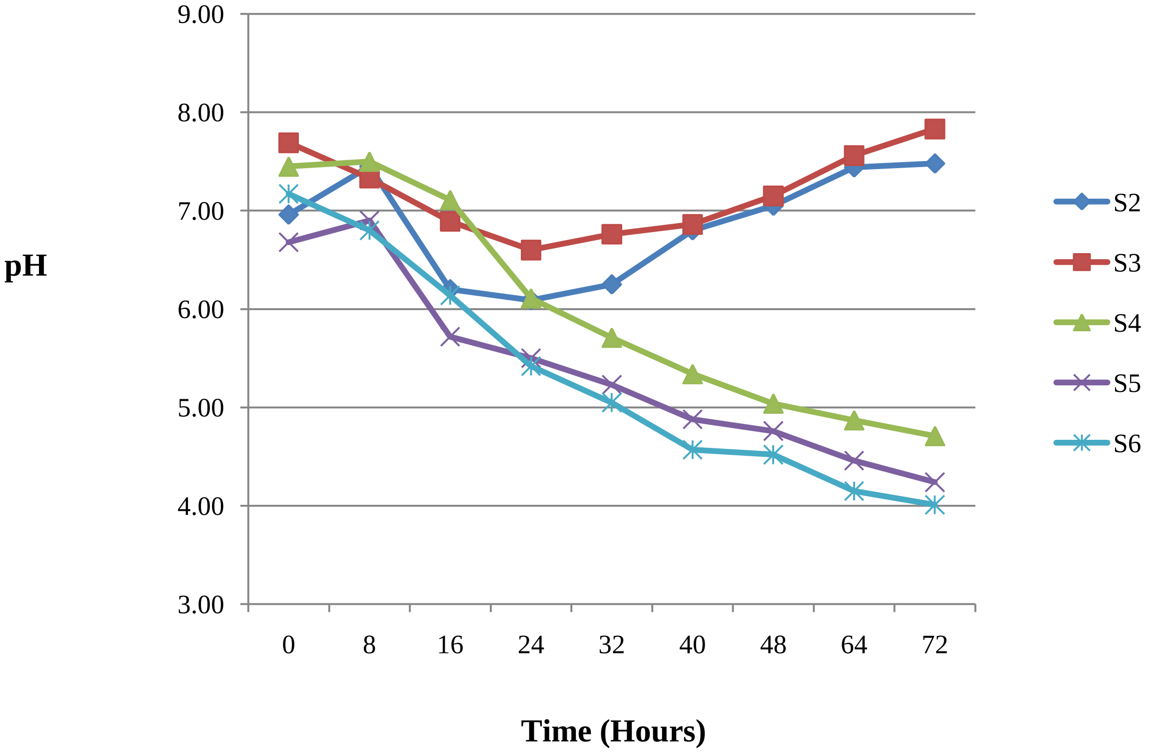

Figure 5-11: pH changes during fermentation

Table 5-1: Characteristics of the LAB isolated from S2 (yellow munkoyo root extract)

Gram		+	+	+	+	+
Catalase		-	-	-	-	-
Cell form		Cocci/ spherical	Cocci/spherical	cocci	cocci	Rods
Cellular arrangement		Small bunches forming chains	Pairs forming chains	Pairs and bunches	Pairs forming chains	pairs
Growth (^{o}C) at	10	+	+	+		+
	15	+	+	+		+
	37	+	+	+		+
	40	+	-	+		+
	45	+	-	+		+
Ammonia from arginine		-	-	+		+
Gas from glucose		-	+	-		+
Reaction in Litmus milk		Acid production (color change) and acid clot	No acid production (color change) with sweet clot (milk coagulation and hydrolysis of caseins	No acid production (color change) and no acid clot		Acid production without acid clot
Growth at different NaCl levels (%)	2	+	+	+		+
	4	+	+	+		+
	6.5	-	+	+		+
Microorganism Identification		N=7 *E. casseliflavus*	N=8 *E. casseliflavus*	N=5 *E. thailandicus*	N=2* Unknown	N=2 *Weissella confusa*

*Difficult to subculture on same media from where the colonies were isolated

Table 5-2: Characteristics of the LAB isolated from S3 (white munkoyo root extract)

Gram		+	+	+	+	+	+	+	+	+
Catalase		-	-	-	-	-	-	-	-	-
Cell form		Very short rods/ cocobacilli	spherical	spherical	spherical	spherical	spherical	cocci	spherical	spherical
Cellular arrangement		pairs	Pairs and singles	Pairs and singles	Pairs and singles	Pairs and singles	Pairs and short chains	Short chains and bunches	Pairs and singles	Pairs, short chains and bunches
Growth (°C) at	10	+	+	+	+	+	+	+	+	+
	15	+	+	+	+	+	+	+	+	+
	37	+	+	+	+	+	+	+	+	+
	40	+	+	+	+	+	+	+	+	+
	45	+	+	+	+	+	+	+	+	+
Ammonia from arginine		+	-	+ on day 6	-	-	-	-	-	-
Gas from glucose		-	-	+	-	-	-	-	+	-
Reaction in Litmus milk		Acid production (color change) and acid clot after 6 days of incubation	Acid production (color change) and acid clot after 6 days of incubation	Color change after 2 days and acid clot after 6 days of incubation	Acid production (color change) and acid clot	No acid production (color change) and no acid clot	Acid production (color change) without acid clot	No acid production (color change) and no acid clot	No acid production (color change) and no acid clot	Acid production (color change) and acid clot
Growth at different	2	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+

NaCl levels (%)	6.5	+	+	+	+	+	-	+	+	+
Microorganism Identification		N=9 *Lactococcus lactis*	N=2 *Enterococcus casseliflavus*	N=3 Unknown	N=1 *Enterococcus casseliflavus*	N=7 *Enterococcus casseliflavus*	N=3 *Enterococcus casseliflavus*	N=7 *Enterococcus gallinarum*	N=1 *Enterococcus casseliflavus*	N=1 *Enterococcus gallinarum*

Table 5-3: Characteristics of the LAB isolated from S5 (white munkoyo root extract)

Gram		+	+	+	+	+	+
Catalase		-	-	-	-	-	-
Cell form		cocci	spherical	rods	Rods	Short rods	Short rods
Cellular arrangement		Pairs and bunches	Pairs and short chains	Singles and pairs	Pairs	Singles and pairs	Pairs chains and singles
Growth (^{o}C) at	10	+	+	+	+	+	+
	15	+	+	+	+	+	+
	37	+	+	+	+	+	+
	40	+	+	+	+	-	-
	45	+	+	+	+	-	-
Ammonia from arginine		+	-	-	+	-	+
Gas from glucose		-	-	+	+	+	-
Reaction in Litmus milk		No acid production (color change) and no acid clot	Acid production without acid clot	No acid production (color change) and no acid clot	Acid production without acid clot	No acid production (color change) and no acid clot	Acid production and acid clot
Growth at different NaCl levels (%)	2	+	+	+	+	+	+
	4	+	+	+	+	+	+
	6.5	+	-	-	+	+	+
Microorganism Identification		N=9 *Enterococcus thailandicus*	N=8 *Enterococcus casseliflavus*	N=3 Unknown	N=8 *Weissella confusa*	N=5 *Leuconostoc lactis*	N=3 *Lactococcus lactis*

Table 5-4: Characteristics of the LAB isolated from S6 (white munkoyo root extract)

Gram		+	+	+	+	+	+	+	+
Catalase		-	-	-	-	-	-	-	-
Cell form		spherical	spherical	spherical	spherical	Spherical	Spherical	spherical	Cocci
Cellular arrangement		Singles, pairs, chains and bunches	pairs	Pairs and singles	Pairs and singles	Pairs and short chains	Pairs	Pairs and short chains	Pairs, chains and bunches
Growth (^{o}C) at	10	+	+	+	+	+	+	+	+
	15	+	+	+	+	+	+	+	+
	37	+	+	+	+	+	+	+	+
	40	+	+	-	+	+	+	-	+
	45	+	-	-	+	+	+	-	+
Ammonia from arginine		-	-	-	-	-	+ after 6 days	-	+
Gas from glucose		-	-	-	-	-	-	+	-
Reaction in Litmus milk		No acid production (color change) and no acid clot	No acid production (color change) and no acid clot	Acid production (color change) with no acid clot	Color change after 1 day and acid clot after 6 days of incubation	Acid production (color change) with no acid clot	Acid production (color change) with no acid clot	No acid production (color change) with sweet clot (milk coagulation and hydrolysis of caseins	Acid production (color change) and acid clot after 6 days of incubation
Growth	2	+	+	+	+	+	+	+	+

at different NaCl levels (%)	4	+	+	+	+	+	+	+	+
	6.5	+	+	+	+	+	+	+	+
Microorganism Identification		N=2 *Enterococcus casseliflavus*	N=13* *Enterococcus casseliflavus*	N=1 *Enterococcus casseliflavus*	N=1 *Enterococcus casseliflavus*	N=3 *Enterococcus casseliflavus*	N=1 *Enterococcus casseliflavus*	N=1 *Enterococcus casseliflavus*	N=4 *Enterococcus faecalis*

Gram		+	+	+	+	+	+	+	+
Catalase		-	-	-	-	-	-	-	-
Cell form		Rods	spherical	spherical	cocci	spherical	Cocci	cocci	cocci
Cellular arrangement		Pairs	Pairs and short chains	Singles and pairs	Bunches	Pairs and short chains	Pairs, singles and bunches	Singles, pairs, chains and bunches	Bunches and chains
Growth (^{o}C) at	10	+	-	+	+	+	+	+	+
	15	+	-	+	+	+	+	+	+
	37	+	+	+	+	+	+	+	+
	40	-	+	+	+	+	+	+	+
	45	-	+	+	+	+	+	+	+
Ammonia from arginine		+	-	+	+	-	+	-	-
Gas from glucose		-	-	-	-	-	-	-	-
Reaction in Litmus milk		No acid production (color change) and no acid	Acid production (color change) with no acid	Acid production (color change) and acid clot	Acid production (color change) and acid clot	Acid production (color change) and acid clot after 6 days of	Acid production (color change) and	No acid production (color change) and no acid clot	Acid production (color change) and

		clot	clot	after 6 days of incubation	after 14 days of incubation	incubation	acid clot after 14 days of incubation		acid clot after 14 days of incubation
Growth at different NaCl levels (%)	2	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+
	6.5	+	-	+	+	+	+	+	+
Microorganism Identification		N=6 *Lactococcus lactis*	N=2 *Enterococcus casseliflavus*	N=1 *Enterococcus casseliflavus*	N=3 *Lactococcus casei*	N=1 *Enterococcus casseliflavus*	N=3 *Lactococcus lactis*	N=1 unknown	N=4 *Enterococcus gallinarum*

Table 5-5: Characteristics of the LAB isolated from S4 (with no munkoyo root extract)

Gram		+	+	+	+
Catalase		-	-	-	-
Cell form		rods	cocci	cocci	rods
Cellular arrangement		Pairs and short chains	Pairs and short chains	Singles, pairs and bunches	pairs
Growth (^{o}C) at	10	+	+	+	+
	?15	+	+	+	+
	37	+	+	+	+
	40	+	+	+	+
	45	+	+	+	+
Ammonia from arginine		-	-	+	+
Gas from glucose		-	-	-	-
Reaction in Litmus milk		Acid production (color change) and acid clot	Acid production (color change) with no acid clot	Acid production (color change) and acid clot after 14 days of incubation	Acid production (color change) and acid clot after 14 days of incubation
Growth at different NaCl levels (%)	2	+	+	+	+
	4	-	+	+	+
	6.5	-	+	+	-
Microorganism Identification		N= 8 *Lactococcus lactis*	N=3 unidentified	N=3 *Lactococcus lactis*	N=1 *Lactococcus lactis*

Table 5-6: Types of yeasts and moulds isolated from S2 (munkoyo root extract) during the fermentation process.

	Yeasts	**Moulds**
yellow munkoyo root extract (**S2**)	*Wickerhamomyces anomalus*	*Penicillium* species
	Rhodotolula mucilaginosa	*Chaetothyriales* species
	Cryptococcus liquifaciens	
	Cryptococcus albidus	
	Pichia fabianii	
	Saccharomyces cerevisiae	
	Sporobolomyces nylandii	
white munkoyo root extract (**S3**)	*Wickerhamomyces anomalus*	*Verticillium* species
	Cryptococcus albidus	*Fusarium* species
	Pichia fabianii	*Aspergillus oryzae*
	Sporobolomyces nylandii	*Penicillium citrinum*
		Penicillium species
chibwantu using yellow munkoyo root extract (**S5**)	*Wickerhamomyces anomalus*	*Penicillium* species
	Cryptococcus laurentii	
	Cryptococcus albidus	
	Rhodotorula mucilaginosa	
	Pichia fabianii	
	Pichia kudriavzevii	
	Saccharomyces cerevisiae	
	Meyerozyma guilliermondii	
	Clavispora lusitaniae	
	Sporobolomyces nylandii	
chibwantu using white munkoyo root extract (**S6**)	*Wickerhamomyces anomalus*	*Aspergillus oryzae*
	Pichia fabianii	
	Pichia ciferri	
	Meyerozyma guilliermondii	
	Sporobolomyces nylandii	
	Rhodotorula mucilaginosa	
chibwantu without munkoyo root extract (**S4**)	*Wickerhamomyces anomalus*	

Chapter 6

Effect of Munkoyo Roots (*Rhynchosia insignis, Rhynchosia heterophylla* and/or *Rhynchosia venulosa*) used in the preparation of a Zambian beverage – Chibwantu, on growth of selected microorganisms

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Chapter 6

6 Effect of Munkoyo Roots (*Rhynchosia* species) used in the preparation of a Zambian beverage – Chibwantu, on growth of selected microorganisms

Abstract

The munkoyo plant (*Rhynchosia* spp) is a sub-shrub found as an under storey plant in the miombo woodlands of Zambia. The root is tuber-like and fibrous, generally yellow or white in colour and known as munkoyo by the local people. The munkoyo roots are used in the preparation of the beverages munkoyo and chibwantu. The effect of the yellow and white munkoyo root extracts and the different concentrations of the root extracts on selected food-borne microorganisms as well as other microorganisms associated with the roots were evaluated. The selected microorganisms consisted of gram positive and gram negative bacteria as well as yeasts. Three different antimicrobial assays were used for the evaluation of the antimicrobial activity. Antimicrobial assay 1; the sensitivity testing of the extracts were determined using the agar diffusion method, assay 2; growth of microorganisms in nutrient broth supplemented with dried root extract was used to determine antimicrobial activity of the munkoyo root extracts and assay 3; growth of microorganisms in different concentrations of munkoyo extract was used to determine antimicrobial activity.

Yields of munkoyo root extracts ranged from 4.12 to 14.4 (%) (w/w) (Table 6-1) with hot water extraction method having the highest yields for both the yellow and white roots, followed by methanolic: water (60:40) extraction and the least with cold water extraction. Higher yields were obtained with the yellow roots compared to the white roots except with hot water extraction.

The results for the antimicrobial activity (Assay 1) showed high activity against *Staphylococcus aureus*, followed by *E. coli, Salmonella* enteric *enteritidis* and *Shigella sonnei*. Gram negative bacteria were more resistant than gram positive bacteria to the

effect of the root extracts. There was less activity against the microorganisms; *Klebsiella variicola*, *Bacillus cereus* and *Bacillus* spp. isolated from the munkoyo roots. Hot water extracts from both the yellow and white munkoyo root types showed activity against *Klebsiella variicola* and methanolic extracts from both the yellow and white munkoyo root types showed activity against *Bacillus cereus* and *Bacillus* spp. There was no effect against *Enterobacter* spp and the yeasts *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens*.

Evaluation of the effect of munkoyo root extracts with assays methods 2 and 3 showed stimulate growth of *E. coli, Salmonella* enteric *enteritidis* and *Shigella sonnei* particularly with the white root.

All the different broth mixtures supplemented with different concentrations of both yellow and white root extracts (Assay 2) inhibited growth of *Staphylococcus aureus* by 24 hours of incubation. *Staphylococcus aureus* growth was not sustained (or was inhibited) with 100% yellow root extract or 100% white root extract for (Assay 3). Bioactive compounds

Broth mixtures supplemented with low concentrations (10 µg/ml) or with high concentrations (40 µg/ml) of either yellow or white root extracts inhibited and broth mixtures supplemented of either yellow or white root extracts stimulated growth of *Bacillus cereus* by 8 hours of incubation. For assay 3, 100% of yellow root extract did not sustain growth of *Bacillus cereus* however 100% of white root extract significantly ($p < 0.01$) sustain growth compared to the growth in nutrient broth. The white root extract showed highest stimulatory effect on the growth of *Bacillus cereus* at the lowest concentration (10%) tested.

The 10% concentration for both the yellow and white root extracts and 25% white root extract showed highest stimulatory effect on the growth of *Lactococcus lactis* subspecies *lactis*. Growth of *Lactococcus lactis* subspecies *lactis* was sustained in 100% yellow and 100% white root extracts though less compared to growth in MRS broth. Growth of test microorganism in 10% yellow and 10% white root extracts was significantly higher ($p < 0.01$) than the control (MRS broth) by the end of the incubation period (Assay 3).

Results for *Saccharomyces cerevisiae* (Assay 3) showed that the lower concentrations of both the yellow and white root extracts (10% and 25%) had a stimulatory effect on growth, however as the concentration of the root extract increased, the yellow root extract showed more influence compared to the white root extract. Both the yellow and white root extracts can sustain growth of yeast but the yellow root extract is more effective compared to the control (YM broth).

