Shelf-life extension of home-made *mahewu* by adding *Aloe vera* powder

By

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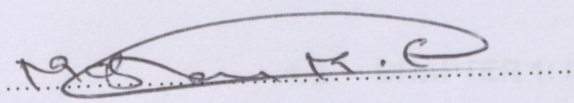
Dedication

This work is dedicated to my family for their love, encouragement and support. I also dedicate this work to my late father who passed on in 2010 and grandmother who passed on in 2011. May their souls rest in peace.

Declaration

I, Mpho Edward Mashau (11543379), hereby declare that this mini-dissertation for the Master of Science (MSc) in Food Science and Technology hereby submitted by me to the Department of Food Science and Technology at the University of Venda, has not previously been submitted by me for a degree at this or any other university or institution of higher learning.



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Abstract

The effect of adding *Aloe vera* (*Aloe barbadensis* Miller) powder (AVP) in the production of home-made *mahewu* was investigated with the intention of extending the shelf-life of the product. *Mahewu* was produced in the laboratory (sample C) and at home (sample B) using standard home-made procedure with the addition of AVP. A control *mahewu* (sample A) was produced without AVP. The extension of shelf-life was determined by following the chemical, microbiological, physical properties at 36 ± 5ºC for 60 days and the sensory properties of the products were also evaluated. Physicochemical analysis revealed decreases in pH ranging between 3.3 and 2.4 from day 15 to 60 days of storage in all three samples. Titratable acidity of all the samples increased significantly ($P \leq 0.05$) throughout the storage period and it ranged between 0.2% and 1.8%. There was a significant difference amongst the samples during day 15 to day 60 with respect to total soluble solids (TSS). A general decrease in TSS was recorded in all three samples during day 45 and 60, while sample B had an increase in TSS in day 30 (3.2). In terms of the colour of the products, there was an increase in the L* value for samples A (68.9) and C (69.9) in day 60 of storage, while sample B had a decrease in L* value (67.8). Samples (B and C) had a decrease in redness during day 60 as compared to day 45 (7.1), while sample A had an increase in day 60 (7.4). In terms of the a* values, there was no significant difference between sample B and C from day 15 to day 60 of storage while sample A was statistically significantly different from the two samples during the same storage days. In terms of the b* values, there was no significant difference in sample B and C during day 15, while sample B was significantly different from samples A and C from day 30 until day 60 of storage at $P<0.05$. There was an increase in numbers of coliform bacteria, lactic acid bacteria and yeast during the storage period of 60 days. The overall acceptability of the three samples in terms of sensory evaluation showed that there was no significant difference between two samples (B and C) with AVP ($P<0.05$), while sample A was significantly different from both samples.

Key words: Maize, *Mahewu*, *Aloe vera*, sensory properties

CHAPTER 1. INTRODUCTION

1.1 Background

Traditional fermentation is a form of food processing, where microbes, for example, lactic acid bacteria (LAB) are utilized (Chelule *et al.*, 2010a). The bacteria use food as a substrate for their propagation. This is a form of food preservation technology used from ancient times. Fermented foods are produced world-wide using various processing techniques, raw materials and microorganisms (Blandino *et al.*, 2003). In general, profound biochemical and nutritional changes occur during the fermentation of cereals. A wide variety of products (Table 1) are obtained as a results of these processes and these can be categorized into gruels (thin porridges), and non-alcoholic beverage, dough/ thick porridge, baked products and alcoholic beverages (Wood, 1998). Gruels and non-alcoholic beverages are fermented cereal products which on processing yield acidic, non-alcoholic and high water content fluidy gruels (porridge). The common cereal gruels include *Ogi* in West Africa, *Akas*a or *Koko* in Ghana, *Uji* in Kenya, *Mahewu* in South Africa and *Abreh* in Sudan (Katangole, 2008).

Several types of foods are traditionally fermented and these contribute substantially to the daily diets of rural communities. These indigenous foods are locally prepared on a small scale in the village homes and their quality depends on the skills of the household occupants, as inherited over the years. These include alcoholic and non-alcoholic beverages, which are mainly cereal-based (Katangole, 2008; Gqaleni *et al.*, 1998). In Africa, few traditional cereal-based fermented beverages are industrialized (Steinkraus, 2004). *Mahewu* (South Africa) and *chibuku* (Zimbabwe), both cereal-based fermented beverages, are rare examples of success. The industrial production of traditional fermented beverages requires scientific investigations into the microorganisms involved in the fermentation, processing equipment and process conditions (Foma *et al.*, 2012).

Table 1. Cereal-based lactic acid fermented products in Africa (Oyewole, 1997)

Raw materials	Fermented product name	Country/region of consumption
Gruels and beverages		
Maize	*Ogi*	Nigerai/West Africa
Sorghum	*Abreh*	Sudan
Millet	*Uji*	Kenya/East Africa
Maize	*Kenkey*	Ghana
Wheat	*Mahewu*	South Africa
Tef	*Humulur*	Sudan
	Mawe	Benin/West Africa
Alcoholic beverages	*Busa*	Kenya/East Africa
	Mbenge	Tanzania
	Bouza	Egypt
	Merisa	Sudan
	Kaffir/kefir	North Africa
	Letting/joule	South Africa
	Umqomboti	South Africa
	Burukutu	West Africa
	Pito	West Africa
	Malawa	Zambia/Uganda
	Kisra	Sudan
Acid leavened breads/pancakes	*Enjera/tef injera*	Ethiopia
	Kishi	Egypt
	Laban zeer	Morocco

Traditional cereal foods play an important role in the diet of the people of African particularly in cereal producing zones. Flour from various cereals is one of the main raw materials used in the production of popular food products with high acceptability, good storage characteristics and affordable cost (Mbata *et al.*, 2009). Fermented cereals are very widely utilized as food in African countries and in fact cereals account for as much as 77% of total caloric consumption. A majority of traditional cereal based foods consumed in Africa are processed by natural fermentation and are particularly important as weaning foods for infants and as dietary staples for adults (Osungbaro and Taiwo, 2009). Cereal beverages are popular in Africa because of the social, religious and therapeutic values associated with them (Nwachukwu *et al.*, 2010).

Mahewu (*amahewu*) is a fermented, non-alcoholic maize beverage/gruel commonly consumed as a staple among black South Africans. Generic *mahewu* is produced industrially, using *Lactobacillus bulgaricus var delbruecki*

or *Lactobacillus brevis* (McMaster *et al.*, 2005). It is a beverage, which is prepared from either thin maize porridge or thick maize porridge (Katangole, 2008). It is an adult-type food, which is commonly used to wean children and is introduced to infants between 4–18 months (Chelule *et al.*, 2010b). The drink is consumed after standing for about 24 h (Gadaga *et al.*, 1999). The commercial *mahewu* is pasteurized before distribution and consumption, and the shelf life of the refrigerated *mahewu* is 21 days, while the shelf life of traditional *mahewu* is less than five days (McMaster *et al.*, 2005; Gadaga *et al.*, 1999).

Aloe barbadensis Miller or *Aloe vera* is a perennial plant of the lily (Liliaceae) family generally known as *Aloe vera* (AV) (Ramachandra and Rao, 2011; Bozzi et al., 2007). Currently the most widely used part of Aloe *Vera* plant *is* the gel, whereas part of its peel is not yet utilized optimally (Narshih *et al.*, 2012). AV is widely used for manufacturing food products, beverages, pharmaceuticals and cosmetics because of its aromatic properties, bitter taste, the cathartic activity of anthraquinones and other pharmacological activities such as emolliency, reduction of inflammation and acceleration of wound healing (Pisalkar *et al.*, 2010). Because of its medicinal and therapeutic values AV is well known as a miracle plant, first aid plant, burn plant and stick of heaven. It helps to cure diabetes, ulcer, and heart disease (Ramachandra and Rao, 2011). A newly developed aloe product as natural health food supplement can attract many health-conscious consumers. AV contains many active ingredients, but the best known is aloin (lo-glucopyranosyl-1,8-dihydroxy-3- (hydroxymethyl)- 9(IOH)-anthracenone). Aloin is a bitter tasting yellow crystal, and it is a C-glycoside derivative of an anthraquinone (Patel and Patel, 2013).

1.2 Statement of the problem

Most traditional, African cereal-based fermented foods deteriorate rapidly and become unacceptable to consumers within one to four days of production (Okafor, 1990). The deleterious changes are primarily due to the objectionable off-flavour or over-souring induced by continued microbial activities after production.

The traditional way of producing *mahewu* requires an extended fermentation time because of the low initial number of desirable lactic acid bacteria. The use of uncontrolled conditions, especially temperature, may also result in the proliferation of undesirable microorganisms which convert lactic acid to undesirable end products which adversely affect the taste and texture of *mahewu* (Gadaga *et al.*, 1999).

1.3 Justification of the study

In Africa, fermented beverages represent improved nutrition and digestibility, and some improvement in stability of foods in regions lacking refrigeration coupled with rising energy costs. The therapeutic value of these foods has been reported and the development of new products for fermented foods, targeted at the growing black consumer market, is required. New products could arise from modifying and improving existing traditional foods such as *mahewu* (McMaster, 2005).

The addition of *Aloe vera* powder may result in *mahewu* product that has a longer shelf life because of its bacterial and antifungal effects. This could/may also improve the nutritional value of *mahewu* because the aloe vera plant contains a number of nutrients such as vitamins, minerals, amino acids, sugars, enzymes, fatty acids and saponins, which have positive effects on the human body and could be used in food formulations as functional ingredient for health benefits.

1.4 Objectives and hypothesis

1.4.1 Overall objective

The overall objective of this study is to improve the shelf-life of home-made *mahewu*. This will be achieved through investigating the effect of adding *Aloe vera* powder (AVP) in the production process of *mahewu*.

1.4.2 Specific objectives are to:

a) Determine the effect of AVP on pH of *mahewu*
b) Determine the effect of AVP on some physical properties of *mahewu*
c) Determine the effect of AVP on the microbiological composition of *mahewu*
d) Evaluate the consumer acceptability of shelf stable *mahewu*

1.5 Hypothesis

Addition of AVP may have a positive or negative effect on the shelf life of traditional *mahewu*.

CHAPTER 2. LITERATURE REVIEW

2.1 Cereal fermentation in Africa

Cereals are used in Africa as staple foods. A large proportion of the world cereal production is processed by fermentation prior to consumption. The enhancement of attractive flavour and texture, and the improved shelf-life and digestibility as a result of fermentation are important reasons for this (Nout, 2009). Like with any other fermentation process, the understanding of the microbial ecology of cereal fermentations need the knowledge of the fermentation substrates, i.e. the grains or seeds of the various cereal plants, as well as the products obtained thereof. This framework includes the characterization of the microbial associations and ecological factors, which govern the fermentation process and arise from the nature of the cereal substrate. In cereal fermentations, endogenous enzymes, bacteria, yeast and moulds play roles either singularly or in combination, and contribute to the creation of a great variety of products (Hammes *et al.*, 2005).

Cereal grains are considered to be one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fibre for people all over the world. However, the nutritional quality of cereals and the sensorial properties of their products are sometimes inferior or poor in comparison with milk and milk products. The reasons behind this are the lower protein content, the deficiency of certain essential amino acids (lysine), the low starch availability, the presence of antinutrients (phytic acidand polyphenols) and the coarse nature of the grains (Katangole, 2008; Blandino *et al.*, 2003). In general, natural fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly- and oligosaccharides. Certain amino acids may be synthesized and the availability of B group vitamins may be improved. Fermentation also provides optimum pH conditions for enzymatic degradation of phytate which is present in cereals in the form of complexes with polivalent cations such as iron, zinc, calcium, magnesium and proteins. Such a reduction in phytate may increase the amount of soluble iron, zinc and calcium several folds (Blandino et al., 2003; Haard *et al.*, 1999; Nout and Motarjemi, 1997).

Cereal porridge has an international acceptance in many regions of the world. In tropical African countries, several fermented and unfermented cereal beverages are consumed daily and form an essential part of the diet. Despite the dawn of science and technology in Africa, the production of fermented cereal foods is still largely a traditional family art done in a crude manner. The production has not increased substantially and shelf life is often short (Raheem, 2006). Most fermented foods, including the major products that are common in the western world, as well as many of those from other sources that are less well characterized, are dependent on lactic acid bacteria (LAB) to mediate the fermentation process (Conway, 1996). Lactic acid fermentation contributes to the safety, nutritional value, shelf life and acceptability of a wide range of cereal based-foods (Blandino *et al.*, 2003; Oyewole, 1997). Cereal fermentation processes are affected by characteristic variables, the control of which is the basis of all technological measures that are used to obtain the various products of a defined quality. According to Hammes *et al.* (2005); Hammes and Ganzle, (1998), these variables affecting cereal fermentation are fermentable substrates, nutrients, growth factors, minerals, buffering capacity, and efficacy of growth-inhibiting principles. Others include water content, degree and moment of comminution of the grains, i.e. before or after soaking or fermentation, duration and temperature of fermentation, components added to the fermenting substrate, such as sugar, salt, hops and oxygen and source of amylolytic activities that are required to gain fermentable sugars from starch or even other polysaccharides.

2.2 Cereal grain products

Cereals are the most important source of the world's food and have significant impact in human diet throughout the world (Sani and Adesulu, 2013; Adebayo *et al.*, 2010). Cereals are used in various ways, and one of these is as fermentable substrates in food preparation (Sacca *et al.*, 2012). In Africa, cereals of major importance are maize (*Zea mays*), sorghum (*Sorghum vulgare*), rice (*Oryza sativa*), and several minor grains such as the millets, especially pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*) (Nout, 2009). Millets are important minor cereals in tropical and subtropical regions and India is the largest producer (Ilango and Antony, 2014).