The results show that both the yellow and white munkoyo roots possess antimicrobial activity against some of the tested microorganisms. It was also observed that both the roots had stimulatory effect on the growth of microorganisms and that the white root showed higher stimulatory effect on bacteria compared to the yellow root and the yellow root a higher stimulatory effect on yeasts compared to the white root. The observed activity is presumed to be due to the different active compounds present in these roots. The results also suggests that hot water and methanolic munkoyo root extracts can be used for isolation of bioactive compounds for use as antimicrobials as well as compounds for use in stimulation of growth of desirable microorganisms such as probiotics.

Key Words: Munkoyo roots, microbial growth, antimicrobial activity.

6.1 Introduction

The use of medicinal plants all over the world predates the introduction of antibiotic and other modern drugs into the African continent (Akinyemi *et al*., 2005). For a long period of time, plants have been a valuable source of natural products for maintaining human health and welfare (Aiyelaagbe and Osamudiamen, 2009; Nascimento *et al*., 2000). Many studies on the use of plant extracts and/or phytochemicals and their use conducted in the last few years are focused on therapeutic treatment. The exploration of plant extracts and their use in food quality and safety has received little attention (Thangadurai *et al*., 2004). Although synthetic antimicrobials are approved in many countries, the recent trend has been for use of natural preservatives (Negi, 2012). Plants contain innumerable constituents and are valuable source of new and biologically active molecules possessing antimicrobial properties (Negi, 2012).

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Microbes can enter the food chain at different steps, are highly versatile and can adapt to the environment allowing survival, growth and production of toxic compounds (Havelaar *et al*., 2010). The microbial safety of foods continues to be a major concern to consumers, food regulatory agencies and industries throughout the world (Negi, 2012). Although strategies have been used traditionally for the control of microbial spoilage in foods, concerns over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreak of food borne diseases, consequently contamination, spoilage and poisoning of food by microorganisms is a problem yet to be brought under adequately control despite the range of robust preservation techniques available (Rauha *et al*., 2000; Negi, 2012).

The munkoyo plant (*Rhynchosia* species) (Simwamba and Elahi, 1986; Zulu *et al*., 1997) is a sub-shrub found as an under storey plant in the miombo woodlands of Zambia. The root is tuber-like and fibrous (FAO, 1993), generally known as munkoyo by the local people. The munkoyo roots are used in the preparation of the beverages munkoyo and chibwantu. They are added for different reasons including quick liquefaction (fermentation) of the porridge during preparation of the beverage, taste and flavor and as sweetener. A study by Zulu *et al*. (1994) showed that the roots had high concentrations of flavonoids and that fermentation had no effect on the flavonoids. Not much work has been done regarding the biological properties of the munkoyo roots. There is a potential that munkoyo roots besides giving color and flavor to the beverage may also have other properties (microbial growth inhibitors or stimulants), thereby contributing to the microbiological quality and safety of the beverages.

This study was to evaluate the effects of munkoyo (yellow and white) root extracts on selected food related microorganisms.

6.2 Materials and Methods

6.2.1 **Munkoyo root samples**

The samples, dry munkoyo root bunches (100 - 150 g) were purchased and collected at market sites (Soweto and City) in Lusaka, as well as from markets in Livingstone and Chingola.

6.2.2 **Preparation of munkoyo root extract**

The aqueous and methanolic extraction

The aqueous and methanolic extractions of the root extracts were prepared with modifications as described by Akinpelu and Onakoya, (2006) and Omojasola and Awe, (2004). The roots (yellow and white types) were cut into small pieces of about 5 - 10 mm in length using a scissors and ground into fine powder using a grinder (Kenwood True, Compact blender BL380 400W). The fine powder (25 g) was soaked in 125 ml of distilled water (cold water extraction) at room temperature, hot water (80^{o}C) (hot water extraction) in the hot water bath at 80^{o}C or 60% methanol : 40% water (methanolic extraction) at room temperature for both the yellow and white roots. The slurry was vigorously shaken periodically for proper extraction. Extraction was done for five hours. The root extracts were decanted and filtered using filter funnels fitted with Whatman No 1 filter papers. The filtrate was then sterilized using a membrane filter (0.20 μm) and the extract solutions were dried using a rotary evaporator (Thermo Speedvac) at room temperature, freeze/vacuum dried (lyophilization) and stored at 4°C until further use.

The yields collected from the yellow root were; cold water extraction 5.32% (w/w), hot water extraction 12.24% (w/w), methanolic extraction 7.92% (w/w) and yields from the white root; cold water extraction 4.36% (w/w), hot water extraction 14.4% (w/w) and methanolic extraction 4.12% (w/w) (Table 6-1).

6.2.3 Preparation of test microorganisms

Pure cultures of *E. coli* (ATCC 10418), *Staphylococcus aureus, Salmonella* enteric *enteritidis* (strain C), *Shigella sonnei*, (strain H) and *Lactococcus lactis* subspecies *lactis* were obtained from the culture collection of the Food Science department of the University of the Free State and *Saccharomyces cerevisiae* from the culture collection of the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State. *Bacillus cereus, Klebsiella variicola, Bacillus* species, *Enterobacter* species, *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens* were isolated from the munkoyo roots.

The bacteria were sub-cultured in nutrient broth (Merck Biolab, Gauteng South Africa), except for *Lactococcus lactis* subspecies *lactis* which was sub-cultured in MRS broth (Fluka). *E. coli, Staphylococcus aureus, Salmonella* enteric *enteritidis* and *Shigella sonnei* were incubated at 37°C for 18 hours. *Bacillus cereus, Klebsiella variicola, Bacillus* species *Enterobacter* species were incubated at 30°C for 18 hours. The yeasts; *Saccharomyces cerevisiae, Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens* were cultured in Yeast Malt (YM) broth and incubated at 30°C for 18 hours. *E. coli, Salmonella* enteric *enteritidis, Shigella sonnei, Klebsiella variicola* and *Enterobacter* species to a concentration of 10^8 cfu/ml, *Staphylococcus aureus, L. lactis* subspecies *lactis, Bacillus cereus, Rhodotorula mucilaginosa* and *Cryptococcus* *liquefaciens* were standardized to a concentration of 10^7 cfu/ml, *Bacillus* species to a concentration of 10^6 cfu/ml and *Saccharomyces cerevisiae* to a concentration of 10^5 cfu/ml.

Standardisation of the test microorganisms

To determine the turbidity of the test microorganisms that approximated bacterial density of 10^8 cfu/ml for *E. coli*, *Salmonella* enteric *enteritidis*, *Shigella sonnei*, *Klebsiella variicola* and *Enterobacter* species, 10^7 cfu/ml for *Staphylococcus aureus*, *L. lactis* subspecies *lactis*, *Bacillus cereus*, *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens* , 10^6 cfu/ml for *Bacillus* species and 10^5 cfu/ml for *Saccharomyces cerevisiae*, the 18 hour test microorganism culture was diluted in sterile broth, same as the one used to culture the test microorganism. The test microorganism culture was diluted to optical density (absorbance) of less than 0.1 and cfu/ml of the dilutions was determined (Table 6.2). Respective dilutions of test microorganism were used as initial inoculums for the experiments.

6.2.4 **Evaluation of the effect of the munkoyo roots on growth of selected microorganisms**

6.2.4.1 Screening for antimicrobial potential using the agar diffusion method - Assay 1

The sensitivity testing of the extracts were determined using the agar diffusion method (Krasemann and Hildenbrand, 1980). The inoculums were pipetted onto the surface of Mueller-Hinton agar (Merck Biolab, Gauteng South Africa) plates. Two concentrations of the root extract were prepared; 100 µg/ml and 500 µg/ml in nutrient broth medium. The concentration of 500 µg/ml was used only for the yeasts *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens*. Afterwards filter paper discs (5mm in diameter) saturated with extracts (50 µl) were placed on the surface of each inoculated plate. The plates were then incubated at 37°C for 24 hours for *E. coli, Staphylococcus aureus, Salmonella* enteric *enteritidis* and *Shigella sonnei* and at 30°C for 24 hours for *Bacillus cereus, Klebsiella variicola, Bacillus* species, *Enterobacter* species, *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens* and further 24 hours for *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens* after which all the plates were observed for zones of inhibition (Table 6-3 and 6-4).

6.2.4.2 Effect of the munkoyo root extract on growth of selected microorganisms- Assay 2

Growth of selected microorganisms in nutrient broth supplemented with root extract was used to determine the effect of the mukoyo root extracts**.** The methanolic (60%) root extract and hot water root extract were incorporated into the nutrient broth at different levels; 10 µg/ml, 20 µg/ml and 40 µg/ml for both yellow and white root extracts and vortex for proper mixing of the nutrient broth with the root extract supplement. The control was 100% nutrient broth with no root extract supplement. 50 µl of the standardized (Table 6-2) test microorganisms was added to test tubes with 6ml of the mixture at different concentrations. *E. coli* and *Staphylococcus aureus* were added to the mixture with methanolic root extract supplement. *Salmonella* enteric *enteritidis*, *Shigella sonnei* and *Bacillus cereus* to the mixture with hot water root extract supplement as the extracts showed increased activity in assay 1. The inoculated mixtures were incubated at 37^{o}C and optical density of each mixture was recorded at three hour interval for 9 hours and after 24 hours of incubation. Three replications were performed for each type of mixture and the experiment was duplicated (n= 3 x 2).

6.2.4.3 Effect of the munkoyo root extract on growth of selected microorganisms- assay 3

Growth of selected microorganisms in respective broth supplemented with munkoyo root extract was used to determine the effect of the munkoyo root extracts. The roots (yellow and white types) were cut into small pieces of about five - 10 mm in length using a scissors and 50 g of each root type was soaked in 300ml of distilled water (room temperature) for three hours. The slurry was vigorously shaken periodically for proper extraction. The root extracts were decanted and filtered using filter funnels fitted with Whatman No 1 filter papers. The filtrate was then sterilized using a membrane filter (0.20 μm). The volumes obtained from the roots after extraction and sterilisation were; yellow 80.0% (v/v) and white 70.3% (v/v). Different concentrations of the root extracts (yellow and white) were prepared (10%, 25%, 50%, 75% and 100%) with nutrient broth for the inoculation of *E. coli, Salmonella* enteric *enteritidis* and *S. sonnei*, *Staphylococcus aureus* and *Bacillus cereus*, MRS broth for the inoculation of *L. lactis* subspecies *lactis* and YM broth for the inoculation of *Saccharomyces cerevisiae*. The test microorganisms were all standardized. The control was 100% nutrient broth. Test microorganisms were first grown in respective broth for 18 hours before standardisation and 50μl of this culture was added to six ml of the different percentages of the root extracts and three replications were performed for each test and the experiment was duplicated.

The effect of the root extracts on growth of test microorganisms was determined by measuring the optical density in the spectrophotometer (HACH DR/2000, Direct reading Spectrophotometer) by comparing the sample readouts against non-inoculated broth. The optical density (absorbance reading at 620 nm) of each sample was documented at three hour intervals for the period of 24 hours.

6.3 Results and Discussions

The munkoyo root extracts yield ranged from 4.12 to 14.4% (w/w) (Table 6-1) with hot water extraction method having the highest yields for both the yellow and white roots. For the aqueous extraction for assay 3, the yields were 70.3% (v/v) for white and 80.0% (v/v) for yellow roots. The yellow roots had higher yields compared to the white roots.

6.3.1 Evaluation of the effect of the munkoyo roots on growth of selected microorganisms

The results of the study show that both the yellow and white munkoyo roots possess some antimicrobial activity against some of the tested microorganisms. From the results, it was also observed that both the roots had some stimulatory activity as well and that the white root showed higher stimulatory activity compared to the yellow root (Fig. 6-1 to 6-11). The observed activity is presumed to be due to the different active compounds present in the munkoyo roots. The data pertaining to the antimicrobial potential of the munkoyo root extracts are presented in Tables 6-2 and 6-3.

6.3.1.1 Microbial assay 1

The degree of antimicrobial activity varied depending on the tested microorganism as well as the extraction method used at a concentration of 100 µg/ml (Table 6-1) The yellow root showed higher activity compared to the white root (Table 6-4). The methanolic extract had higher activity followed by the hot water extraction and cold water extraction had the least activity for both the yellow and white roots (Table 6-4). The highest antimicrobial activity was shown against *Staphylococcus aureus*, then *E. coli*, *Salmonella* enteric *enteritidis* and *Shigella sonnei*. Other studies (Agatemor, 2009; Nwinyi *et al*., 2009) have reported similar observations that gram negative bacteria are more resistant than gram positive bacteria to antimicrobial agents. There was less activity against the microorganisms isolated from the roots. The methanolic extracts showed activity against *Bacillus cereus* and *Bacillus* species, with the yellow root extract showing significant activity. For *Klebsiella variicola* some insignificant activity was shown from hot water extracts of both the yellow and white roots. All the different root extracts showed no antimicrobial activity against *Enterobacter* species, *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens.*

6.3.1.2 Microbial assay 2

E. coli (methanolic (60%) extraction)

Antimicrobial activity of the methanolic munkoyo root extract was only observed within the first three hours of incubation and thereafter growth for all the parameters tested was stimulated to almost 3-fold that of the control as shown in Fig. 6-1.

For the extract from the white munkoyo roots, there was a significant growth increase ($p < 0.01$) with use of increased concentrations of the extract. With addition 10 μg/ml of white munkoyo root extract an absorbance reading of 0.505 was observed. Absorbance readings for mixtures with 20 μg/ml and 40 μg/ ml of white munkoyo root extracts were 0.674 and 0.806 respectively. With the addition of the yellow root extract however the contrary was observed. At the lowest concentration of 10 μg/ml of yellow munkoyo root extract the highest absorbance reading of 0.515 was observed while at 20 μg/ml and 40 μg/ml, the readings of 0.466 and 0.497, respectively, were observed. The control (nutrient broth) absorbance reading was 0.251.

Staphylococcus aureus (methanolic extraction)

Antimicrobial activity of munkoyo root extract was observed for all the parameters tested, except for the mixture with yellow root extract supplement at 20 μg/ml concentration, which showed growth closely related to that of the control (Fig. 6-2).

Salmonella enteric *enteritidis* (Hot water extraction)

Antimicrobial activity was observed within the first six hours and thereafter growth was stimulated. After 24 hours of incubation all the parameters had increased by more than 3-fold that of the control, except for the mixture with yellow munkoyo root extract supplement at concentration 10 μg/ml; which had the least stimulation in growth as shown in Fig. 6-3. The extract from the white munkoyo roots had significantly higher effects of stimulation ($p < 0.01$) than the extract from the yellow munkoyo roots at all the concentrations tested.

Shigella sonnei (Hot water extraction)

Antimicrobial activity was observed for the whole period of incubation (24 hours) particularly for the mixture with yellow root extract supplement at a concentration 20 µg/ml. There was higher inhibitory activity from the yellow root extract compared with the white. However, after nine hours of incubation, growth from the mixtures with white extract had significantly higher stimulation in growth compared to the mixtures with yellow extract as shown in Fig. 6-4.

Bacillus cereus (Hot water extraction)

Antimicrobial activity against the test microorganism (Table 6-5) was observed, for the different mixtures supplemented with different concentrations of the yellow or white root extract, within the first three hours of incubation as optical density readings were less compared to the control (nutrient broth with no root extract supplement) (Fig. 6-5). At 8 hours of incubation, broth supplemented with 40 µg/ml of yellow munkoyo root extract continued to show antimicrobial activity against the test microorganism. However, for broth supplemented with 20 µg/ml of yellow munkoyo root extract, 10 µg/ml of yellow munkoyo root extract and 10 µg/ml of white munkoyo root extract, growth increased compared to the control.

6.3.1.3 Microbial assay 3

E. coli

Both the yellow and white root extracts (100%) allowed growth of the microorganism however, for the yellow extract growth was lower than the control (100% nutrient broth). The results indicate that the extracts have compounds that allow growth of *E. coli*. In general, within the first three hours of incubation, absorbance readings were lower than control readings, except for 10% and 25% of both the yellow and white root extracts. Thereafter there was stimulation of growth particularly from the white root extracts (Fig. 6-6).

Salmonella enteric *enteritidis*

Within the first three hours of incubation, growth of the test microorganism in different concentrations of the munkoyo root extracts was closely related to growth in the control (100% nutrient broth). After three hours of incubation, growth of the test microorganism in 100% yellow and 100% white root extracts was less compared to growth in control. The other different concentrations of root extracts from both the yellow and white roots stimulated growth of the test microorganism when compared with the control; particularly the white root extracts (Fig. 6-7).

Shigella sonnei

The growth trend of *Shigella sonnei* was similar to that of *E. coli* at three hours of incubation, growth of test microorganism in different concentrations of the munkoyo root extracts was less compared to the control and for 100% of the yellow and 100% white root extracts growth remained less compared to the control. After three hours of incubation, there was stimulated growth for the other different concentrations of root extracts from both the yellow and white roots particularly from the white root extracts (Fig. 6-8).

Staphylococcus aureus

There was almost no growth in both the yellow and white root extracts (100%) and at 75% and 50% yellow root extracts growth was below that of the control. Within the first nine hours of incubation, growth for the white root extracts at 75% and 50% was below the control and thereafter increased above that of the control (Fig. 6-9). Overall, growth of *Staphylococcus aureus* in the different percentages of root extract was lower compared to all the other tested microorganisms (Table 6-6).

Lactococcus lactis subspecies *lactis*

From the results, there was growth of *Lactococcus lactis* subspecies *lactis* in 100% of the munkoyo root extracts; therefore both the yellow and white munkoyo roots have properties that can sustain growth of the test microorganism. Low concentrations of the munkoyo root extract (10%) for both the yellow and white munkoyo roots had the highest growth, significantly higher than MRS broth ($p < 0.01$). Generally the white munkoyo root showed more stimulatory activity compared to the yellow. Fig. 6-10 shows the growth patterns of *Lactococcus lactis* subspecies *lactis* at different concentration of the munkoyo root extracts.

Saccharomyces cerevisiae

The yellow munkoyo root had significant growth stimulation of *Saccharomyces cerevisiae*. 100% of the yellow root extract increase growth significantly higher ($p < 0.01$) than the control (YM broth) towards the end of the incubation period. Even though 100% white munkoyo root extract showed lower influence on growth of test microorganism compared to 100% yellow munkoyo root extract and the control (YM broth), the extract still could sustain growth of the yeast. At lower concentration of 10% munkoyo root extract, both the yellow and white munkoyo root extracts had similar effects, but as the concentration of the munkoyo root extract increased, the yellow root extract had higher effect compared to white root extract. This could explain the association of the yellow root with diverse yeasts compared to the white root (unpublished results). Fig. 6-11 shows the growth patterns of *Saccharomyces cerevisiae* at different concentration of the munkoyo root extracts.

Bacillus cereus

There was minimal growth of test microorganism in the 100% yellow root extract. For the 100% white root extract, growth of test microorganism was less compared to the control in the initial stages of incubation however, after nine hours of incubation, growth increased, exceeding that of the control.

For all (25%, 50% and 75%) concentrations of the yellow root extracts tested, growth was less than that of the control and only increased after three hours of incubation. For the other parameters, all the different concentrations of the white root extract and the 10% yellow root extract, growth was higher than that of the control from the initial stage of inoculation. The growth patterns with the different percentages of the root extracts varied for *Bacillus cereus* compared to the other tested microorganisms (Fig. 6-12).

For the other parameters, the different percentages of the white root extract and 10% of yellow root extract tested, growth was above that of the control from start of growth. However, within the first three hours of incubation, for 25%, 50% and 75% of the yellow root extracts, growth was below that of the control and increase only after three hours.

6.4 Conclusions

The study indicates that both the root types (yellow and white) possess different active compounds effecting different antimicrobial and microbial growth stimulation patterns. A possible application of munkoyo roots extracts as natural antimicrobials and or as supplements for probiotic organisms is possible.

Particularly the yellow root methanolic extract possess antimicrobial properties with inhibitory effects against *Staphylococcus aureus*. A broader range of gram positive microorganisms should be tested. Munkoyo root extracts stimulated growth of test microorganisms including *E. coli*, *Lactococcus lactis* subspp *lactis* and yeast *Saccharomyces cerevisiae*. Different concentrations of the extracts affected growth of microorganisms differently. The results indicate that munkoyo root extracts can stimulate growth of different types of microorganisms such as bacteria (gram positive and gram negative), yeasts and probably other types of microorganisms.

Munkoyo root stimulation of both the desirable microorganisms such as LAB as well as the undesirable microorganisms such as coliforms and spoilage yeasts compromise the quality of beverages prepared with the roots. The results explain the increased growth of microorganisms observed during fermentation of chibwantu beverage (Chapter 5) a possible contribution to the quick fermentation as well as spoilage of the beverage.

Nonetheless, the results form a strong basis for further studies on the active components of the roots for possible use in acceleration of fermentation processes (stimulation of growth of essential microorganism such as lactic acid bacteria used as starter cultures) for preservation of foods and improved food quality and safety.

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Table 6-1: Yields of munkoyo root extracts

Munkoyo root type extracts (% (w/w))	**Extraction method**		
	Methanolic (60%) extraction	**Hot water extraction**	**Cold water extraction**
Yellow	7.92	12.24	5.32
White	4.12	14.40	4.36

Table 6-2: Standardisation of test microorganisms

Test microorganism	**Optical Density (absorbance (620nm))**	**CFU/ml**
E. coli	0.083	2.3 x 10^{8}
Klebsiella Variicola	0.068	2.8 x 10^{8}
Enterobacter species	0.077	6.0 x 10^{8}
Salmonella enteric *enteritidis*	0.068	6.24 x 10^{8}
Shigella sonnei	0.067	6.12 x 10^{8}
Rhodotorula mucilaginosa	0.050	1.8 x 10^{7}
Crptococcus liquefaciens	0.083	1.7 x 10^{7}
Bacillus cereus	0.090	3.6 x 10^{7}
Staphyloccocus aureus	0.051	1.0 x 10^{7}
Lactococcus lactis subsp *lactis*	0.045	3.4 x 10^{7}
Bacillus species	0.037	3.8 x 10^{6}
Saccaromyces cerevisiae	0.051	5.2 x 10^{5}

Table 6-3: Antimicrobial activity of munkoyo root extracts against selected test microorganisms.

Test Microorganism	Amount of root extract (µg/ml)	CFU/ml	Mean Zones of inhibition (mm) n=5					
			Extraction method					
			W MeOH	W Cw	W Hw	Y MeOH	Y Cw	Y Hw
E.coli	100	1.5 x10^{8}	10	11	10	13	8	10
Klebsiella Variicola	100	2.8 x10^{8}	0	0	6	0	0	6
Enterobacter species	100	6.0 x 10^{8}	0	0	0	0	0	0
Bacillus species	100	3.8 x10^{6}	8	0	0	12	0	0
Bacillus cereus	100	3.6 x10^{7}	6	0	0	10	0	0
Staphyloccocus aureus	100	1.0 x10^{7}	7	0	6	17	8	10
Salmonella enteric *enteritidis*	100	5.6 x10^{8}	10	0	9	9	8	10
Shigella sonnei	100	7.4 x10^{8}	9	6	9	7	7	9
Rhodotorula mucilaginosa	100	1.8 x10^{7}	0	0	0	0	0	0
Crptococcus liquefaciens	100	1.0 x10^{7}	0	0	0	0	0	0
Rhodotorula mucilaginosa	500	1.8 x10^{7}	0	0	0	0	0	0
Crptococcus liquefaciens	500	1.0 x10^{7}	0	0	0	0	0	0

WMeOH = White root methanolic extraction (60% methanol)
Wcw = White root cold water extraction
WHw = White root hot water extraction (80^{o}C)
YMeOH = Yellow root methanolic extraction (60% methanol)
Ycw = Yellow root cold water extraction
YHw = Yellow root hot water extraction (80^{o}C)

Table 6-4: Mean Zones of inhibition (mm) of test microorganisms using different munkoyo root extraction methods.

Plant extract	Extraction method			
	Methanolic	Hot water	Cold water	Total
Yellow	9.0 ± 5.8	6.1 ± 4.8	4.2 ± 4.0	**6.4 ± 2.4**
White	6.2 ± 3.8	5.1 ± 4.1	2.7 ± 4.2	**4.7 ± 1.8**
Mean	**7.6 ± 5.0**	**5.6 ± 4.3**	**3.5 ± 4.1**	

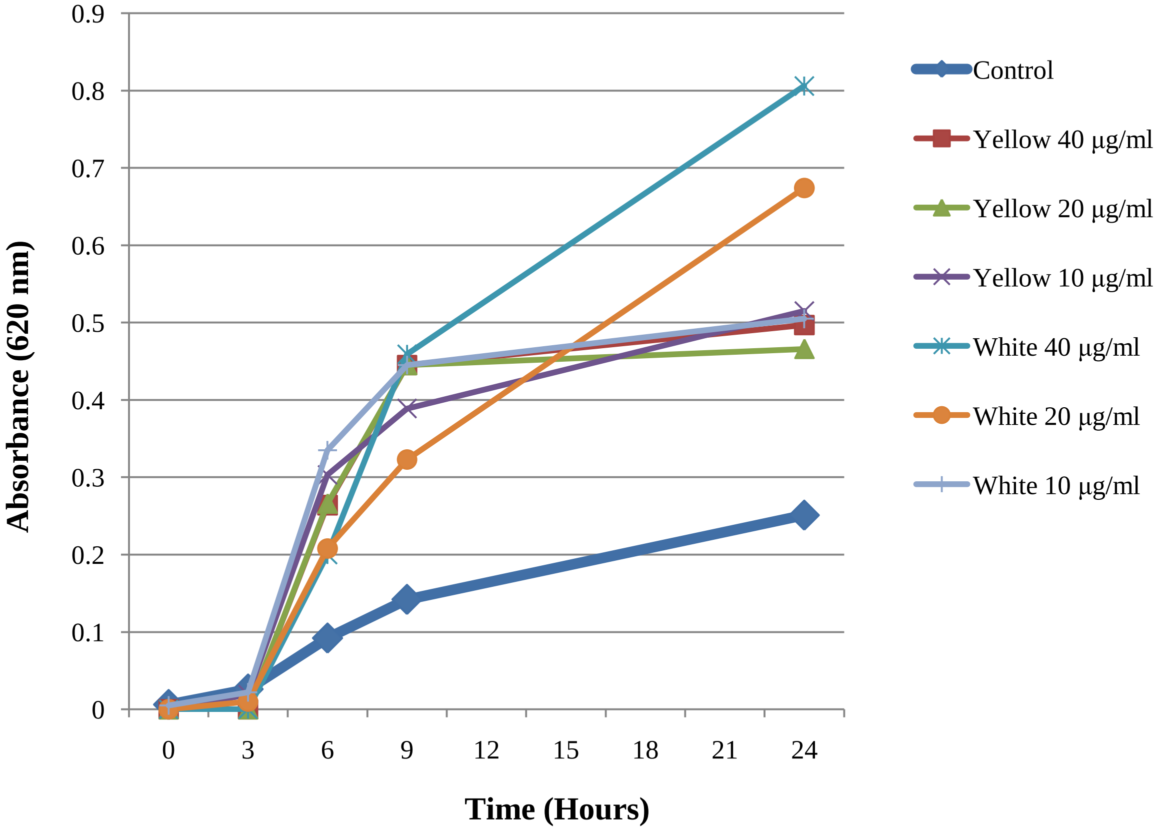

Figure 6-1: Effect of supplementing nutrient broth with different concentrations of methanolic (60%) munkoyo root extracts on the growth of *E. coli*

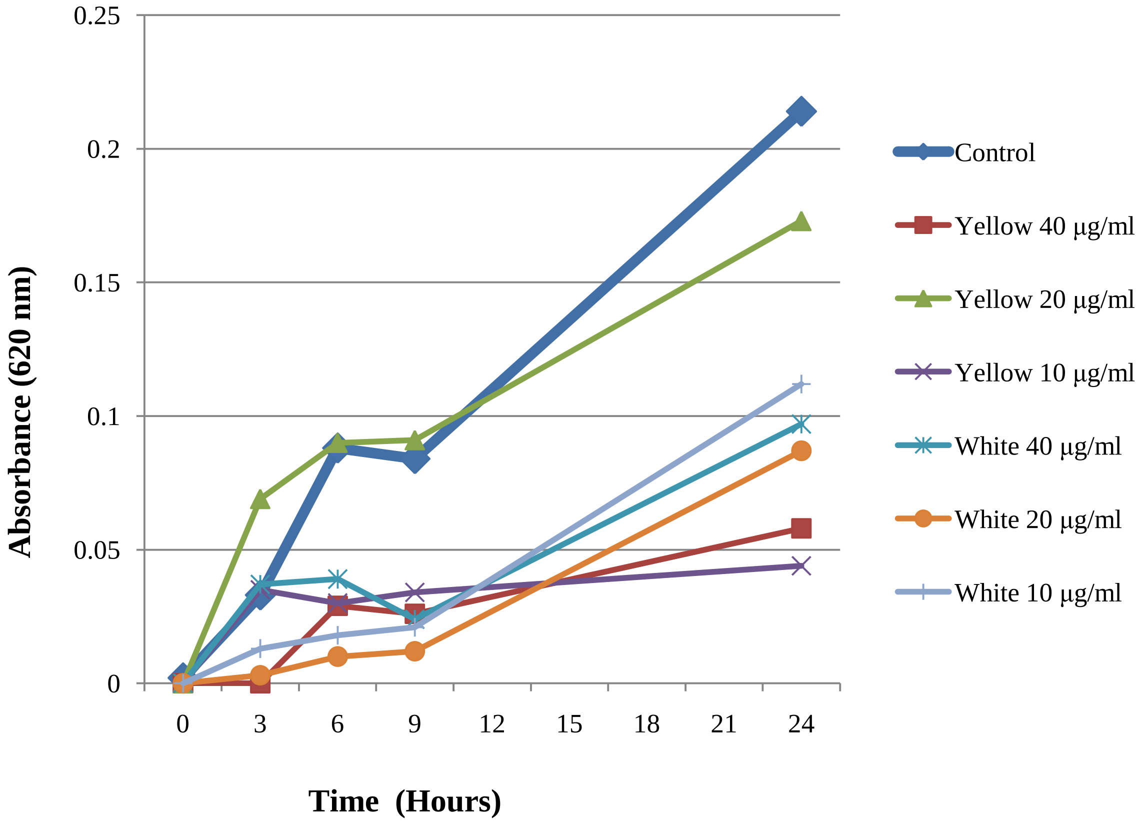

Figure 6-2: Effect of supplementing nutrient broth with different concentrations of methanolic (60%) munkoyo root extracts on the growth of *Staphylococcus aureus*

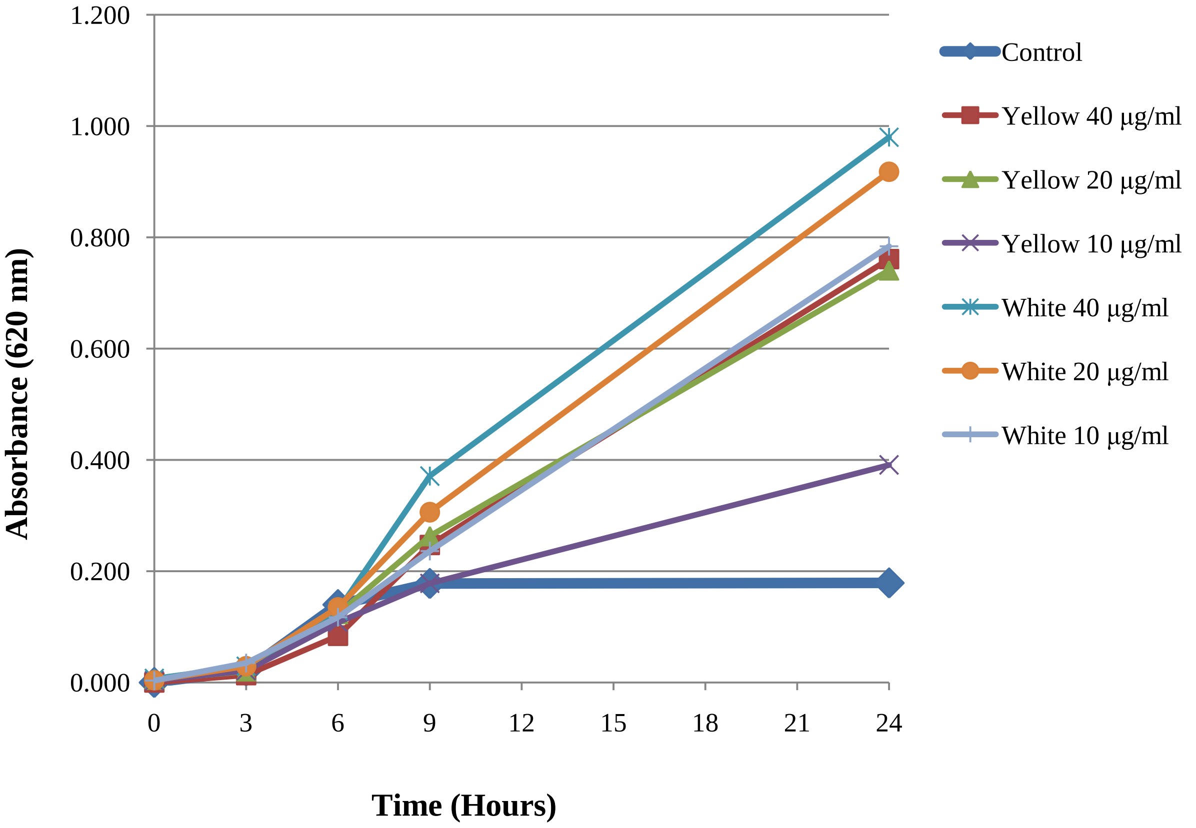

Figure 6-3: Effect of supplementing nutrient broth with different concentrations of hot water extracted munkoyo root extracts on the growth of *Salmonella* enteric *enteritidis*