Cereals account for as much as 77% of total calorie consumption in African countries (Alais and Linden, 1999; Norman *et al.,* 1999). A majority of traditional cereal-based beverages are consumed in Africa and mainly processed by natural fermentation (Norman *et al.*, 1999). Maize (*Zea mays*) also known as corn, belongs to the tribe Maydeae of *Poaceae* (*Gramineae*), commonly known as grass family (Chaudhary *et al.*, 2014). Maize has a major influence on the global use of land, human diet and the quality of human lives. It has a high energy value and is used for human food,animal feed and fuel (Gwirtz and Garcia-Casal, 2014; Ongol *et al.*, 2013; Brockway, 2001) and it is a good source of dietary fibre and protein, while being very low in fat and sodium (Sangwan *et al.*, 2014). Many cereals share the same compounds and basic structure. A single kernel can be divided into many different constituents on the macroscopic level as illustrated in Figure 1 A. The structural components of maize kernels can be divided into 4 classes denoted as *endosperm*, *germ*, *pericarp* and *pedicel.* These components have different composition as listed in Table 2 below (Arngren and Pedersen, 2009). The endosperm consists of 72–73 % starch embedded in a protein matrix that makes the maize an excellent substrate for fermentation. Maize is processed, fermented and consumed in various ways. It is usually ground and pounded followed by boiling, baking or frying. Alternatively, the whole grain may be boiled or roasted prior to fermentation (Sangwan *et al.*,

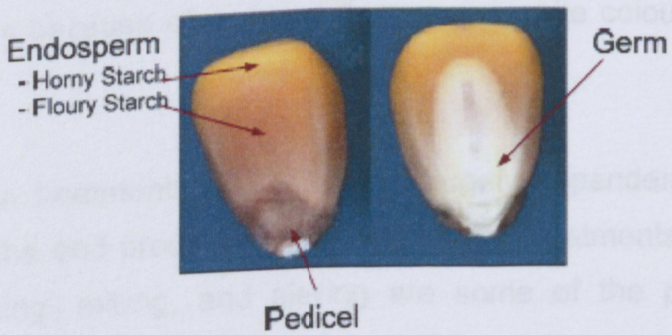
2014). Various food products are produced from maize such as *mahewu, pozol, ogi, banku, Kenkey* and *koko* (Fowoyo and Ogunbanwo, 2010).

Table 2. Constituents (%) of maize kernels

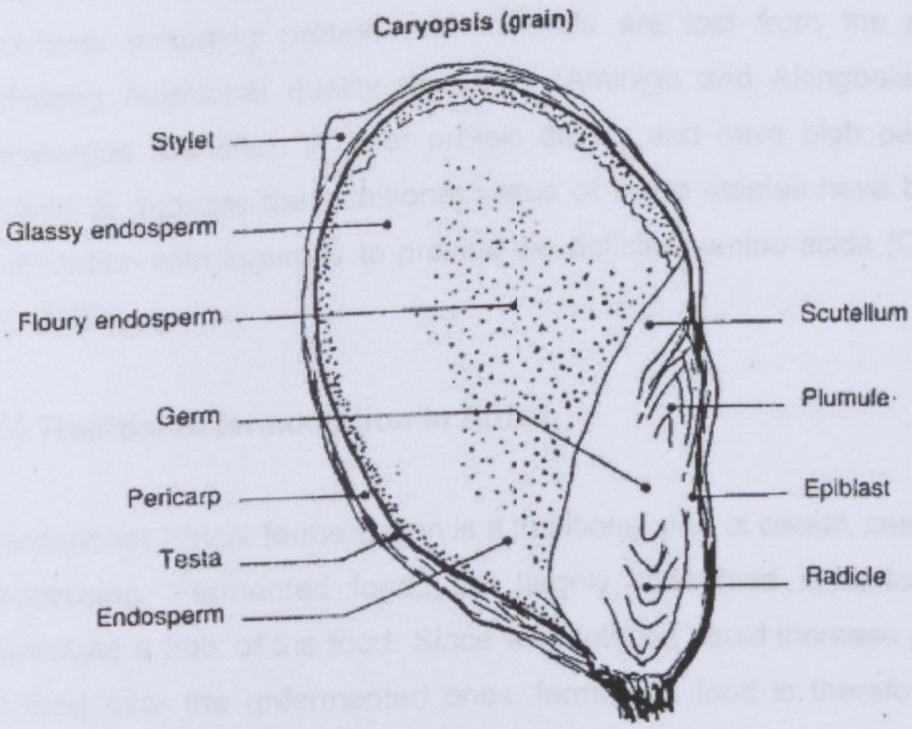
Chemical composition	Pericarp	Endosperm	Germ
Protein	3.7	8.0	18.4
Oil	1.0	0.8	33.2
Crude fibre	86.7	2.7	8.8
Ash	0.8	0.3	10.5
Starch	7.3	87.6	8.3
Sugar	0.34	0.62	10.8

Non-alcoholic maize fermentation, using lactic acid bacteria (LAB), is among the oldest forms of secondary food processing biotechnology and has played a vital role in the production of many of the fermented beverages consumed today (Chelule *et al.*, 2010b). Several maize based fermented products, such as *ogi* in West Africa, *Togwa* in East Africa, b*anku* in Ghana, *kenkey* in Ghana, *mahewu* in South Africa and *mawe* in Benin have been documented. A variety of cereals are used either singly or mixed to produce a number of fermented beverages (Mbata *et al.*, 2009; Mugula *et al.*, 2003). Sorghum is one of the cereals cultivated in the tropical regions of Africa (Kolawole *et al.*, 2007). It has a structure which is broadly similar to that of other cereals (Figure 1 B). The major components of the grain are the pericarp (outer covering), the testa between pericarp and endosperm (which may or may not be present), the endosperm, and the embryo. It is a large variable genus with many cultivars (Mbajiuka *et al.*, 2010). It constitutes a major source of energy and protein for people in Asia and Africa and serves as a staple food of many of the world's poorest and the least privileged people (Michodjehoun-Mestres, *et al.*, 2005). According to Ahmed *et al.*, (1995), sorghum products have poor nutritional value due to their deficiency in lysine, threonine and tryptophan and presence of anti-nutritional factors such as tannins and phytates that interact with proteins, vitamins and minerals, thus restricting their bio-availability. However,

various techniques have been investigated to improve the protein digestibility and mineral availability of sorghum by reducing its tannin and phytate content. These include malting, fermentation and cooking (Mbajiuka *et al.*, 2010, Elkhalifa *et al.*, 2005).



A. Structure of maize kernel



B. Structure of sorghum kernel

Figure 1. A. Structure of a maize kernel (Arngren and Pedersen, 2009). B. Structure of sorghum kernel (Sautier and O'Deye, 1989)

Fermentation is widely used traditionally for processing sorghum into fermented products (Achi, 2005; Isabel *et al.*, 2005). Their low pH confers the advantage of microbiological safety. Sorghum based foods include burukutu, pito, bogobe,

kisra, injera etc. They are mainly fermented; some are non-alcoholic while others are alcoholic beverages (Mbajiuka *et al.*, 2010, Booney, 2000). Sorghum is potentially suitable for use in composite flours (Hugo *et al.*, 2003). Sorghum flour can have a definite advantage over maize and other tropical cereals in composite flours because of its bland flavour and white colour (Rooney *et al.*, 1997).