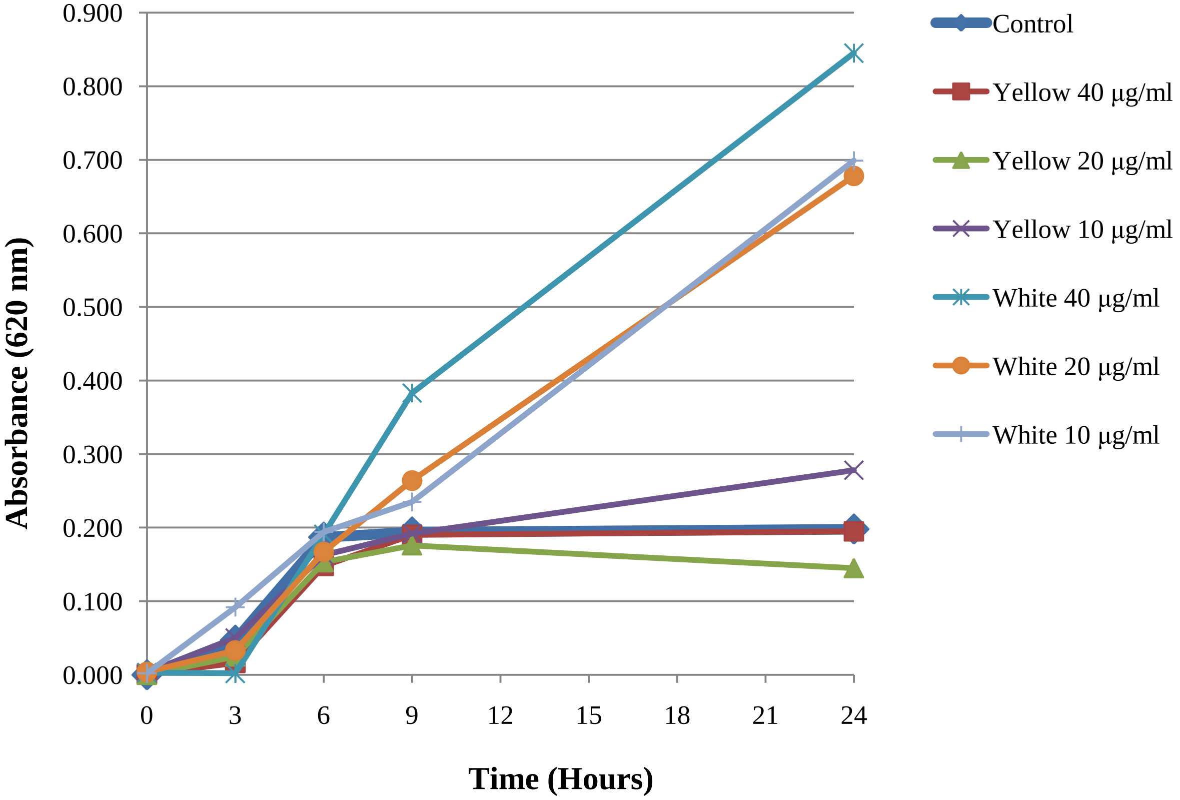

Figure 6-4: Effect of supplementing nutrient broth with different concentrations of hot water munkoyo root extracts on the growth of *Shigella sonnei*

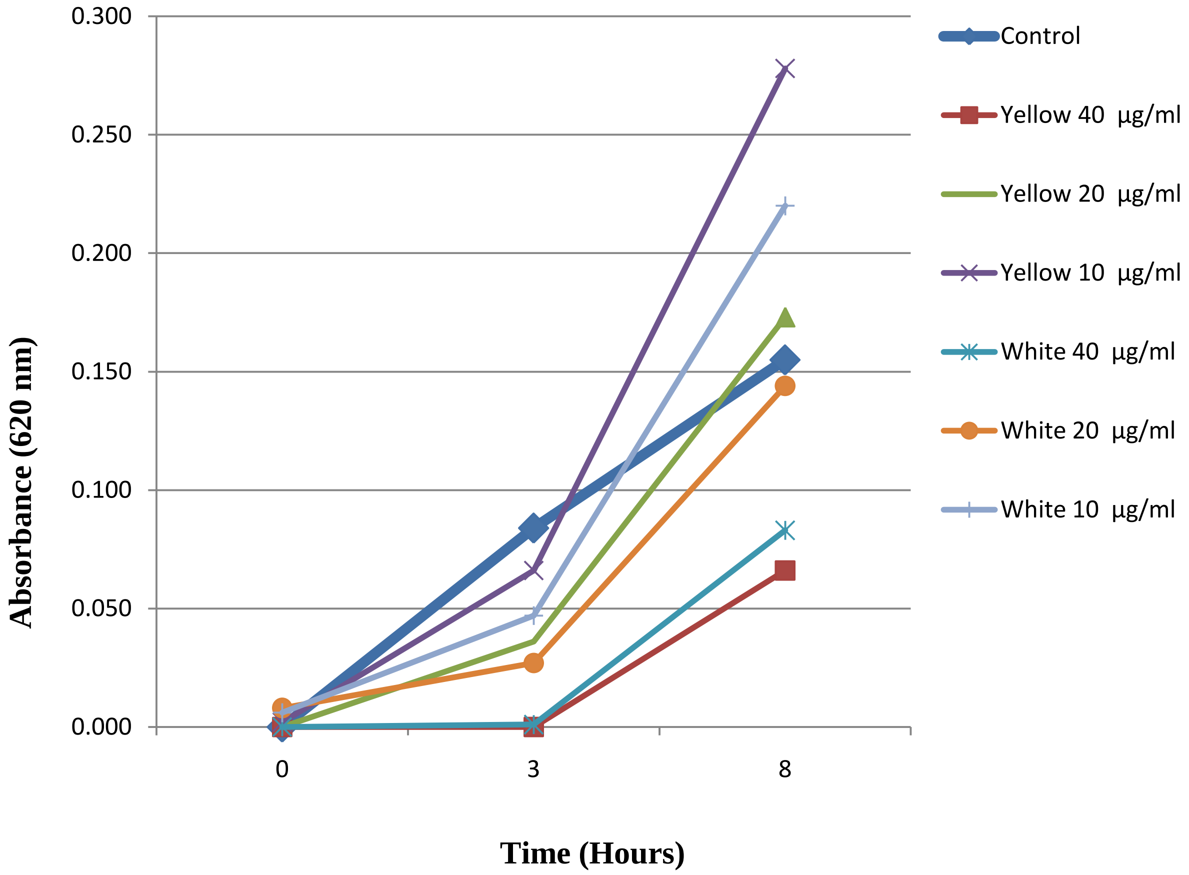

Figure 6-5: Effect of supplementing nutrient broth with different concentrations of hot water munkoyo root extracts on the growth of *Bacillus cereus*

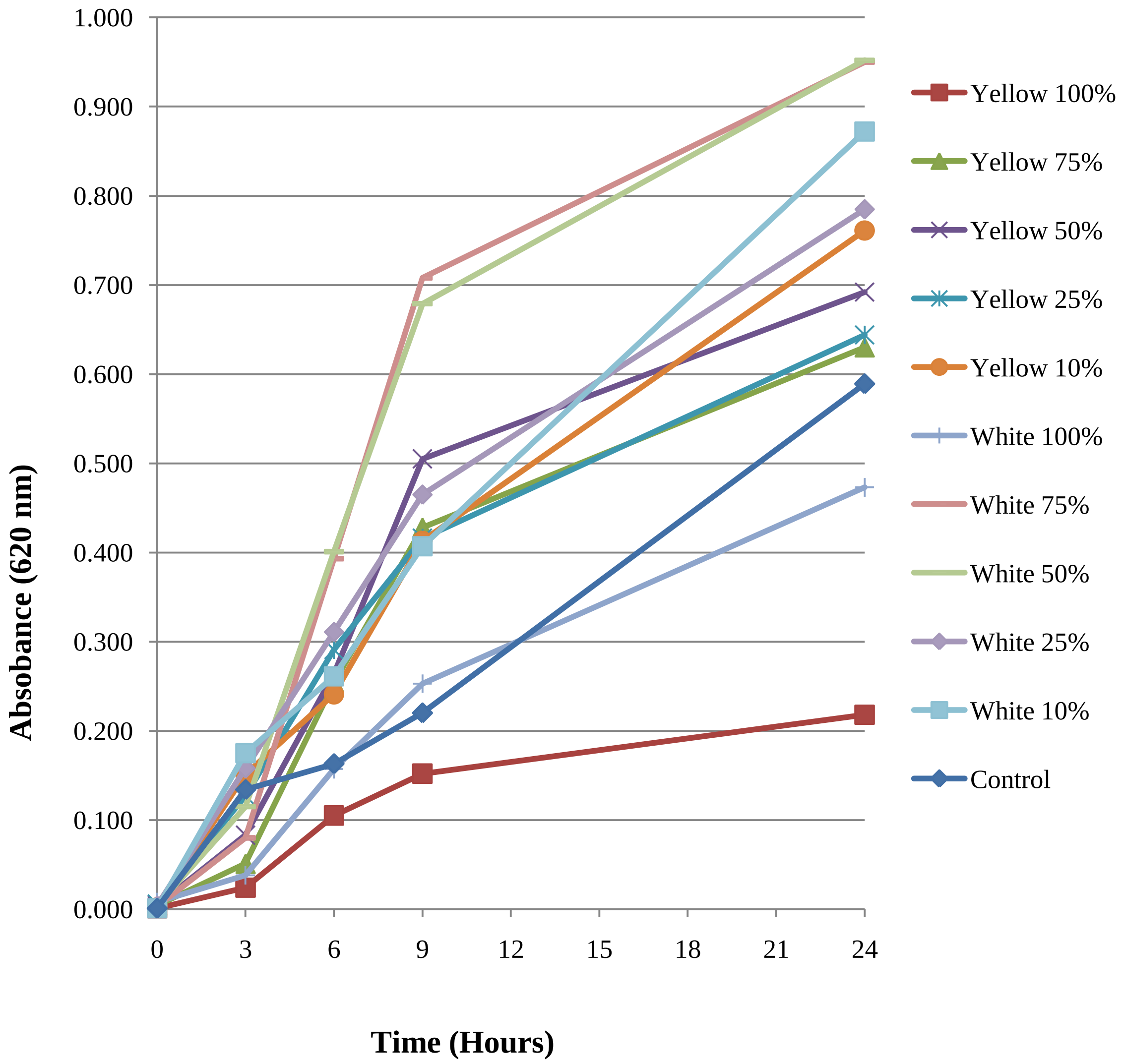

Figure 6-6: Growth patterns of *E. coli* in different concentration (%) of munkoyo root extracts.

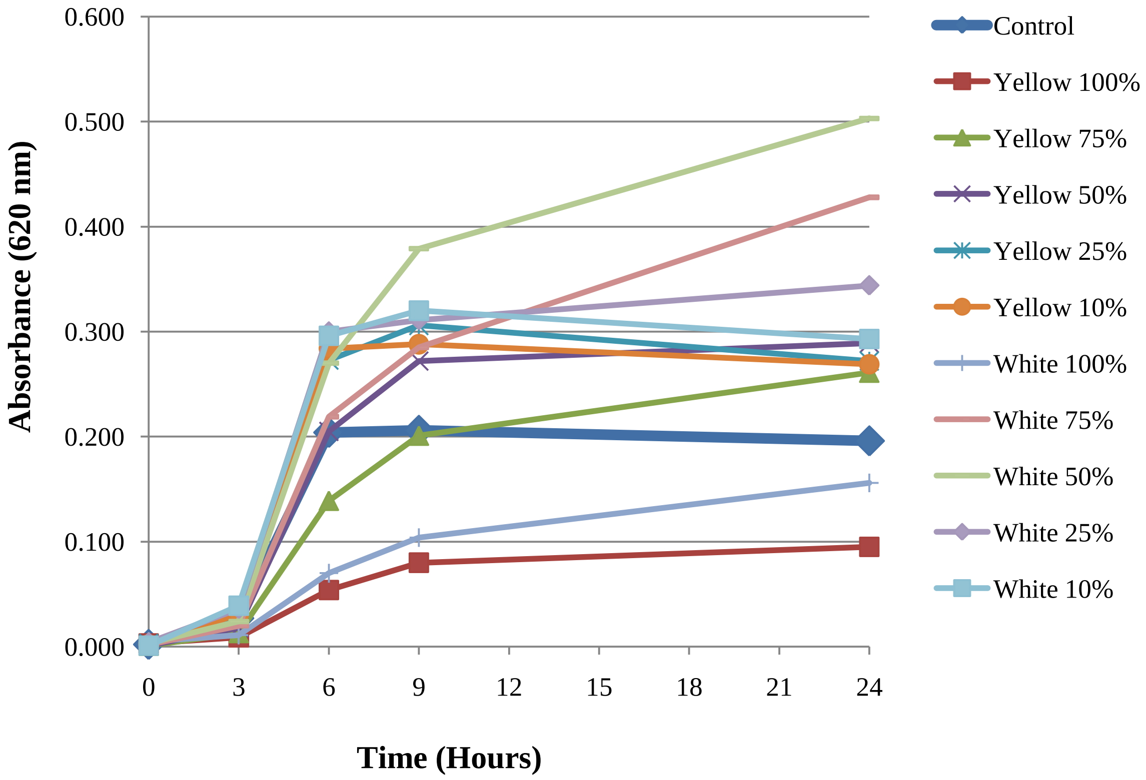

Figure 6-7: Growth patterns of *Salmonella* enteric *enteritidis* in different concentration (%) of munkoyo root extracts.

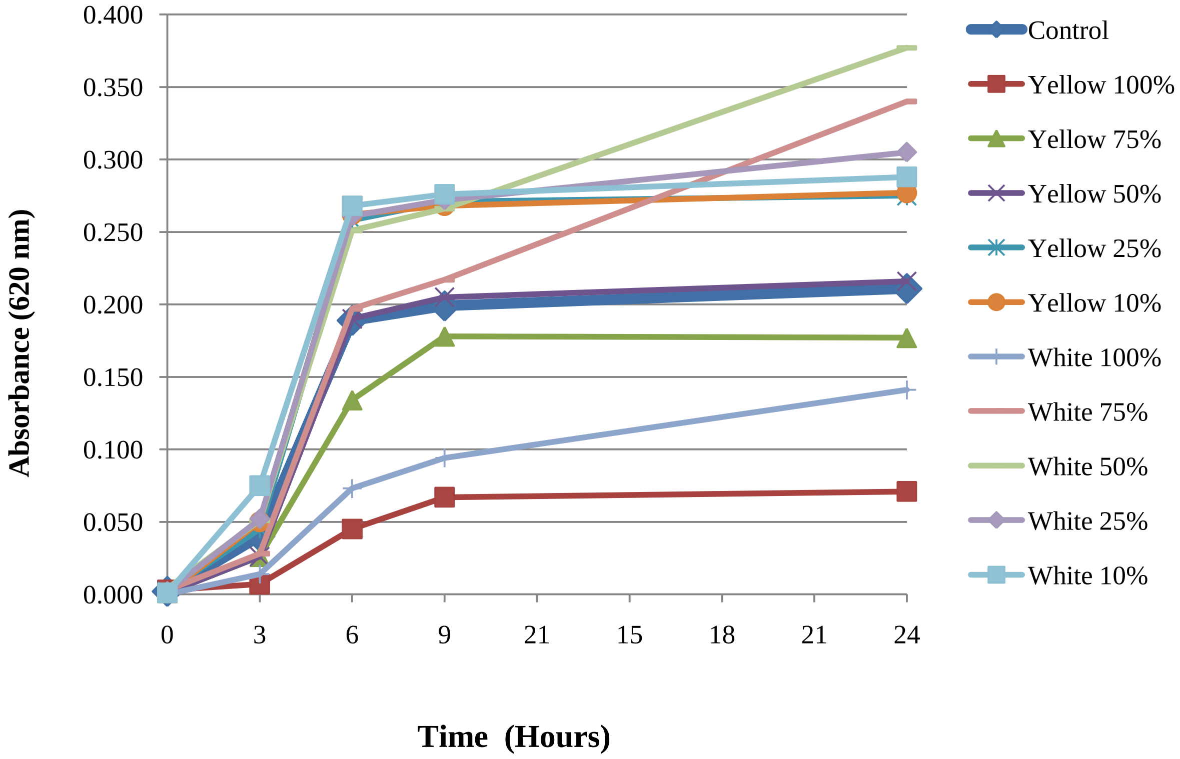

Figure 6-8: Growth patterns of *Shigella sonnei* in different concentration (%) of munkoyo root extracts.

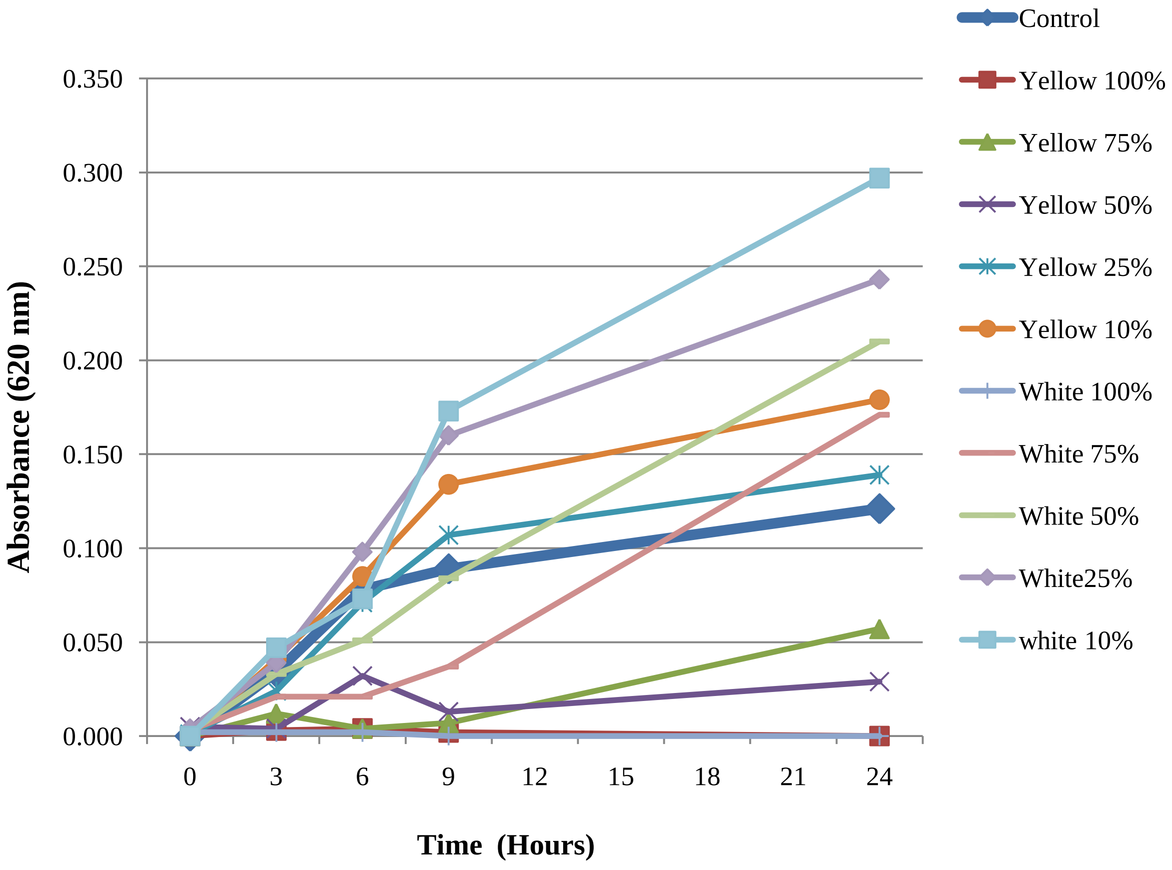

Figure 6-9: Growth patterns of *Staphylococcus aureus* in different concentration (%) of munkoyo root extracts.

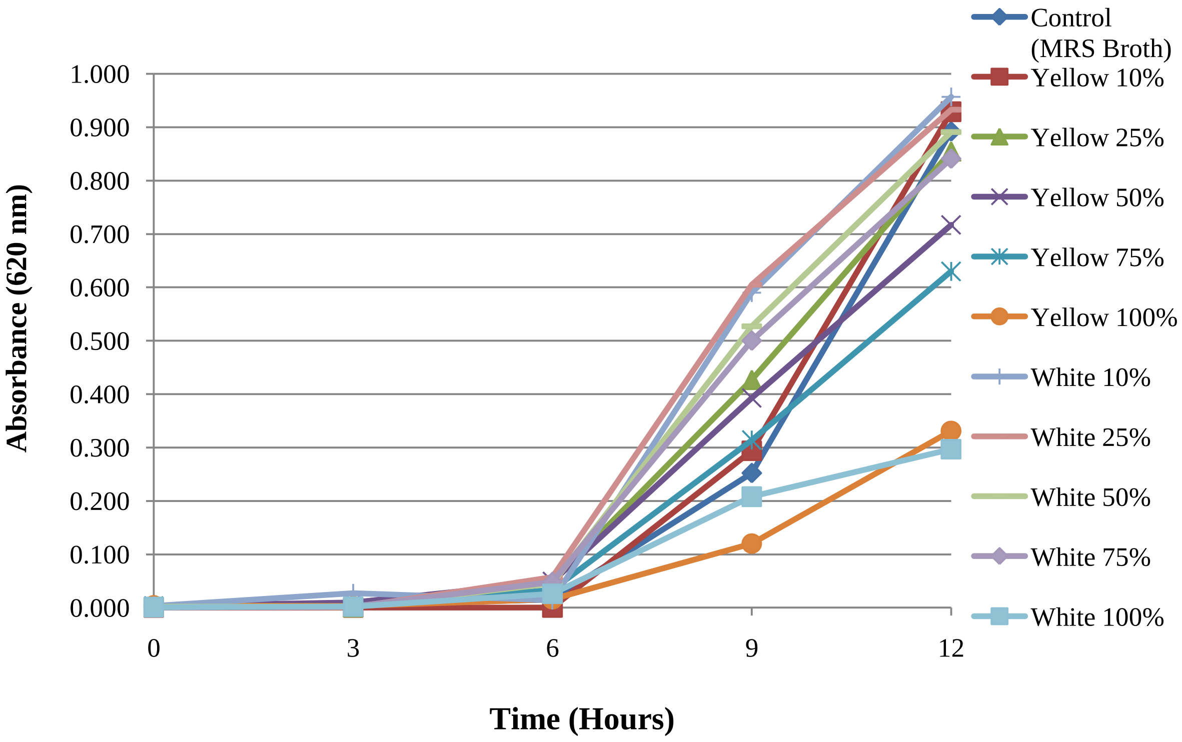

Figure 6-10: Growth patterns of *Lactococcus lactis* subspecies *lactis* in different concentration (%) of munkoyo root extracts.

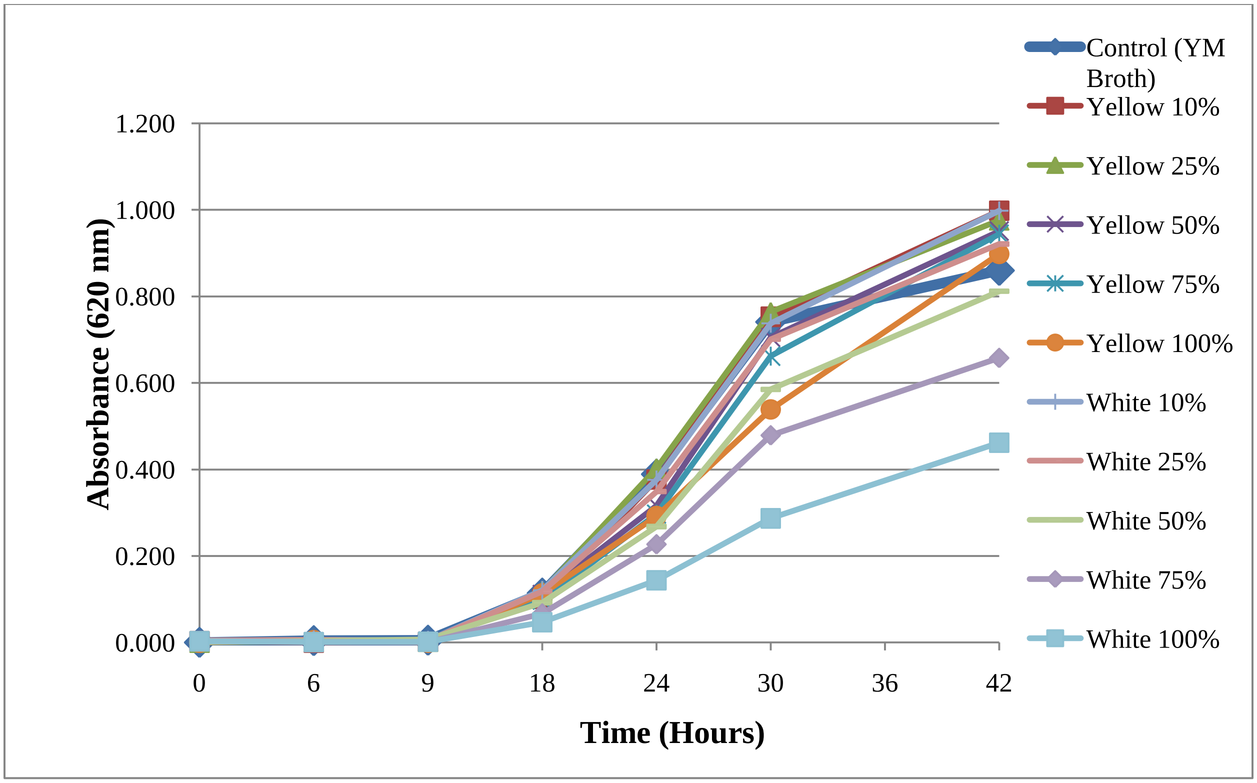

Figure 6-11: Growth patterns of *Saccharomyces cerevisiae* in different concentration (%) of munkoyo root extracts.

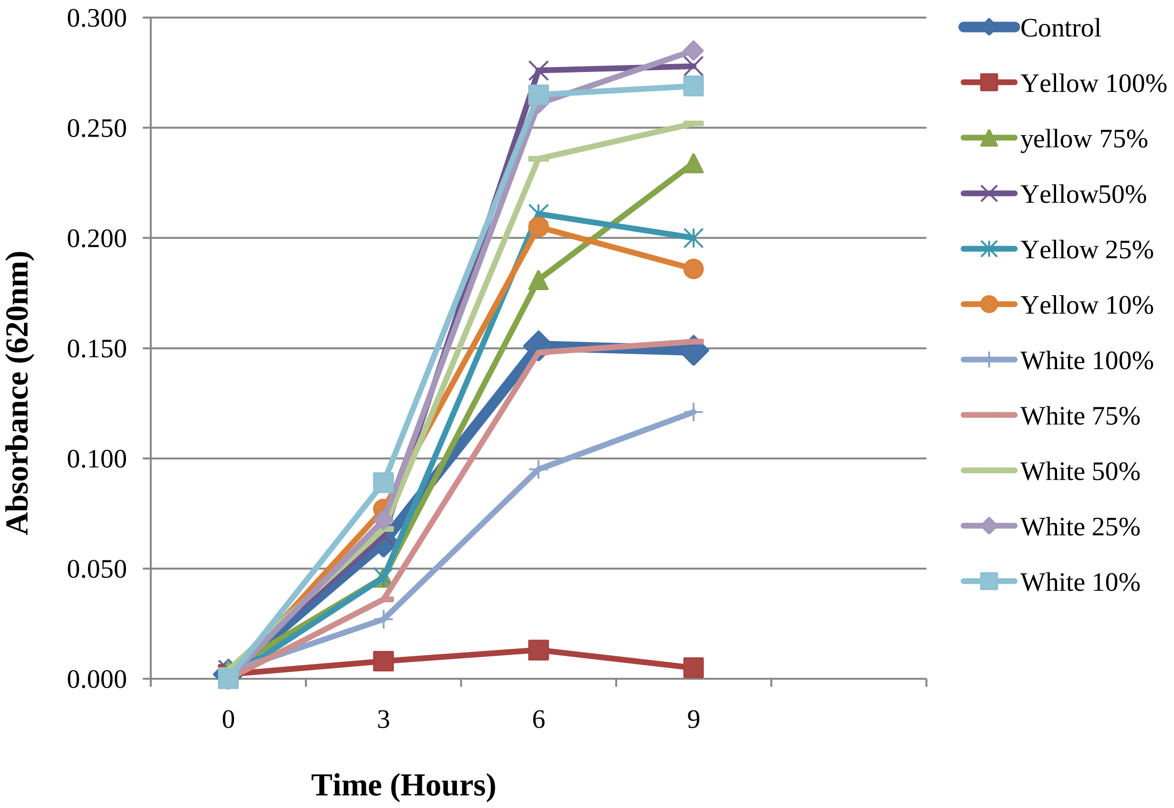

Figure 6-12: Growth patterns of *Bacillus cereus* in different concentration (%) of munkoyo root extracts

Chapter 7

Survival of selected food borne pathogenic microorganisms during fermentation of a Zambian beverage- Chibwantu

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Chapter 7

7 Survival of selected food borne pathogenic microorganisms during fermentation of a Zambian beverage- Chibwantu

Abstract

Fermentation is one of the oldest and effective ways of preserving foods and beverages. Fermented cereal based foods (gruels) are common weaning foods in Zambia. There are a number of studies that have been carried out on the survival abilities of pathogenic bacteria in cereal based fermented foods and results have shown that cereal gruels with pH < 4.0 significantly suppress the growth of food borne pathogens (*Salmonella* spp., *Shigella* spp., *E. coli*, *Campylobacter jejuni*, *Staphylococcus aureus* and *Bacillus cereus*). These food borne pathogens are among the causative agents of diarrheal diseases in infants and children through consumption of contaminated food. Diarrhea is the 3rd leading cause of clinic visits and death for children under five years of age in Zambia after pneumonia and malaria and is the major cause of complications in children with severe acute malnutrition.

Chibwantu is a cereal based fermented beverage prepared and consumed among the rural and urban population of Zambia. It is also used as a weaning and/or complementary food for children. The growth pattern and the survival of selected pathogenic microorganisms (*Staphylococcus aureus*, *E. coli*, *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei*) during the natural fermentation of chibwantu prepared using the yellow and white munkoyo roots and during storage of the beverage at room (16^oC - 25^oC) and refrigeration ($+4^oC$) temperatures were studied.

During the study of the survival of *Staphylococcus aureus* in chibwantu fermentation the pH of the gruel prepared using the white munkoyo root extract decreased almost two fold from pH 7.68 to pH 4.85 after 24 hours and to pH 4.27 after 48 hours compared to pH of the gruel prepared using the yellow munkoyo root extract. When using yellow munkoyo root extract, the pH 7.18 decreased to pH 5.61 after 24 hours and to pH 5.02 at 48 hours. However, there was no significant differences ($p<0.05$) in the pH (sampling done at six hour intervals) between the control gruel without the test microorganism and the gruel with test microorganisms. *Staphylococcus aureus* had no influence on the pH of the gruel.

During natural fermentation of the gruel prepared using the yellow munkoyo root extract the microbial counts of *Staphylococcus aureus* decreased. The decrease was minimal at the initial stages of fermentation (inoculation up to 24 hours at six hours interval). The microbial count_decreased from 5.76 log cfu/ml at inoculation to 5.08 log cfu/ml at 24 hours and to 3.80 log fcu/ml at 48 hours of fermentation. For the gruel prepared using the white munkoyo root extract, the microbial count of *Staphylococcus aureus* increased at the initial stages (inoculation up to 12 hours) from 6.02 log cfu/ml to 6.46 log cfu/ml at 24 hours and then decreased to 6.08 log cfu/ml at 48 hours. Under refrigeration temperatures, the gruel prepared using the white munkoyo root extract had higher inhibitory effect when compared to the gruel prepared using the yellow munkoyo root extract. For the gruels prepared with white munkoyo root extract and inoculated with test microorganisms at the initial stage of the fermentation process, the inhibitory effect was almost 16 fold higher compared to the gruel prepared using the yellow munkoyo root extract after one day of storage. For the gruels inoculated with test microorganisms after 24 hours of the fermentation process, there was more variability in microbial counts for the gruel prepared with yellow munkoyo root extract compared to the gruel prepared with white munkoyo root extract.

During the study of the survival of *E. coli* in chibwantu, fermentation of the gruel using both types of the munkoyo root extracts had inhibitory effects on *E. coli*.

However, the microbial counts increased at the initial stages (from inoculation up to 12 hours) of fermentation for the gruel prepared using the white munkoyo root extract.

Gruel prepared using both types of the munkoyo root extracts also had inhibitory effects on *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei. Salmonella* enteric *enteritidis* was not detected after six hours of the fermentation process and *Shigella sonnei* was only detected at the initial stage of the fermentation process.

In addition to the influence of pH, the yellow and white munkoyo roots have properties that influence the growth patterns and survival of enteropathogenic microorganisms during the fermentation process.

Key Words: Enteropathogenic microorganisms, fermentation, munkoyo roots, chibwantu

7.1 Introduction

Diarrhea is the 3rd leading cause of clinic visits and death for children under five years of age in Zambia after pneumonia and malaria and the second globally (CIDRZ, 2012) contributing to high rates of malnutrition. Diarrhea is also the major cause of complications in children with severe acute malnutrition (Irena *et al*., 2011; Talbert *et al*., 2012), thereby increasing the risk of death. With the spread of the Aids pandemic, diarrhea in adults has also become a major burden on the families of the sufferer. The study by Kelly *et al*. (1996) showed that 97% of hospital patients with diarrhea of over one month's duration were HIV- seropositive.

The causative agents of diarrheal diseases in infants and children include infectious pathogens such as *E. coli* (enteropathogenic, enterotoxigenic, enteroinvasive), *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *C. coli*, toxin-producing strains of *Bacillus cereus* and *Clostridium perfringens* and rotavirus (Thapar and Sanderson, 2004; Kingamkono *et al*., 1999; Kunene *et al*., 1999). Another microorganism, *Vibrio cholera*, is the major cause of epidemic diarrhea especially where sanitation is compromised (Thapar and Sanderson, 2004).

There are a number of studies that have been carried out on the survival abilities of pathogenic bacteria in cereal fermented foods (Simango and Rukure, 1992; Svanberg *et al*., 1992; Kingamkono *et al*., 1994; Kunene *et al*., 1999 and Tetteh *et al*., 2004) and results have shown that cereal gruels with pH < 4.0 significantly suppress the growth of food borne pathogens (*Salmonella* spp., *Shigella* spp., *E. coli*, *Campylobacter jejuni*, *Staphylococcus aureus* and *Bacillus cereus*). Svanberg *et al*. (1992) indicate that the possible mechanisms of the antagonistic effects of the fermenting microorganisms (e.g. lactic acid bacteria (LAB)) are;

i. organic acid production (lactic, propionic and acetic acid; pH< 4.0)
ii. competition for nutrients
iii. hydrogen peroxide formation
iv. bacteriocins
v. antibiotic like substances

Cereal based foods (gruels) are common weaning and/or complementary foods for children in Zambia. Chibwantu is among the favorite indigenous beverages produced at household level with traditional techniques. Chibwantu is a fermented food product consumed while still actively fermenting.

The aims of the current work were to study the growth pattern of selected pathogenic microorganisms during the fermentation of chibwantu prepared using the yellow munkoyo roots and white munkoyo roots and the survival of the selected pathogenic microorganisms during storage of chibwantu at room temperature (16^{o}C - 25^{o}C) and refrigeration temperature (+4^{o}C).

7.2 Materials and Methods

7.2.1 Samples

The samples, maize grit and munkoyo root bunches (yellow and white), of approximately 100 - 150 g per bunch, were purchased. Maize grit was purchased and collected at market sites (Soweto and City) in Lusaka. Munkoyo roots were purchased and collected at market sites (Soweto and City) in Lusaka, as well as at markets in Livingstone and Chingola.