Pre-fermentation treatments of cereals are largely dependent on the type of cereal and on the end product desired. Generally, treatments such as drying, washing, steeping, milling, and sieving are some of the processing steps applied in the preparation of these fermented cereal beverages (Osungbaro and Taiwo, 2009). During manufacture of these fermented cereal beverages, nutrients including protein and minerals are lost from the grains thereby affecting nutritional quality adversely (Aminigo and Akingbala, 2004). Such beverages are often of poor protein quality and have high paste properties. Efforts to improve the nutritional status of these staples have been based on fortification with legumes to provide the deficient amino acids (Osundahunsi *et al.*, 2003).

2.3 Traditional fermentation in Africa

Throughout Africa, fermentation is a traditional part of cereal, cassava and dairy processing. Fermented foods are largely consumed in Africa, where they constitute a bulk of the food. Since fermentation could increase protein content of food over the unfermented ones, fermented food is therefore desirable in developing countries like South Africa where protein deficiency is a problem (Adebayo *et al.*, 2013). A diversity of fermented products, which include porridges, beverages (alcoholic and non-alcoholic), breads and pancakes, fermented meat, fish, vegetables, dairy products and condiments, are produced in developing countries (Owusu-Kwarteng *et al.*, 2010; Steinkraus, 1996).

Fermentation is one of the oldest technologies and most important traditional food processing and preservation technique (Kalui *et al.*, 2010). Food fermentations involve the use of microorganisms and enzymes for the

production of foods with distinct quality attributes that are quite different from the original agricultural raw material (Lei and Jakobsen, 2004). In addition, fermentation provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product (Blandino *et al.*, 2003).

Fermentation may also lead to the detoxification and destruction of undesirable factors present in raw foods such as phytates, tannins and polyphenols. Several traditional fermented products have been documented in different African countries and include non-alcoholic beverages, alcoholic beverages, breads, pancakes, porridges, cheeses and milk (Gadaga *et al.*, 1999). Cereal-based fermented beverages have a long history in Africa and are traditionally used as staple foods and consumed in certain geographical regions in Africa (Loponen and Sibakov, 2013). They are major contributors to energy intake in developing countries (Guyot, 2012). However, the absence of a writing culture in most of Africa makes their origins difficult to trace. By the medieval ages, when most of northern and western Africa was conquered by the Muslim Arabs, many records of the presence of fermented beverages were made by the Arab travellers, mostly merchants and geographers (Odunfa and Oyewole, 1998). In Africa, cereal grains such as maize, sorghum and millet are a common substrate for producing various fermented products. The grains are malted, milled and fermented to produce thin gruels and non-alcoholic beverages known by various names in different parts of Africa (Bvochora, 1999). The preparation of many traditional or indigenous cereal-based beverages is normally carried out by natural fermentation involving mixed cultures of bacteria, yeast and/or fungi. The most common fermenting bacteria are of the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Micrococcus* and *Bacillus*. The yeasts most frequently found are of the genera *Saccharomyces* and *Candida*, though *Zygosaccharomyces*, *Geotrichum* and *Torulopsis* have also been identified in some beverages (Gotcheva *et al.*, 2000).

Yeasts have been reported to be involved in the production of several different types of indigenous fermented beverages (Gadaga *et al.*, 2001; Hounhouigan *et al.*, 1999; Blanco *et al.*, 1999). Despite their presence, the role of yeasts in

these products is often poorly investigated. The most dominant yeast species associated with African indigenous fermented beverages is *Saccharomyces cerevisiae* (Jespersen, 2003).

2.4 Aims of cereal fermentation

Traditional fermented cereal foods are known all over the world, especially in developing countries where they are valued for the taste, aroma, bioactive components, and texture (Sacca *et al.*, 2012). A multitude of fermented products made from cereals have been created in the history of human nutrition. According to Hammes *et al.* (2005); Johnson, (2000); Oelke and Boedicker, (2000); Haard *et al.* (1999); Hammes and Ganzle, (1998) and Nout (1994), the fermentation steps aim to achieve conditioning for wet milling by steeping of maize and wild rice, affecting sensory properties (aroma, taste, colour, texture), saccharification by use of *koji* prior to alcoholic fermentation or producing sweetened rice, preservation, which relies mainly on acidification and/or alcohol production, enhancing food safety by inhibition of pathogens, e.g. *Burkholderia gladioli* that had caused Bongkrek poisoning in products made from pre-soaked corn, *Staphylococcus aureus* and *Bacillus cereus* causing enterotoxicoses. Other aims of fermentation steps include improving the nutritive value by removing antinutritive compounds (e.g. phytate, enzyme inhibitors andpolyphenols), and enhancing the bioavailability of components by, e.g. affecting physio-chemical properties of starch and associations of fibre constituents with vitamins, minerals or proteins, removal of undesired compounds such as mycotoxins, endogenous toxins, cyanogenic compounds, flatulence producing carbohydrates, reducing energy required for cooking and achieving the condition of bakeability as it is required for producing leavened rye bread.

2.5 Lactic acid fermentation in Africa

Lactic acid bacteria (LAB) are generally mesophilic but can grow at temperatures as low as 5°C or as high as 45°C. Similarly, while the majority of strains grow at pH 4.0 – 4.5, some are active at pH 9.6 and others at pH 3.2. Strains are generally weakly proteolytic and lipolytic and require preformed amino acids, purine and pyrimidine bases and B vitamins for growth. All LAB produce lactic acid from hexoses and since they lack functional heme linked electron transport chains and a functional Krebs cycle, they obtain energy via substrate level phosphorylation (Blandino *et al.*, 2003; Caplice and Fitzgerald, 1999). According to Blandino *et al.* (2003), the term LAB is used to describe a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, usually non-motile, that utilize carbohydrates fermentatively and form lactic acid as the major end product (Table 3).

Table 3. Genera of lactic acid bacteria (LAB) involved in cereal fermentation

Genera of LAB	Cell form	Catalase Activity	Gram (±) Reaction
Lactobacillus	Rods (Bacilli; cocobacilli)	-	+
Streptococcus	Spheres in chains (Cocci)	-	+
Pediococcus	Spheres in tetrads (Cocci)	-	+
Lactococcus	Cocci	-	+
Leuconostoc	Spheres in chains (Cocci)	-	+
Bifidobacterium	Branched rods	-	+
Carnobacterium	Cocci	-	+
Enterococcus	Cocci	-	+
Sporolactobacillus	Rod	-	+
Lactosphaera	Cocci	-	+
Oenococcus	Cocci	-	+
Vagocuccus	Cocci	-	+
Aerococcus	Cocci	-	+
Weisella	Cocci	-	+

(McKay and Baldwin, 1990; Oberman and Libudzisz, 1996; Suskovic *et al.*, 1997)

LAB fermentation is a common way of preparing food traditionally in Africa. Some of the traditionally fermented foods in Africa include maize porridge, alcoholic beverages and dairy products. Some of the main reasons for the fermentation practice using LAB is to increase palatability and improve the quality of beverages by increasing the availability of proteins and vitamins. Furthermore, LAB confers preservative and detoxifying effects on beverages as well. When used regularly, LAB fermented beverages boost the immune system and strengthen the body in the fight against pathogenic bacterial infections. Thus, LAB fermentation is not only of a major economic importance, but it also promotes human health in Africa (Chelule *et al.*, 2010a).

LAB fermentation is a safe, economical and traditional method of food preservation foremost, as well as having the additional benefits of flavour, texture and retention of nutrients. Additionally, LAB fermentation is known to render cereal-based foods and beverages safe, in a chemical food additive-free, consumer-friendly manner, from an antinutrient and toxigenic perspective (Waters *et al.*, 2013). Lactic acid fermentations include those in which the fermentable sugars are converted to lactic acid by lactic acid organisms such as *Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Pediocccus cerevisiae, Streptococcus thermophilus, Streptococcus lactis, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus citrovorum, Bifidobacterium bifidus*, and so on. This single category is responsible for processing and preserving vast quantities of human beverages and insuring its safety. In addition, the lactic acid fermentations provide the consumer with a wide variety of flavours, aromas and textures to enrich the human diet (Steinkraus, 2002). It has been shown that lactic acid fermented beverages with pH 4.0 inhibit the growth of *B. cereus* and other pathogens (Byaruhunga, 1998). The different ways in which LAB inhibit pathogens have been reviewed by Lindgren and Dobrogosz (1990). Most of the work done on the inhibition of pathogens in lactic acid fermented beverages has concentrated on the effect of pH and acidity, however, it is possible that apart from the low pH and acidity, other inhibitory factors like the production of bacteriocins and lowering of the redox potential by lactic acid bacteria could be involved in the inhibition of *B. cereus* (Olsen *et al.*, 1995). According to Oyewole (1997), lactic acid

fermentation processes in Africa have survived throughout the centuries because of the following benefits that this technology possesses: (i) it serves as a household technology for improving food safety, (ii) it serves as a low-cost method of food preservation, and (iii) it contributes to the improvement of nutritional value and digestibility of raw materials.

LAB fermentation of beverages is also known to confer a variety of important therapeutic benefits on consumers, including anticarcinogenic activity (Chelule *et al.*, 2010b; Hirayama, 1999). For example, detoxification of natural toxicants, such as cyanogenic glycosides and mycotoxins, has been proposed (Chelule *et al.*, 2010b; Mokoena *et al.*, 2005). The therapeutic benefits associated with LAB include prophylaxis against some types of intestinal infections, increased tolerance to lactose-containing beverages and possible prevention of cancer initiation (Chelule *et al.*, 2010b).

2.6 Cereal fermented and non-alcoholic beverages

These are fermented cereal products which on processing yield acidic, nonalcoholic fluidly gruels with a high water content (porridge). The common cereal gruels include '*ogi*' in West Africa, '*akasa*' or '*koko*' in Ghana, '*uji*' in Kenya, '*mahewu*' or '*magou*' in South Africa and '*abreh*' in Sudan (Katangole 2008; Wood, 1998). A range of fermented non-alcoholic cereal based beverages and foods in Africa are listed in Table 4.

2.6.1. *Mahewu*

Mahewu (*amahewu*) is an example of a non-alcoholic sour beverage made from corn meal, consumed in Africa and some Arabian Gulf countries. It is an adult type of food, although it is commonly used to wean children. In South Africa it is known by various names. In Zulu it is known as '*amahewu*', the Xhosas call it '*amarehwu*', the Swazis, '*emahewu*', the Pedis, '*metogo*', Sothos, '*machleu*', while the Vendas call it '*mabundu*' (Nyadzi, 2007). The most commonly used term is *mahewu* (Katangole, 2008; Steinkraus, 2004). It is prepared from maize porridge, which is mixed with water. Sorghum, millet malt or wheat flour is then

added and left to ferment (Odunfa *et al.*, 2001). Alternatively, it can be prepared by mashing left-over pap into a slurry and then ferment it overnight (Gadaga *et al.*, 1999). The fermentation is a spontaneous process carried out by the natural flora of the malt at ambient temperature (Gadaga *et al.,* 1999).

Mahewu is a popular fermented non-alcoholic beverage that is readily available on food retail stores in South Africa, Botswana and in the southern African region as a whole (Matsheka *et al.*, 2013). The consumption of *mahewu* in South Africa is seasonal and peak sales are registered during summer months from October to March. The winter can bring a decline of 50-60% in *mahewu* consumption, with the worst months being May through July. The total market for *mahewu* was about 146 million litres in 1984 of which about 97 million litres was packed and 49 million litres delivered in bulk. The annual per capita consumption of the commercial product can be estimated at 12-14 litre per black adult (Steinkraus, 2004). *Mahewu* is known to offer some advantages over *ogi* in that the initial wild fermentation by fungi, etc. is eliminated by boiling both the maize meal and water for steeping. Furthermore, it is pre-cooked and requires only mixing prior to consumption. *Mahewu* consists of coarse maize particles while ogi contains very fine pasty maize particles (Haard *et al.*, 1999).

Table 4. Fermented non-alcoholic cereal based gruels and beverages in sub-Saharan Africa

Product	Region	Countries	Substrate	Microorganisms	References
Mahewu	Southern Africa	South Africa, Botswana and Zimbabwe	Maize, water	*Lactococcus lactis subsp. Lactis*	Blandino *et al.*, 2003
Incwancwa	Southern Africa	South Africa	Maize , water	Lactobacillus species	Gqaleni*et al.*, 1998
Ogi (Akamu)	West Africa	Nigeria, Ghana	Maize, Sorghum, millet, water	*Lactobacillus* species , Aerobacter and yeasts	Osungbaro and Taiwo, 2009
Togwa	East Africa	Tanzania	Maize, finger millet malt, water	Lactobacillus species, *Issatchenkiaorientalis and Saccharomyes cerevisiae*	Mugula*et al.*, 2003
Borde (shamita)	East Africa	Ethiopia	Maize, wheat, barley, water	Lactobacillus species, *Enterobacteriaceae*	Antenehe*et al.*, 2011, Abegaz, 2007
Munkoyo	Southern Africa	Zambia	Maize, *Rhynchosiaheterophylla* root extract, water	Unknown	Zulu *et al.*, 1997
Uji	East Africa	Kenya, Uganda	Maize, Sorghum, millet, water	*Leuconostoc mesenteriodes, Lactobacillus platarum,Pediococcus acidilactici* and *P. pentosaceus*	Blandino*et al.*, 2003 and Nout, 2009
Kunu	West Africa	Nigeria, Cameroon, Niger	Sorghum, millet, water	*Bacillus subtilis, Micrococcus sp, Staphylococcus Aureus*	Adebayo *et al.*, 2010, *Gaffa et al.*, 2001, 2002

2.7 Production of *mahewu* – cereal non-alcoholic fermented beverage

In Southern Africa, *mahewu* is a traditional fermented beverage that is prepared using maize. It contains little or nc alcohol, has a pH of about 3.5 and is popular among the black people of southern Africa (Byaruhunga, 1998).