7.2.2 Bacterial strains

E. coli (ATCC 10418), *Salmonella* enteric *enteritidis* (strain C), *Shigella sonnei* (strain H) and *Staphylococcus aureus* were from the culture collection of the Food Science Department of the University of the Free State.

7.2.3 Preparation of chibwantu (cereal gruel)

The traditional process of preparation for Chibwantu is that thick or thin maize grit porridge is prepared and the roots (munkoyo) and/or extract added to the warm porridge and left to ferment spontaneously. Fermentation occurs at ambient temperatures (25 – 30 ^{o} C) in 24 - 48 hours (Lovelace, 1977; Simwamba and Elahi, 1986; Zulu *et al*., 1997).

7.2.3.1 Cereal gruel (Chibwantu)

To prepare the maize grit porridge, 1700 ml of tap water was measured into a cooking pot and allowed to boil on a hot plate. 300 g of maize grit washed with tap water was mixed with the boiling water. The porridge was allowed to cook; first 30 minutes on high heat setting and the last 30 minutes on low/medium heat setting, while stirring periodically to allow for proper mixing and prevent the grit from burning at the bottom of the pot and then allowed to cool down.

To prepare the munkoyo root extract, 50 g of munkoyo root (yellow or white type) was weighed and soaked in one litre of water (ratio 1:20) (tap water which had been boiled and allowed to cool) for two hours and 30 minutes.

To prepare for fermentation of the gruel, four portions 500 g each of the cooked maize grit porridge was weighed into plastic fermentation jars. To each of two portions of the maize grit porridge, 500 ml of the white munkoyo root extract was added and to the other two portions, 500 ml of the yellow munkoyo root extract, to each, was added. The different mixtures were mixed vigorously.

7.2.3.2 Inoculation of the test bacteria

7.2.3.2.1 Room temperature fermentation

The sample gruel was prepared to a volume of one litre. The test bacteria were sub-cultured in nutrient broth for 18 hours and then one ml of the stock culture was inoculated into the gruel, vigorously mixed for thorough distribution and the gruel allowed to ferment at room temperature for 48 hours while appropriate samples (25 ml) were taken at six hour intervals for determination of pH, microbial count, detection and confirmation of the test bacteria. The concentration of the test bacteria were 10^9 cfu/ml for *Staphylococcus aureus*, 10^8 cfu/ml for *E. coli, Salmonella* enteric *enteritidis*, and *Shigella sonnei.* The control was sample gruel not inoculated with test bacteria for both the white and yellow root gruels. The fermentation process was done in triplicate (n=3).

7.2.3.2.2 Refrigeration storage

The sample gruel was prepared to a volume of one litre and allowed to ferment at room temperature for 24 hours. The test bacteria were sub-cultured in nutrient broth for 18 hours and then 1ml was inoculated into the fermented gruel, vigorously mixed for thorough distribution of the inoculum and stored at refrigeration temperature (4^oC) for two to three days. Samples of 25 ml were taken at start of the fermentation process, and after every 24 hour for determination of pH. Microbial count and confirmation of the test bacteria was done immediately after inoculation with test bacteria and every 24 hours for two to three days. The concentration of the test bacteria were 10^7 cfu/ml for *Staphylococcus aureus*, and *E. coli,* 10^8 cfu/ml for *Salmonella* enteric *enteritidis* and *Shigella sonnei.* The control was sample gruel not inoculated with test bacteria for both the white and yellow root gruels. The process was repeated three times (n=3).

7.2.4 **Enumeration and detection of test bacteria**

Baird-Parker Agar (Merck, Gauteng, South Africa), supplemented with egg-york tellurite emulsion (5% v/v) incubated at 37^oC for 48 hours was used for the enumeration of *Staphylococcus aureus*, Violet Red Bile Agar-MUG (VRBA-MUG) (Merck, Gauteng, South Africa) incubated aerobically at 37^oC for 24 hours for enumeration of *E. coli.* Confirmation was done by observing for fluorescent colonies under long wave UV light (BAM, 1995), and suspected colonies were inoculated into tryptone water and incubated at 37^oC for 48 hours. Kovacs' reagent was used to detect the production of indole from tryptophan (BAM, 1995).

Detection of *Salmonella*

For the non-selective enrichment, added 25 ml of the sample gruel to 225 ml of buffered peptone water (pre-enrichment medium) and incubated at 37°C for 24 hours. For selective enrichment, added 0.1 ml of culture to 10 ml of Rappaport-Vassiliadis (RV) soy broth and 1 ml of culture to 10 ml of (i) Rappaport-Vassiliadis (RV) soy broth and (ii) Selenite cystine broth. Incubated the Selenite cystine broth was at 37°C for 18 - 24 hours and the Rappaport-Vassiliadis (RV) soy broth as shown in the table below

i. Tube 1: 0.1 ml culture in 10 ml RV soy broth incubated at 37°C for 18 - 24 hrs.
ii. Tube 2: 1 ml culture in 10 ml RV soy broth incubated at 37°C for 18 - 24 hrs.
iii. Tube 3: 0.1 ml culture in 10 ml RV soy broth incubated at 41.5°C for 18 - 24 hrs.
iv. Tube 4: 1 ml culture in 10 ml RV soy broth incubated at 41.5°C for 18 - 24 Hrs.

Selective isolation

All the inoculated tubes were streaked on Xylose Lysine Desoxycholate (XLD) agar and Brilliant Green (BG) agar and incubated at 37°C for 24 hours.
(BAM, 1995; Global Salm-Surv, 2003)

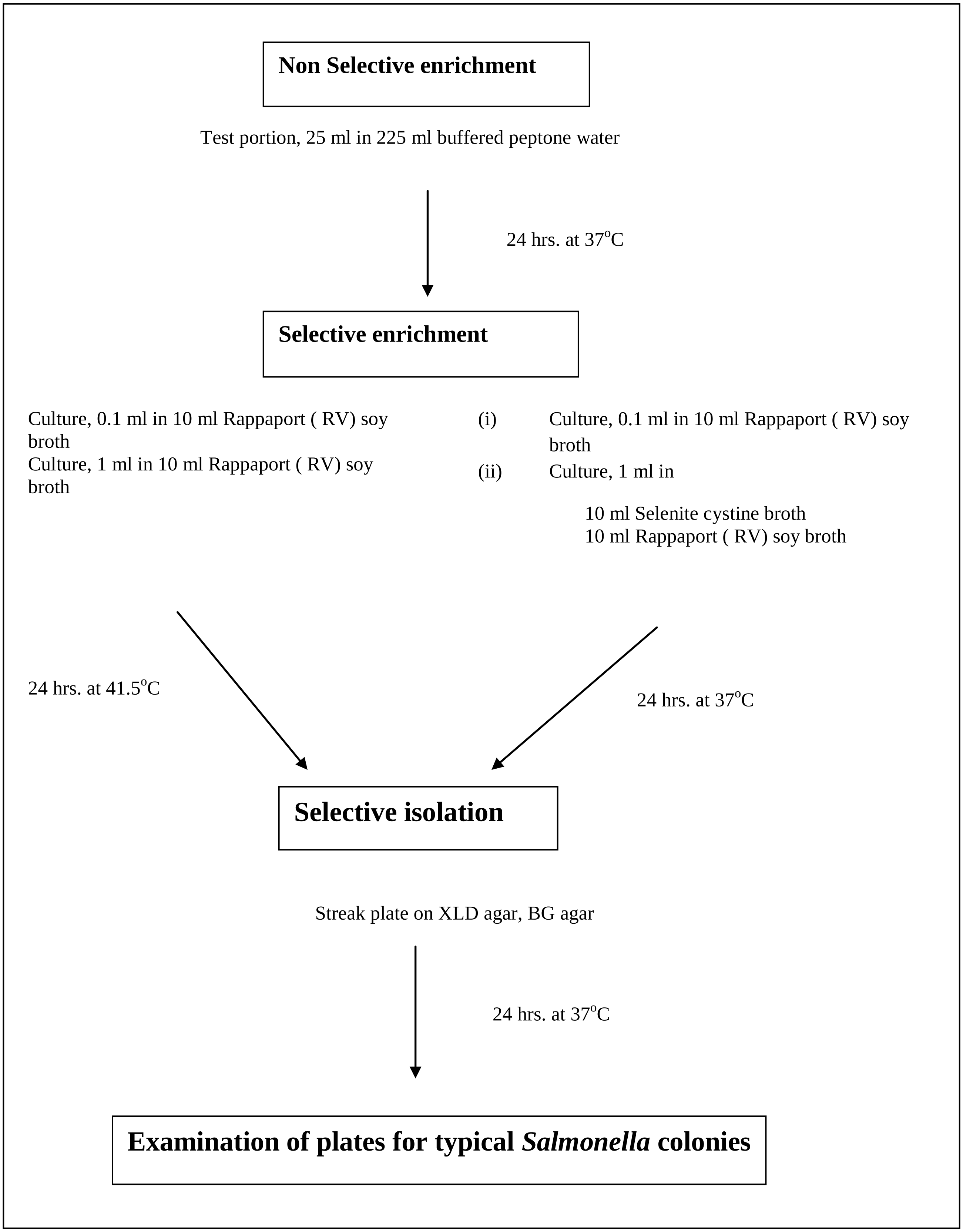

Figure 7-1: The flow diagram for Salmonella isolation

Detection of *Shigella sonnei*

For the nonselective enrichment, added 25 ml of the sample gruel to 225 ml of buffered peptone water (pre-enrichment medium), incubated at 37^{o}C for 24 hours, added 0.1 ml of the culture to 10 ml of the Enterobacteriaceae Enrichment Broth, and incubated at 37^{o}C for 24 hours for selective enrichment. For selective isolation of the test microorganism, all the inoculated tubes were streaked on Salmonella Shigella (SS) agar and XLD agar and incubated at 37^{o}C for 24 hours and then examined (Merck, 1981; BAM, 1995).

pH

The pH was determined using a pH meter (Cyberscan pH 510 by Eutech Instruments). The pH meter was calibrated using standard buffer solutions (Merck) at pH 4.0 and pH 7.0.

7.3 Results and Discussion

Enumeration of *Staphylococcus aureus* during natural fermentation at room temperature

Inoculation of Staphylococcus aureus at the initial stage of the natural fermentation process

The mean pH of the gruel prepared using the white munkoyo root extract decreased more than the gruel prepared using the yellow munkoyo root extract for both gruels with the test microorganisms and without the test microorganisms (Table 7-1). Microbial counts were decreasing with fermentation time for the gruel prepared using the yellow munkoyo root extract. The initial count was 5.76 log cfu/ml, then 5.08 log cfu /ml after 24 hours of fermentation and 3.80 log cfu /ml after 48 hours.

For the gruel prepared using the white munkoyo root extract, there was an increase in microbial counts in the initial stages (from inoculation up to 12 hours) of the fermentation process, however, then the counts decreased, slower as compared to the gruel prepared using the yellow munkoyo root extract. The initial count was 6.02 log cfu/ml, 6.64 log cfu /ml after 24 hours of fermentation and 6.08 log cfu /ml after 48 hours. The observe initial increase in microbial counts for the gruel prepared using the white munkoyo root extract could be due to some properties (compounds) within the white roots that are not available in the yellow root and have an effect on the test microorganism.

For both the gruels prepared, using either the yellow or the white munkoyo root extracts, there were generally small changes in microbial counts over the period of 24 hours of fermentation. During that period of fermentation the microbial counts decrease for the gruel associated with the yellow munkoyo roots and increased with the gruel associated with the white munkoyo roots. The decrease or increase in microbial counts respectively was less than one log cfu units, and resulted in no significant difference ($p< 0.05$). The results could imply that the yellow and white munkoyo roots had properties which influenced the growth of the pathogen in different ways during fermentation of the gruel.

Inoculation of Staphylococcus aureus at the initial stage of fermentation and storage at refrigeration temperature (4^{o}C)

For the gruel prepared using the yellow munkoyo root extract, the microbial counts had decreased at 24 hours of fermentation from 4. 48 log cfu/ml to 2.95 log cfu/ml. After one day of storage at refrigeration temperature (4^{o}C), the count decreased slightly to 2.78 log cfu/ml and by day three of storage the test microorganism could not be detected. The microbial count increased slightly after 24 hours of fermentation from 4.40 cfu/ml to 4.64 log cfu/ml for the gruel prepared using the white munkoyo root extract. After one day of storage at refrigeration temperature (4^{o}C) decreased significantly to 2.00 log cfu/ml that was > 2.5 log units and by day two of storage the test microorganism was not detectable.

Under refrigeration temperatures, the gruel prepared using the white munkoyo root extract had more than three times greater inhibitory effect compared to the gruel prepared using the yellow munkoyo root extract. Further indication of the different properties associated with the white and yellow munkoyo roots.

Inoculation of Staphylococcus aureus after 24 hours of fermentation and storage at refrigeration temperature (4^oC)

Staphylococcus aureus reduced significantly immediately after inoculation into the already fermented gruel prepared using the yellow munkoyo root extract. The initial count was 4.30 log cfu/ml and after storage under refrigeration for a day there was not much change in the count (4. 51 log cfu/ml). Then there was a decrease in microbial count, at day three of storage the count was 2.00 log cfu/ml. *Staphylococcus aureus* was not detected at day four of storage.

For the gruel prepared using the white munkoyo root extract, the initial count immediately after inoculation in the already fermented gruel was 5.62 log cfu/ml higher compared to the initial mean count for the gruel prepared using the yellow munkoyo root extract. The counts decreased after day two of storage and *Staphylococcus aureus* was not detected at day four of storage.

Even with inoculation of *Staphylococcus aureus* after 24 hours of gruel fermentation and storage under refrigeration temperatures (4^oC), the gruel prepared using the white munkoyo root extract had higher inhibitory effect compared to the gruel prepared using the yellow munkoyo root extract.

Tables 7-1 and 7-2 summarised the changes in pH and the growth patterns of *Staphylococcus aureus* in gruel prepared using the yellow and white munkoyo root extract.

Enumeration of *E. coli*

Inoculation of the test microorganism (E. coli) at the initial stage of the natural fermentation process

The pH of the gruel prepared using the white munkoyo root extract decreased more than the gruel prepared using the yellow munkoyo root extract for both the gruel with the test microorganisms and without the test microorganisms. Microbial counts were decreasing with fermentation time and could not be detected by 18 hours from the gruel prepared using the yellow munkoyo root extract. For the gruel prepared using the white munkoyo root extract, the counts increased in the initial stages (from inoculation up to 12 hours) of fermentation. The microbial count decreased sharply from 5.51 log cfu/ml at 12 hours to less than one log cfu/ml at 18 hours of fermentation and at 24 hours, the test microorganism was not detectable.

Fermentation of the gruel using both yellow and white types of the munkoyo root extracts had inhibitory effects on *E. coli* even though the microbial counts increased in the early stages (from inoculation up to 12 hours) of fermentation for the gruel prepared using the white munkoyo root extract. Chibwantu beverage is generally fermented for a period of 18 - 24 hours, others ferment the beverage to almost two days (48 hour) before consumption. If contamination with *E. coli* occurred by means of unhygienic utensils, substrates or hands, during the beverage preparation process, the pathogen could be cleared by the time of consumption.

Inoculation of E. coli after 24 hours of fermentation and storage at refrigeration temperature (4^{o}C)

E. coli was not detected immediately after inoculation into the already fermented gruel prepared using the yellow munkoyo root extract and for the already fermented gruel prepared using the white munkoyo root extract, the initial mean count was 3.00 log cfu/ml and after refrigeration (4^{o}C) for a day no test microorganism was detected. The gruel prepared using the yellow munkoyo root extract had higher inhibitory effect against *E. coli* compared to the one prepared using the white munkoyo root extract.

Tables 7-3 and 7-4 summarised the changes in pH and the growth patterns of *E. coli* in gruel prepared using the yellow and white munkoyo root extract.

Detection of *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei*

For *Salmonella* enteric *enteritidis* at 6 hours of fermentation the pathogen was not detected and for *Shigella sonnei* the pathogen was only detected at the initial stage of the fermentation process (Table 7-5 and 7-6).

The munkoyo roots are associated with microorganisms that are capable of growth in all the enrichment, different selective enrichment and isolation media used for detection of *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei*.

7.4 Conclusion

The pH of the gruel prepared using the white munkoyo root extract decreased more than the gruel prepared using the yellow munkoyo root extract for the parameters tested. However, the gruel prepared using the yellow munkoyo root extract showed higher inhibitory effect against the tested pathogens *Staphyloccocus aureus*, *E. coli*, *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei*, particularly *Staphyloccocus aureus, E. coli*. The results indicated that pH was not the only factor in the inhibitory effect of the fermented gruel, but the different munkoyo root extracts contributed to the effects observed. For *Staphylococcus aureus*, the gruel prepared using the white munkoyo root extract showed higher effect with refrigeration at 4^{o}C.

Since the munkoyo roots were purchased from the local markets for the study, the sources of variation between the yellow and white type of munkoyo roots could have been due to factors including:

- Differences in microbiota, chemical composition and other unknown features within the munkoyo root types

- Source of the munkoyo roots (region from where the roots were collected)
- Processing methods during preparation of the munkoyo roots for sale
- Storage conditions at the local markets

The results indicate that use of munkoyo roots (yellow or white type) during preparation of chibwantu has inhibitory effects on *Staphyloccocus aureus*, *E. coli*, *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei*, thereby contributing to the safety of the beverage.