It is traditionally prepared by adding one part of maize meal to 9 parts of boiling water. The suspension is cooked for ten minutes, allowed to cool and then transferred to a fermentation container. Cooking of the maize leads to gelatinization of the starch. Gelatinization is characterized by swelling of the starch granules, leaching of the starch components (especially the amylose) and increase in the viscosity of the porridge and increased susceptibility to enzymatic digestion (Chelule *et al.*, 2010a; Byaruhunga, 1998). At this stage, wheat flour (about 5% of the maize meal used) is added to serve as a source of inoculums. The fermentation is a spontaneous process carried out by the natural flora of the malt at ambient temperature (20-30°C). The predominant microorganisms in the spontaneous fermentation of the African *mahewu* belong to *Lactococcus lactis subsp. Lactis*. (Blandino *et al.*, 2003).

Mahewu can be stored in a cool place for 20-25 days and serves as a refreshing energy drink both for adults and children (Chelule *et al.*, 2010a). Figure 2 is a flow diagram showing how this product is traditionally produced.

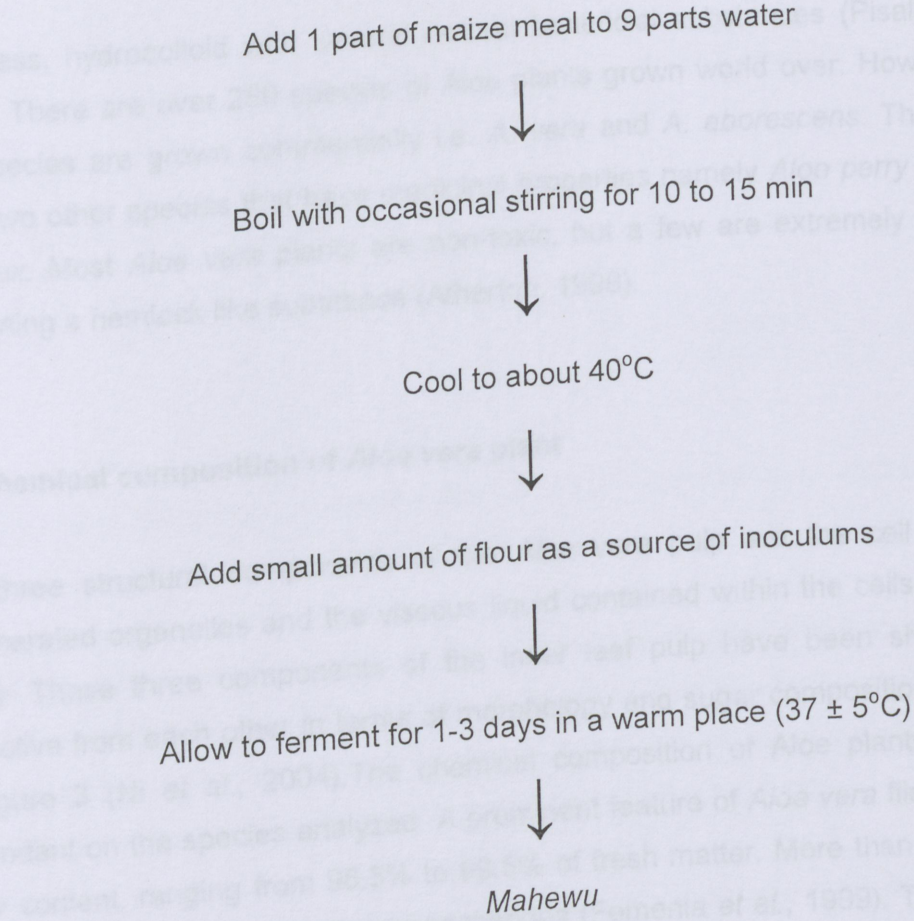


Figure 2. Production of home-made *mahewu,* a South African fermented maize beverage (Chelule *et al.*, 2010a).

2.8 Botany of *Aloe vera* plant

Aloe vera or *Aloe barbadensis* Miller is a spiky cactus-like xerophytes. It is a clump forming perennial plant with thick fibrous root which produces large basal leaves, usually 12–16 per plant, weighing up to 1.5 kg when mature. The leaves are up to 50 cm long and 8–10 cm across at the base, tapering to a point, with saw-like teeth along their margins (Ahlawat and Khatkar, 2011). The leaves are formed by a thick epidermis (skin) covered with cuticle surrounding the mesophyll, which can be differentiated into chlorenchyma cells and thinner walled cells forming the parenchyma (fillet). The parenchyma cells contain a transparent mucilaginous jelly, which is referred to as *Aloe vera* gel. The gel contains 97–98% of water and more than 60% of dry matter is made up of polysaccharides. The gel is a colourless,

odourless, hydrocolloid with several natural beneficial substances (Pisalkar *et al.*, 2010). There are over 250 species of Aloe plants grown world over. However, only two species are grown commercially i.e. *A. vera* and *A. aborescens*. There are at least two other species that have medicinal properties namely *Aloe perry baker* and *A. ferox*. Most *Aloe vera* plants are non-toxic, but a few are extremely poisonous containing a hemlock like substance (Atherton, 1998).

2.9 Chemical composition of *Aloe vera* plant

The three structural components of the *Aloe vera* pulp are the cell walls, the degenerated organelles and the viscous liquid contained within the cells (Hamman, 2008). These three components of the inner leaf pulp have been shown to be distinctive from each other in terms of morphology and sugar composition as shown in Figure 3 (Ni *et al.*, 2004).The chemical composition of Aloe plants is largely dependant on the species analyzed. A prominent feature of *Aloe vera* filet is its high water content, ranging from 98.5% to 99.5% of fresh matter. More than 60% of the remaining solid is made up of polysaccharides (Femenia *et al.*, 1999). There are as many as 200 different types of molecules in *aloe vera* (Davis, 1997). The *Aloe vera* leaf gel contains about 98% water (Bozzi *et al.*, 2007). The total solid content of *Aloe vera* gel is 0.66% and soluble solids are 0.56% with some seasonal fluctuations. On dry matter basis, aloe gel consists of polysaccharides (55%), sugars (17%), minerals (16%), proteins (7%), lipids (4%) and phenolic compounds (1%). The gel contains many vitamins including the important antioxidant vitamins A, C and E. Vitamin B_1 (thiamine), niacin, Vitamin B_2 (riboflavin), choline and folic acid are also present (Lawless and Allen, 2000). Novel components of *Aloe vera* plant along with their health benefits are shown in Table 5.