7.5 References

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Table 7-1: Changes in pH of gruel and *Staphylococcus aureus* microbial counts (cfu/ml) during fermentation process at room temperature (n=3)

Fermentation time (hrs)	Means pH				Pathogen microbial counts (Log cfu/ml)			
	Control gruel (no pathogen)		Gruel with pathogen		Control gruel (no pathogen)		Gruel with pathogen	
	Yellow	White	Yellow	White	Yellow	White	Yellow	White
0	7.26	7.66	7.18	7.68	0.00	0.00	5.76	6.02
6	6.91	6.82	6.60	6.76	0.00	0.00	6.22	6.60
12	6.40	5.89	6.11	5.72	0.00	0.00	6.13	6.26
18	5.97	5.44	5.88	5.29	0.00	0.00	5.82	6.79
24	5.90	5.06	5.61	4.85	0.00	0.00	5.08	6.64
30	5.74	4.76	5.44	4.67	0.00	0.00	4.83	6.54
42	5.34	4.49	5.08	4.39	0.00	0.00	3.83	6.37
48	5.23	4.31	5.02	4.27	0.00	0.00	3.80	6.08

Table 7-2: Changes in pH of gruel and *Staphylococcus aureus* microbial counts during fermentation process and storage at refrigeration temperature (4^{o}C) (inoculation of pathogen was done at the start of fermentation and after 24hrs of fermentation) (n=3).

Fermentation time (hrs and storage in days)		Means pH (Gruel with pathogen)		Pathogen microbial counts (Log cfu/ml)					
				Control gruel (no pathogen)		Gruel with pathogen (inoculation of pathogen at start of the fermentation process)		Gruel with pathogen (inoculation of pathogen after 24hrs of the fermentation process)	
		Yellow	White	Yellow	White	Yellow	White	Yellow	White
0 hr		7.23	8.05	0.00	0.00	4.48	4.40	n/a	n/a
24 hrs		5.41	4.98	0.00	0.00	2.95	4.64	4.30	5.62
Storage at Refrigeration	**Day 1**	**4.77**	**4.91**	**0.00**	**0.00**	**2.78**	**2.00**	**4.51**	**4.53**
	Day 2	**4.54**	**4.98**	**0.00**	**0.00**	**2.18**	**0.00**	**3.97**	**4.40**
	Day 3	**4.42**	**4.87**	**0.00**	**0.00**	**0.00**	**0.00**	**2.00**	**2.00**
	Day 4	**4.31**	**4.87**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

n/a: not applicable

Table 7-3: Changes in pH of gruel and *E. coli* microbial counts during fermentation process at room temperature (n=3)

Fermentation time (hrs)	Means pH				Pathogen microbial counts (Log cfu/ml)			
	Control gruel (no pathogen)		Gruel with pathogen		Control gruel (no pathogen)		Gruel with pathogen	
	Yellow	White	Yellow	White	Yellow	White	Yellow	White
0	6.86	6.72	6.52	6.70	0.00	0.00	4.38	5.27
6	6.62	6.65	6.32	6.55	0.00	0.00	<2.00	5.83
12	5.95	6.62	5.75	6.54	0.00	0.00	<2.00	5.51
18	5.88	5.15	5.73	5.08	0.00	0.00	<1.00	<1.00
24	5.64	5.06	5.63	5.01	0.00	0.00	0.00	0.00

Table 7-4: Changes in pH of gruel and *E. coli* microbial counts during fermentation process and storage at refrigeration temperature (4^oC) (inoculation of pathogen was done only after 24hrs of fermentation) (n=3).

Fermentation time (hrs and storage in days)		Means pH (Gruel with pathogen)		Pathogen microbial counts (Log cfu/ml)			
				Control gruel (no pathogen)		Gruel with pathogen	
		Yellow	White	Yellow	White	Yellow	White
0 hr		6.86	6.92	0.00	0.00	n/a	n/a
24 hrs		5.83	4.98	0.00	0.00	0.00	3.00
Storage at Refrigeration	**Day 1**	**5.81**	**4.84**	**-**	-	**0.00**	**0.00**

n/a: not applicable.

Table 7-5: Detection of *Salmonella* in sample gruel (room temperature)

Gruel with pathogen	**Selective medium**	**Isolation medium**	**Fermentation Time (hrs)**			
			0	6	12	18
Y (Gruel with yellow munkoyo root extract)	(Sc)	BGA	-	-	-	-
		XLD	+	+	-	-
	(RV) 1	BGA	-	-	-	-
		XLD	-	-	-	-
	(RV) 2	BGA	-	-	-	-
		XLD	+	-	-	-
	(RV) 3	BGA	+	-	-	-
		XLD	-	-	-	-
	(RV) 4	BGA	-	-	-	-
		XLD	+	-	-	-
W (Gruel with white munkoyo root extract)	(Sc)	BGA	-	-	-	-
		XLD	-	-	-	-
	(RV) 1	BGA	-	-	-	-
		XLD	-	-	-	-
	(RV) 2	BGA	-	-	-	-
		XLD	-	-	-	-
	(RV) 3	BGA	-	-	-	-
		XLD	-	-	-	-
	(RV) 4	BGA	-	-	-	-
		XLD	-	-	-	-

+: Detected, **-:** Not Detected, **PC:** Positive Control, **NC:** Negative Control, **Y:** Gruel with Yellow munkoyo root extract, **W:** Gruel with White munkoyo root extract, **Sc:** Selenite cystine broth, **RV:** Rappaport-Vassiliadis soy broth, **BGA:** Brilliant green agar, **XLD:** Xylose lysine desoxycholate agar, **RV 1:** 0.1ml of culture inoculated into 10ml (RV) soy broth incubated at 37^{o}C, **RV 2:** 1ml of culture inoculated into 10ml (RV) soy broth incubated at 37^{o}C, **RV 3:** 0.1ml of culture inoculated into 10ml (RV) soy broth incubated at 41.5^{o}C, **RV 4:** 1ml of culture inoculated into 10ml (RV) soy broth incubated at 41.5^{o}C.

For the positive control, there was growth of the test microorganism on both types of media used BGA and XLD as well as the selective enrichment and no growth on the negative controls.

Table 7-6: Detection of *Shigella sonnei* from sample gruel

Gruel with pathogen	**Selective medium**	**Isolation method**	**Fermentation time (hrs)**			
			0	6	12	18
Y (Gruel with yellow munkoyo root extract)	(XLD)	Spread	+	-	-	-
		Streak	+	-	-	-
	(SSA)	Spread	+	-	-	-
		Streak	+	-	-	-
W (Gruel with white munkoyo root extract)	(XLD)	Spread	-	-	-	-
		Streak	+	-	-	-
	(SSA)	Spread	+	-	-	-
		Streak	+	-	-	-

+: Detected, **-:** Not Detected, **PC:** Positive Control, **NC:** Negative Control, **Y:** Gruel with Yellow munkoyo root extract, **W:** Gruel with White munkoyo root extract, **SSA:** Salmonella Shigella agar, **XLD:** Xylose lysine desoxycholate agar.

For the positive control, there was growth of the test microorganism on both types of media used XLD and SSA and no growth on the negative controls.

Chapter 8

General Discussions and Conclusions

Chapter 8

8 General Discussions and Conclusions

8.1 Discussion

The objective of the PhD study was to evaluate the microbiological quality and safety of Chibwantu. Since there is inadequate information on cereal based fermented food products prepared using munkoyo roots and the little information available is so scattered, the first specific objective of the study was to:

1. Gather information on the production and utilisation of indigenous cereal based fermented of food products in Lusaka and Chongwe districts of Lusaka province to help establish the relevance of these foods in households and the country at large – with special emphasis on munkoyo and chibwantu beverages.

The other specific objectives of the study were to:

2. Isolate and characterize the microorganisms associated with the Munkoyo Roots and Maize grit used during the preparation Chibwantu.
3. Isolate and characterize the essential microorganisms involved during the fermentation of Chibwantu.
4. Study the survival of selected food borne enteropathogenic microorganisms during fermentation of Chibwantu
5. Investigate the effect of munkoyo roots (*Rhynchosia* species) on growth of selected microorganisms.

A survey was conducted in Lusaka and Chongwe districts of Lusaka province, Zambia to gather information on production and utilisation of indigenous cereal based fermented of food products with emphasis on munkoyo and chibwantu beverages prepared using the munkoyo roots (the focus of the study) and also establish the relevance of these foods to the country. The study was conducted in Lusaka province because it is culturally diverse, people are originally from different parts of the country and ethnic groups and the province has the highest share of the country's population.

Results of the survey showed that these foods are prepared in homes for home consumption, to sell and use on special occasions. The most often prepared of the indigenous cereal based fermented food products are the beverages, especially the non-alcoholic such as munkoyo, chibwantu, mahewu and thobwa. These beverages in particular are found in local markets and as street vended foods; as alternative cheaper energy drinks compared to the branded ones available on the market. Not all respondents prepared cereal based fermented beverages. One of the reasons was that they bought these products at markets, as it is readily commercially available. In addition, literature shows that street vended foods currently make a significant contribution to the food intake of large segments of the population of most African countries (FAO/WHO, 2005; Muleta and Ashenafi, 2001; Ekanem, 1998).

Chibwantu and munkoyo beverages are prepared using munkoyo roots and majority of the respondents who prepared these beverages use munkoyo roots making it an important ingredient for the fermentation process of these beverages. Munkoyo roots are mainly bought at local markets. The roots are collected from the miombo woodland forests by local people with indigenous knowledge on identification of the edible munkoyo plant (*Rhynchosia* spp.) and to the author's knowledge the roots are not cultivated commercially for marketing purposes. Different utensils are used for the fermentation process particularly the plastic buckets and containers and if the beverages are for sale, used plastic bottles of water and soft drinks are washed and used for packaging. Measuring cups are also used or the customer brings along a packaging utensil. There is consequently a variety of areas that could be a source of health hazards especially microbial hazards associated with preparation and consumption of chibwantu and munkoyo beverages prepared using munkoyo roots.

Most handlers of local market and street vended foods in Africa and the developing world at large, are largely ignorant of basic food safety issues (Ekanem, 1998) and trading is conducted in places that may have poor sanitation (FAO/ WHO, 2005). Therefore despite the many advantages that selling foods in local markets, stalls and street vending offer, there are also many health risks that are associated as foods may become contaminated by either spoilage or pathogenic microorganisms (Muleta and Ashenafi, 2001).

Traditional preparation of munkoyo and chibwantu beverages, is generally similar within the study area, however, there are widely varied sources of raw materials, activities performed at each of the steps during the preparation process, packaging and the general handling of raw material and final products, from different households, thereby, contributing to the variation in quality and safety of these beverages. Quality and safety of fermented food products is dependent on factors such as quality of raw material, initial levels of contamination, level of hygiene and sanitation and fermentation conditions, e.g. temperature (Motarjem, 2002; Sengun and Karapinar, 2012) which are difficult to control particularly when foods are prepared under household conditions.

Enumeration of coliforms, LAB, yeast and moulds was used to determine the quality of the main raw materials used in the preparation of chibwantu (maize grit and munkoyo roots). Microscopic examination, conventional biochemical and physiological tests and sequencing of the 16S rRNA and 18S rRNA genes were used to identify the microorganisms associated with the munkoyo roots.

Results of microbiological analyses showed that munkoyo roots had significantly higher microbial counts ($p < 0.01$) compared to the maize grit, besides the maize grit is cooked for a long time (more than 30 minutes at high cooking temperatures) and hence many microorganisms are destroyed. The main concern for this study was with the munkoyo roots that are added after cooking of the cereal, just before the fermentation process.

The mean total aerobic mesophilic counts for the yellow roots was 6.12 ± 1.82 log cfu/g and white roots 7.02 ± 1.28 log cfu/g. The mean microbial counts for total aerobic mesophiles, presumptive LAB and coliforms were not significantly different ($p > 0.01$) for both the yellow and white roots. Generally, counts on white roots were higher than on yellow roots however, the differences were not statistically different ($p > 0.01$). The dominant LAB on the yellow roots was *Enterococcus faecalis* (71%) and the other was *Enterococcus casseliflavus* (29%). The only LAB identified on white roots was *Enterococcus casseliflavus* (100%). Diverse yeasts was found on yellow roots compared to the white roots and the dominant yeast on the yellow roots was *Wickerhamomyces anomalus*; > 80% occurrence of all yeasts and moulds. Only *Aspergillus oryzae* and few *Fusarium* species occurred on the roots. *Wickerhamomyces anomalus, Cryptococcus laurentii, Cryptococcus liquefaciens* occurred frequently on the white roots and they accounted for > 85% occurrence of all yeasts and moulds. The diversity of moulds was found on the white roots was higher compared to the yellow roots. The dominant coliforms were *Enterobacter* and *Klebsiella* species. None of the pathogenic microorganisms *E. coli*, *Staphylococcus aureus* and *Salmonella* species were detected on both types of the roots. The information obtained demonstrates that there is a diversity of microorganisms on munkoyo roots. To the author's best of knowledge no information in literature is available on the microorganisms associated with the roots. This study is the first to identify the microorganisms associated with munkoyo roots and indigenous cereal based fermented of food products containing these roots.

The microorganisms associated with the munkoyo roots (LAB, coliforms, yeasts and moulds) were the main microorganisms encountered during fermentation of chibwantu with munkoyo roots. The root extract was also fermented for comparison purposes. During the fermentation process of chibwantu and the munkoyo roots extracts there were no significant differences ($p > 0.05$) in mean microbial counts at different intervals for the aerobic mesophiles, LAB and coliforms from the different parameters tested. The parameters tested were yellow munkoyo root extract, white munkoyo root extract, chibwantu using the yellow munkoyo root extract and chibwantu using the white munkoyo root extract. Increased numbers in types of microorganisms (LAB and yeasts) were encountered during the fermentation of the munkoyo root extract compared to the dry (raw) roots, however, the microorganisms isolated during fermentation of the extract were also isolated during the fermentation of chibwantu using the respective munkoyo root extracts.

Growth characteristics of the LAB isolates; growth at different temperatures, tolerance of different levels of salt, gas production from glucose, reduction of litmus before clotting in milk and production of ammonia from arginine varied including the isolates that were identified as belonging to the same species after sequencing of the 16S rRNA genes, especially those associated with the white munkoyo root.

Lactic acid bateria isolated during the fermentation process with yellow root extract were *Enterococcus casseliflavus, Enterococcus thailandicus, Weissella confusa* and some unidentified LAB. *Enterococcus gallinarum, Enterococcus casseliflavus*, *Lactococcus lactis* and some unknown LAB were isolate from fermentation process with the white root extract. *Enterococcus thailandicus, Enterococcus casseliflavus, Enterococcus gallinarum, Leuconostoc lactis, Weissella confusa, Lactococcus lactis* and some unknown LAB were isolated from chibwantu fermentation using the yellow munkoyo root extract and *Enterococcus gallinarum, Enterococcus casseliflavus, Lactobacillus casei, Lactococcus lactis* and *Weissella confusa* from chibwantu using the white munkoyo root extract. The *Enterococcus* species particularly *Enterococcus casseliflavus* were found to have the highest rate of occurrence during the fermentation process.

These findings for munkoyo roots or their extracts and chibwantu with yellow or white munkoyo roots differs from maize-based fermented products studied before. From literature dealing with natural fermentation of cereals particularly maize as raw materials, the most commonly associated LAB appears to be *Lactobacillus* species particularly *Lactobacillus plantarum*, others are *Pediococcus* species, *Leuconostoc* species, *Lactococcus lactis* and *Weissella confusa* (Steinkraus1983; Holzapfel, 1997; Mensah, 1997; Odunfa, 1988; Odunfa and Adeyeye, 1985; FAO, 1999; Abegaz *et al*., 2002; Blandino *et al*., 2003; Muyanja, 2003; Lei, 2006; Nout, 2009; Osungbaro, 2009). *Enterococcus gallinarum* and *Enterococcus casseliflavus* have not been reported to be among the dominant microorganisms in naturally fermented cereal based products particularly the beverages; however *Enterococcus* species have been found to occur in plant based fermented foods. Studies by Olusapo *et al*. (2002) reported occurrence of *Enterococcus faecium* in ogi and kunun-zaki cereal based foods from Nigeria and by Muyanja *et al*. (2003) in household and laboratory produced bushera, a cereal based fermented beverage from Uganda. Oladipo *et al*. (2013), reported occurrence of *Enterococcus* species in traditional Nigerian fermented vegetable condiments (Ugba, Okphe, Iru, Dawadawa and Ogiri).

Studies of naturally fermented cereal based foods have reported that coliforms increased in the initial stages of fermentation and then decreased (Muyanja *et al*., 2003; Kunyanga *et al*., 2009; Lawal *et al*., 2009) to even undetectable levels (Mugula *et al*., 2003; Kunyanga *et al*., 2009; Mbata *et al*., 2009; Kalui *et al*., 2010). In contrast with those studies, coliforms were found during fermentation of munkoyo root extract and chibwantu prepared using both the yellow and white munkoyo roots, in the present study, however, the mean counts increased until the end of the fermentation process (after 72 hours) for all the tested parameters.

Munkoyo roots possess properties that promote growth of coliforms and probably other microorganisms even with decreased pH of growth medium. The coliforms during fermentation of the gruels and munkoyo root extracts were not affected by change in pH. Gruel pH decreased with fermentation. Munkoyo root extracts pH decreased in the initial stages of the fermentation process and after 24 hours increased until the end of the fermentation process at 72 hours. The coliform counts from the gruel compare to that of munkoyo root extracts were not significantly different ($p > 0.05$). There was also no significant difference ($p > 0.01$) in the total aerobic counts and presumptive LAB.