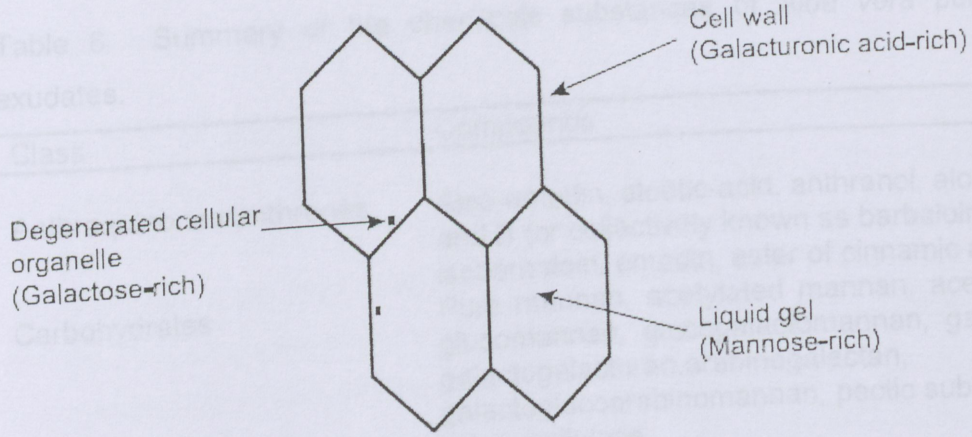


Figure 3. Schematic representation of *Aloe vera* leaf pulp structure and its component (Ni *et al.*, 2004).

Wang (1993) reported that potassium and chloride concentration appeared to be excessive in *Aloe vera* juice in comparison to most plant products whereas the sodium content was found lesser in quantity. Calcium, magnesium, copper, zinc, chromium and iron were also found in the aloe products.

Table 5. Novel components of *Aloe vera* plant along with their health benefits (Ahlawat and Khatkar, 2011)

Chemical component	Health benefits
Acemannan	Accelerate wound healing, modulate immune system, antineoplastic and antiviral effects
Alprogen	Anti-allergic
C-glycosyl chromone	Anti-inflammatory
Bradykinase	Anti-inflammatory
Magnesium lactate	Anti-allergic
Salicylic acid	Analgesic, anti-inflammatory

Major chemicals substances occurring in *Aloe vera* pulp and exudate are summarized in Table 6 below. It is suggested that the growth stage of the aloe plant plays a vital role in the composition and antioxidant activity (Hu *et al.*, 2003). *Aloe vera* juice also has antibacterial properties against Gram- positive bacteria (Alemdar and Agaoglu, 2009).

Table 6. Summary of the chemicals substances of *Aloe vera* pulp and exudates.

Class	Compounds
Anthraquinones/anthrones	Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid
Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan,arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose
Chromones	8-C-glucosyl-(2′-O-cinnamoyl)-7-O methylaloediol A, 8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-7-O-methyl-(S)-aloesol,8-C-glucosyl-7- Omethylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoaloesin A
Enzymes	Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxidase, cyclooxygenase, lipase, oxidase, phosphoenol, pyruvate carboxylase, superoxide dismutase
Minerals	Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, zinc
Lipids and miscellaneous organic compounds	Arachidonic acid, γ-linolenic acid, steroids (campestrol, cholesterol, β-sitosterol), triglicerides, triterpenoid, gibberillin, lignins, uric acid
Amino acids	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, valine, phenylalanine, proline, threonine, tyrosine
Proteins	Lectins, lectin-like substance
Saccharides	Mannose, glucose, L-rhamnose, aldopentose
Vitamins	B1, B2, B6, C, β-carotene, choline, folic acid, α-tocopherol

(Ni and Tizard, 2004; Choi and Chung, 2003; Dagne *et al.*, 2000; Femenia *et al.*, 1999)

2.10 Use of *Aloe vera* in food industry

Aloe vera is an industrial crop and, in the food industry, it has been utilized for the preparation of beverages like tea, milk, ice-cream and confectionary (Seoshin *et al.*, 1995). Currently, there is an increasing interest in the use of *Aloe vera* gel in the food industry, as a source of functional food in beverages and ice creams (Ramachandra and Rao, 2011; Moore and MacAnalley, 1995). A few examples of food product applications (Table 7) of *Aloe vera* are in soft drink (with electrolytes), diet drink with soluble fiber, hangover drink, healthy vegetable juice, tropical fruit juice, yogurt and yogurt drinks, jelly desserts with chunks of aloe, instant granules, gums for sore or bleeding gums, *Aloe vera* candy, *Aloe vera* sorbet with citrus juice and smoothies (Eshun and He, 2004; Hamman 2008; Hastuti 1999; Singh and Singh, 2009).

Table 7. Food applications of *Aloe vera* products.

Aloe vera products	Food applications
Concentrate	Squash, jam, jellies, aloe vera concentrate can also be mixed with tea, water or juice
Gel fillet	Candies, bar, munch, chewing gum, instant aloe vera tea granules, aloe vera gum for sore or bleeding gums, candy type aloe vitamins, aloe vera fruit smoothies
Juice	Ready to serve drink, health drink, soft drink, laxative drink, sherbet, sports drink (with electrolytes), diet drink with soluble fiber, hangover drink with B vitamins, amino acids and acetaminophen, healthy vegetable juice mix, yoghurts, aloe vera mix for whiskey or other alcohol, white bread with aloe vera, and cucumber juice with aloe vera
Powder	Yoghurt, curd, 'lassi', ice-cream, and aloe vera 'laddu'

Eshun and He 2004; Hamman 2008; Hastuti 1999; Singh and Singh 2009)

The processing of *Aloe vera* gel, derived from the leaf pulp of the plant, has become a big industry worldwide due to its application in food industry. The gel processed either by traditional hand filleting and/or whole leaf process can be

reduced to powder form which improves shelf-life compared to liquid products and eliminate the cost of transport and storage (Ahlawat and Khatkar, 2011). It is utilized in functional foods especially for the preparation of health drinks with no laxative effects. It is also used in other food products including milk, ice cream, confectionery, etc. The gel is also used as flavoring component and preservative in some food (Christaki and Florou-Paneri, 2010). Thus, a simple and efficient processing technique needs to be developed especially for the aloe beverage industry to improve product quality and safety by preserving the bioactive chemicals naturally present in the intact *Aloe vera* leaf (Eshun and He, 2004). Nevertheless, processing techniques used to obtain *Aloe vera* gel are very important to ensure product quality and to maintain almost all the bioactive components (He *et al.*, 2005).

The potential use of *Aloe vera* products often involves some type of processing, like heating, dehydration and grinding (Chang *et al.*, 2006). Unfortunately, because of improper processing procedure aloe products contain very little or virtually no active ingredients (Ramachandra and Rao, 2006), so it has become very important to develop a better method of preservation for increasing the shelf life and maintaining the quality of *Aloe vera* gel. *Aloe vera* gel powder is being used by food processing industries in preparation of yogurt and other food products (Smitha and Pratima, 2007). The physiological activity of *Aloe vera* polysaccharides has been widely reported (Ramachandra and Rao, 2006). Glucomannan and acemannan were proved to accelerate wound healing, activate macrophages, stimulate the immune system and have antibacterial and antiviral effects (Bozzi *et al.*, 2007). Besides medicinal values, this plant contains a number of nutrients such as vitamins, minerals, amino acids, sugars, enzymes, fatty acids and saponins, which have positive effects on the human body and could therefore be used in food formulations as functional ingredients for health benefits (Choi and Chung, 2003). In food industry, AV is used as a resource of functional food (Ramachandra and Rao, 2011).