The dominant coliforms were *Enterobacter* and *Klebsiella* species. This is an important factor in the resulting quality and spoilage of the beverage prepared using these roots. Odugbemi *et al*. (1993) reported increased levels of coliforms during storage of cooked ogi for 9 hours and Essien *et al*. (2011) reported presence of coliforms in hawked kunun, a traditional non-alcoholic beverage prepared from cereals such as sorghum, maize, millet or rice. Other bacteria that occurred during fermentation include *Bacillus cereus*, *Bacillus* species and *Micrococci* species.

Pathogens have been isolated from some cereal based fermented foods (Simango and Rukure, 1992; Mensah, 1997; Kunene *et al*., 1999; Olusapo *et al*., 2002; Essien *et al*., 2011) indicating that pathogens are capable of growing in the food or surviving the fermentation process. Pathogens that are found in fermented foods come from the respective raw materials or from the handlers (Gadaga *et al*., 2004). During the natural fermentation of chibwantu gruel using the yellow and white munkoyo roots the enteropathogenic microorganisms, *Staphylococcus aureus*, *E. coli*, *Salmonella* and *Shigella* species were not detected. In the present study, survival of *Staphylococcus aureus*, *E. coli*, *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei* was investigated during the natural fermentation of chibwantu prepared using both the yellow and white munkoyo root extract.

During natural fermentation (at room temperature) of chibwantu gruel prepared using the yellow munkoyo root extract, *Staphylococcus aureus* at counts of 10^7 cfu/ml was inoculated into the gruel. *Staphylococcus aureus* counts decreased gradually, however, for the chibwantu gruel prepared using the white munkoyo root extract, the pathogen increased in the initial stage of fermentation (0-12 hours) and then decreased, but the decrease was not significant. The pH of gruel prepared using the white munkoyo root extract decreased more; from 7.68 to 4.85 by 24 hours and then to 4.27 by 48 hours compared to the gruel prepared using the yellow munkoyo root extract, from 7.18 to 5.61 by 24 hours and then to 5.02 by 48 hours, therefore the effect of the fermentation of the gruel on survival of the pathogen could be affected more with the munkoyo roots or other factors than the pH. Studies show reduction in the level of the pathogen with fermentation of the cereal gruel (Nout *et al*., 1989; Mbugua and Njenga, 1992; Svanberg *et al*., 1992). Kingamkono *et al*. (1995) reported elimination of the pathogen by 12 hours of fermentation. When the 24 hours pre-fermented chibwantu gruel and already inoculated with test pathogen was refrigerated at 4^{o}C, the test pathogen decreased slightly by day 1 of storage for the gruel prepared using the yellow munkoyo root extract and it was eliminated by day 3 of storage. In contrast counts for the gruel, prepared using the white munkoyo root extract, which increased during pre-fermentation decreased significantly (from 4.64 log cfu/ml to 2.00 log cfu/ml) by day 1 of storage and was not detected by day 2 of storage. After 24 hours of pre-fermentation of the gruel, it was inoculated with test pathogen. The gruel prepared using the yellow munkoyo root extract had high inhibitory effect on the initial test pathogen counts (4.30 log cfu/ml) compared to the gruel prepared using the white munkoyo root extract (5.62 log cfu/ml). However, the gruel prepared using the white munkoyo root extract showed siginificant inhibitory effect during storage. Counts of 10^5 – 10^8 cfu/ml may be required and a sufficient period of time to result in the formation of enough enterotoxins to cause food borne gastroenteritis (Jablonski and Bohach, 1997).

Inhibition of *E. coli* was observed during fermentation of chibwantu gruel using both types of the munkoyo root extracts even though the mean counts increased in the early stages (0 to

12 hours) of fermentation for the gruel prepared using the white munkoyo root extract. The test pathogen could not be detected immediately after inoculation into the already fermented gruel prepared using the yellow munkoyo root extract and for the already fermented gruel prepared using the white munkoyo root extract, the initial mean count was 3.00 log cfu/ml and after refrigeration (4^{o}C) for a day no test pathogens were detected. Overall, the gruel prepared using the yellow munkoyo root extract showed more effect compared to the one prepared using the white munkoyo root extract.

Gruel prepared using both types of the munkoyo root extracts also had inhibitory effects on *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei*. The test pathogens, *Salmonella* enteric *enteritidis* could not be detected from the start of the fermentation process for the gruel prepared using the white munkoyo root extract and could not be detected at 6 hours but at the start for the gruel prepared using yellow munkoyo root extract. *Shigella sonnei* could only be detected at the start of the fermentation process for gruel prepared using both types of the munkoyo root extracts. The observed results could be due to effect of properties associated with munkoyo root and/or competition with microorganisms associated with munkoyo roots which were capable of increased growth in selective media for growth of *Salmonella* enteric *enteritidis* (strain C) and *Shigella sonnei*.

Results of many studies on cereal based fermented foods generally suggest that these foods have bacteriostatic and bacteriocidal properties that reduce the ability of pathogens to survive in the food product consequently unlikely to play a major role in the transmission of enteropathogens (Nout *et al*., 1989; Mbugua and Njenga, 1992; Simango and Rukure, 1992; Svanberg *et al*., 1992; Kingamkono *et al*., 1995; Annan-Prah and Agyeman, 1997; Tetteh *et al*., 2004). Fermentation may therefore be an important preparation technique in preventing the transmission of enteropathogens introduced during food preparation and handling.

There are no studies to the author's knowledge up to date on the benefit or effect of the addition of munkoyo (*Rhynchosia* species) yellow or white roots or extracts in the preparation of chibwantu and munkoyo beverages, on microbial growth. This prompted the study to investigate the effect of these roots on the growth patterns of selected microorganisms, by first establishing if the roots had antimicrobial and secondly microbial growth stimulatory properties. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids quinines and flavonoids which have been found in vitro to have antimicrobial properties (Cowan, 1999). Zulu *et al*. (1994) found that munkoyo roots (*Rhynchosia insignis* and *R heterophylla*) had high concentrations of flavanoids. There are many published studies and reviews on the antimicrobial properties of plant products with emphasis on phytochemicals which could be developed for treatment of infectious diseases (Nascimento *et al*., 2000; Omojasola and Awe, 2004; Akinpelu and Onakoya, 2006; Cowan, 1999) and preservation of food both raw and processed (Zaika *et al*., 1983; Lis-Balchin and Deans, 1997; Smith-Palmer *et al*., 1998; Hammer *et al*., 1999; Rauha *et al*., 2000).

In the present study, dried munkoyo root (aqueous, cold and hot, as well as methanolic (60%)) extracts were prepared and tested with the agar diffusion method to determine the sensitivity of selected microorganisms to these extracts (assay 1). Results showed the high inhibitory activity against *Staphylococcus aureus*; less against microorganisms; *E. coli*, *Salmonella* enteric *enteritidis* and *Shigella sonnei* from our culture collection. Other studies in literature have also shown inhibition of these microorganisms by plant extracts (Hammer *et al*., 1999; Nascimento *et al*., 2000; Rauha *et al*., 2000; Kim *et al*., 2011). For the microorganisms isolated from the roots themselves, there was no activity against *Enterobacter* species, *Rhodotorula mucilaginosa* and *Cryptococcus liquefaciens*. For *Bacillus cereus* and *Bacillus* species, the inhibitory effect was only observed with the methanolic (60%) extracts - with more effect from the yellow extract compared to the white root extract. For *Klebsiella variicola,* minimal inhibitory effect was observed - only with the hot water extracts and there was no difference in effect between the yellow and white root extracts. The inhibitory effect of munkoyo roots varied with test microorganism and extraction method of the roots.

The study further investigated the growth pattern of microorganisms in appropriate growth media supplemented with the root extract. Two assays were employed:

1. Growth of microorganisms in appropriate growth media supplemented with dried root extract; obtained through aqueous and methanolic extraction (assay 2)

2. Growth of microorganisms in different concentrations of the aqueous root extract (assay 3)

The dried munkoyo root extracts yield ranged from 4.12 to 14.4% (w/w) with hot water extraction method having the highest yields for both the yellow and white roots. For the aqueous extraction the yields were 70.3% (v/v) for white and 80.0% (v/v) for yellow roots. The yellow roots generally had higher yields compared to the white roots.

The munkoyo roots have significant stimulatory effect on growth of microorganisms. Bacteriostatic effect was observed within the first 3 to 6 hours and after it was overcome, all the test gram negative bacteria; *E. coli*, *Salmonella* enteric *enteritidis* and *Shigella sonnei* had significantly increased growth compared to the controls for both assays 2 and 3. However for the gram positive bacteria; *Staphylococcus aureus* growth was mostly inhibited and *Bacillus cereus* growth was stimulated after 3 hours of the incubation period for assay 2. When comparing the gram positive bacteria to the gram negative bacteria growth stimulation was very minimal. For assay 3, growth of the gram positive bacteria varied depending on the concentration of the root extract and the type of root. Both the root types were capable of stimulating growth of the yeast, *Saccharomyces cerevisiae*. The yellow munkoyo root extract had significant growth stimulation of *Saccharomyces cerevisiae*, 100% of the yellow root extract increased growth significantly ($p < 0.01$) compared to the control (YM broth). This could explain the association of the yellow root with diverse yeasts compared to the white root.

Generally, the results showed that the munkoyo roots possess compounds that can influence and sustain growth of microorganisms; the white roots showed more stimulatory activity compared to the yellow roots and the yellow roots more inhibitory effect compared to the white roots.

From the present studies it is certain that the munkoyo roots contribute immensely to the growth characteristics of both desirable and undesirable microorganisms during the fermentation process of chibwantu and munkoyo beverages thus explaining their very short shelf life.

There has been a growing interest in the use of indigenous fermented food products because of the microbial inhibitory properties of the organic acids produced (mostly lactic acid) and other inhibitors such as hydrogen peroxide, diacetyl, alcohols, carbon dioxide, and bacteriocins) as well as their synergistic inhibitory effects, during the fermentation process (Rodgers, 2001; Caplice, 1999; Helander, 1997). However, munkoyo roots possess properties that influence and sustain growth of microorganisms, therefore, in the case of indigenous fermented food products prepared using munkoyo roots, there is need for further investigation of the munkoyo roots particularly investigation of specific component of the roots that inhibit or stimulate growth and how these can be used to develop fermented food products with improved quality and safety.

8.2 Conclusions and perspectives

The main conclusions derived from this PhD study are summarized below

- Indigenous cereal based fermented food products are prepared in Zambian households using varied traditional methods and are used for home consumption, to sell and on special occasions.
- The food products are prepared in rural, peri - urban and urban areas and are used for different purposes which include weaning and complementary foods for children.
- The types of indigenous cereal based fermented foods and beverages prepared and utilise in Lusaka province of Zambia include munkoyo, chibwantu, thobwa, mahewu, sour porridge, nshima from fermented maize meal, seven days (gankhata), katata and kachasu and the commercial alcoholic opaque beverages such as chibuku.
- The foods contribute to the nutritional, economical and medical needs of the consumers, thereby making them very relevant to the country at large.
- The raw materials (maize grit and munkoyo roots) used in the preparation of the beverage chibwantu consist of coliforms, LAB, yeasts and moulds, particularly the munkoyo roots.
- The yellow munkoyo roots had higher diversity in occurrence of yeasts and the white munkoyo roots had higher diversity in occurrence of moulds.
- *Saccharomyces cerevisiae* was the dominant yeast associated with maize grit accounting for more than 80% of yeasts and moulds.
- The groups of microorganisms; coliforms, LAB, yeasts and moulds, associated with the raw materials (maize grit and munkoyo roots) used in the preparation of the beverage chibwantu, particularly from the roots were also responsible for the fermentation of the beverage.
- Some microorganisms that could not be identified from the raw materials were encountered during the fermentation process of chibwantu. Coliforms, LAB and yeasts dominated during the fermentation process.
- *Staphylococcus aureus*, *E. coli*, *Salmonella* and *Shigella* species were not encountered during natural fermentation of chibwantu and growth of some selected

enteropathogenic microorganisms is significantly suppressed during the fermentation process and storage.

- Both the yellow and white munkoyo roots possess some antimicrobial activity against some enteropathogenic microorganisms. The roots also possess microbial growth inhibitory and stimulatory activity.

Indigenous cereal based fermented food products are prepared in many parts of Africa and information on their preparation is passed on from generation to generation. The foods offer many advantages which include enhanced sensory properties, improved nutritional value and food safety and longer shelf life compared to the non-fermented cereals, however, in addition to the above mentioned advantages the food products are also prepared as an expression of culture and lifestyle. The cereal based fermented beverages are mainly prepared using malt and/or other cereals as sources of amylase or fermentable substrate and studies on these food products have been carried out and information on their preparation, nutritional value, antimicrobial potential has been documented and is readily available. Some of the beverages such as mahewu in Zambia and other countries in the southern part of Africa have even been commercialized. However, for indigenous cereal based fermented food products such as chibwantu and munkoyo that are prepared using the munkoyo roots, information available is very inadequate even though these foods contribute significantly to the welfare of the consumers.

Studies on effects of adding munkoyo roots during preparation of these foods on nutritional value, probiotic potential and other beneficial and detrimental effects that would be associated with their use for preparation of these food products is essential for increased consumption and commercialisation. There is more research work that is needed in exploring the inhibitory and stimulatory effects of these roots (extracts) on growth of microorganisms to establish ways in which the munkoyo roots can possibly be utilise as food preservatives thereby enhancing food safety. One research direction could be a possible use of the root extracts in stimulation of starter cultures during the fermentation process of foods.

Since majority of the consumers prepare the beverages in homes and purchase the roots from local market, there is also need for proper preparation, handling, packaging and commercialisation of the munkoyo roots. The storage conditions at markets are usually poor and mostly the roots are not packaged in any form, except only after purchase. As cases of food poisoning due to mistaken use of poisonous species of the roots occurred, studies on taxonomy and characterization of the roots would be useful for both traders and consumers.

The beverages, munkoyo and chibwantu, prepared from these roots are sold in the local markets and also street vended; in some instances inappropriately packaged e.g. in used water and soft drink plastic bottles. There is a need to establish the safety of chibwantu and munkoyo beverages sold in the local markets as well as street vended to help regulatory agencies take appropriate steps to protect consumers. In addition, there is a need for educating food handlers, particularly those who prepare these foods in homes and food vendors on food hygiene and proper storage in an aim to prevent food borne diseases. Fermented food products have a very good safety record yet there is still a lot of work needed on fermented products prepared from munkoyo roots and the munkoyo root or plant itself to be able to establish the quality and safety or health benefits conclusively.

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Summary

Summary

Fermentation has been used for over three thousand years as an effective and inexpensive means to preserve the quality and safety of foods. In Africa most of the traditional foods are fermented at household level before consumption. The cereals are fermented, and then cooked prior to consumption and this offers added safety advantages because pathogenic microorganisms are inactivated thereby increasing shelf life of product. However, some cereals are cooked first and then other sources of fermentable carbohydrates and amylase such as other cereals (malts) or roots from *Rhynchosia* spp. (generally called munkoyo roots) are added and then fermented prior to consumption. Chibwantu and munkoyo beverages, Zambia's important cereal based fermented foods are prepared in such a way. The substrates added after cooking of cereals are a major concern in terms of product quality and safety.

In literature there is limited information on munkoyo roots. Knowledge on quality of these roots, their effect on quality and microbiological safety of the fermented food products prepared by their use is essential for the development of improved products for increased consumption, commercial production and marketing. The present research work aimed at contributing to closing up this knowledge gap by evaluating the microbiological quality and safety of chibwantu.

This was done by first gathering information on the production and utilisation of indigenous cereal based fermented food products in Lusaka and Chongwe districts of Lusaka province, Zambia with special emphasis on chibwantu and munkoyo beverages prepared using munkoyo roots. Second to isolate and characterize the microorganisms associated with the munkoyo roots and maize grit used during the preparation of chibwantu and to establish whether the same microorganisms are involved during the fermentation process of the beverage. Third to study the survival of selected food borne enteropathogenic microorganisms during fermentation of chibwantu beverage and finally to investigate the effect of munkoyo roots on growth of selected microorganisms.

From the information gathered during this study, it was found that different types of indigenous cereal based fermented foods especially the beverages are prepared in Zambian households. The foods contribute to the nutritional, economical and medical needs of the consumers, thereby making them relevant to the consumers and the country at large.

The raw materials; maize grit and munkoyo roots, used in the preparation of the beverage chibwantu are associated with coliforms, lactic acid bacteria (LAB), yeasts and moulds, particularly the munkoyo roots. The groups of microorganisms; coliforms, LAB, Yeasts and moulds, associated with the raw materials used in the preparation of the beverage chibwantu, particularly from the roots, were also responsible for the fermentation of the beverage. Some microorganisms that could not be identified from the raw materials were also encountered during the fermentation process of chibwantu. *Staphylococcus aureus*, *E. coli*, *Salmonella* and *Shigella* species were not encountered during natural fermentation of chibwantu and growth of selected enteropathogenic microorganisms was significantly suppressed during the fermentation process and storage.

Both the yellow and white munkoyo roots possess some antimicrobial activity against some enteropathogenic microorganisms as well as microbial growth stimulatory activity. This study forms basis for further studies on the active components of the roots for possible use in preservation for improved food quality and safety.

The quality of the beverage is varied due to different methods of preparation and the microorganisms involved during fermentation which are dependent on the quality of the munkoyo roots. There is still a lot of work needed on the munkoyo roots and the fermented products prepared from them to be able to establish their safety convincingly. However, fermented foods generally have a very good safety record and chibwantu should be relatively safe to consume.

Key words: Fermentation, cereal based foods, munkoyo roots, food quality and safety