2.11 *Aloe vera* powder and its food application

In the dehydration method, the pure intact *Aloe vera* gel fillets are first washed to remove traces of aloin. The fillets are placed in a humidity chamber where the desired levels of relative humidity and temperature are maintained and then dried with the passage of hot air. This material is ground to powder and packed (Ramachandra and Rao, 2008).

Qmatrix drying is a novel proprietary method of dehydration of *Aloe vera* enabling dehydration while maintaining its integrity with respect to flavour, colour and bioactivity. It is comparable to freeze drying in quality aspects, but without the high operational cost. In freeze drying, gel fillet is lyophilized at −88 °C and 0.01 mm Hg pressure for 65 h and ground to powder of moisture content below 4% (Ahlawat and Khatkar, 2011).

Qian (2002) prepared freeze dried powder from ultra-filtration and reverse osmosis of concentrated *Aloe vera* gel. Gautam and Awasthi (2007) prepared *Aloe vera* leaf powder by cutting leaves in small pieces, blending in a mixer and drying in a tray drier at 50 °C for 12 h. before grinding into powder in a mixer grinder. AVP can be used in curd, lassi, ice-creams, and in the preparation of yoghurts (Ahlawat and Khatkar, 2011).

CHAPTER 3. MATERIALS AND METHODS

3.1 Materials

Maize meal (5 kg) and sorghum flour (5 kg) were purchased from Shoprite in Thohoyandou, South Africa. AVP (250 g) was supplied by Khutsong Natural Herbs and Treatment Centre, Makwarela location, Thohoyandou area. Distilled water from the Department of Food Science and Technology, University of Venda and reagents from Merck were used to perform the experiment.

Equipment: Stainless steel pots (x3), 500 ml plastic bottles (x20) – purchased from Nampak, stove, electric kettle, water bath GFL 1083 (Optolabor), Beckman pH Meter 145 model, glass beakers, colorimeter (Hunter lab), plastic petri dishes, 100-ml volumetric flasks, filter papers, crucibles, desiccators , 250 and 300 ml Beakers, magnetic stirrer and stirring bars, Atago refractometer (3T), vertical autoclave EA-630 (Instrulab), auto vortex mixer MT 19 (Chiltern), forced circulation incubator FSIE (Labcon) and analytical balance.

3.2 Design and plan of experiment

The experiment was conducted at the Department of Food Science and Technology, University of Venda, Thohoyandou. The experimental treatments were arranged in a completely randomised design and replicated five times with the following treatments: addition of AVP in the samples, temperature of incubation for microbiological analysis and storage temperature of samples at 36 ± 5°C for 60 days.

3.2.1 Production of *mahewu*

Maize meal and sorghum flour were soaked in water for 24 h. and cooked into porridge of 8-10 % solid content. The porridge was cooled and allowed to ferment at temperatures between 25 – 30°C for 3 days. The porridge was re-cooked and AVP (10 g) added before the product was packaged in 500 ml bottles. This was the basic procedure which was followed for preparation of

mahewu in the laboratory (Sample C) and at home (Sample B). A control *mahewu* was produced in the laboratory without adding AVP (Sample A).

3.2.2 Shelf-life of *mahewu* for determination of improvement

The effect of adding AVP in the production of *mahewu* was investigated using the method in section 3.2.1. A control *mahewu* (Sample A) was produced without adding AVP. *Mahewu* prepared at home (Sample B) and in the laboratory (Sample C) were used for shelf-life observation. The experiments were replicated three times. Microbiological, organoleptic and chemical profiles of the three types of *mahewu* were compared.

3.2.3 Shelf-life study of mahewu samples

Fermented *mahewu* from all the three samples were stored at 36 ± 5ºC for 60 days. From all the three samples, 10 ml of the sample was withdrawn at 15-day intervals starting at zero until 60 days for the purposes of chemical and microbiological analyses.

3.2.4. Chemical analysis of mahewu samples

After every 15 days during storage, equal amounts of samples from the two stored *mahewu* (AVP added) as well as the control sample were analyzed for change in pH and also the amount of acid present by titration.

3.2.5 Determination of the total lactic acid in different *mahewu* samples

The total amount of acid present in each sample during 15-day intervals was determined by titration (AOAC, 1998), whereby 2 g of sample was measured in triplicate into 250 ml flasks, 8 ml of distilled water and three drops of phenolphthalein indicator were added with thorough mixing. The mixture was titrated against 0.1N NaOH to a pink colour. The amount of acid produced was calculated as percent lactic acid according to the formula:

% lactic acid

$$= \frac{\text{ml of 0.1M NaOH x normality of NaOH x mol weight of acid}}{\text{ml of sample x 10}}$$

3.2.6 Microbiological analysis of *mahewu* samples

The number of lactic acid bacteria (LAB), yeasts and moulds and also coliform bacteria were monitored in all fermented *mahewu* samples at the end of each of the 15 days storage cycles starting from 0 day until a total storage period of 60 days was achieved.

3.2.6.1 Preparation of dilutions

Tenfold serial dilution of the samples was made by aseptically transferring 1 ml of sample into sterile 9 ml, ¼ strength Ringer solution and thoroughly mixing and thus resulting in a dilution of 10^{-1} (IDF, 1992). Further tenfold dilution was made by transferring 1 ml of successive serial dilutions into test tubes containing 9 ml of sterile saline until a required dilution was reached.

3.2.6.2 LAB count

The de Man, Ragusa and Sharpe (MRS) agar (Merck) was used for the enumeration of LAB according to a method of Jay (2000). This medium was prepared according to the manufacturer's instructions and left to cool to a temperature of approximately 55°C ± 5°C. Appropriately marked sterile petri dishes were inoculated with 100 µl of the appropriate tenfold serial dilution of the samples and this was done in triplicate. MRS agar (15 ml), at room temperature, was then added to the petri dishes. The plates were mixed by swirling on a flat horizontal surface before allowing solidifying for about 30 min. The plates were anaerobically incubated in an inverted position at 30°C for a period of 72 h. The colonies were counted and expressed as number of colony forming units per milliliter (cfu/ml) of sample.

3.2.6.3 Fungal count

Potato dextrose agar (PDA) (Merck) was used for the enumeration of yeasts and moulds in the samples A, B and C according to the method of Jay (2000). The medium was prepared according to the manufacturer's instruction and 12 to 15 ml was equally distributed into marked petri plates. The plates were allowed to dry for 30 min. The dried plates were inoculated with 100 µl of the relevant serial dilutions of each sample. Each inoculums was spread over the surface of the PDA plates using a sterile (dipped in ethanol and flamed) bent glass rod. The plates were incubated in an inverted position at 25ºC for 120 h. After incubation, the colonies were counted and expressed as number of colony forming units per milliliter (cfu/ ml) of sample.

3.2.6.4 Coliform count

The Violet red bile agar (VRBA) (Merck) was used for the enumeration of coliforms in the samples A, B and C according to the method of Jay (2000). The medium was prepared according to the manufacturer's instructions and distributed into marked petri dishes. From appropriate serial dilutions, 100 µl amount of each sample was inoculated onto VRBA plates, the procedure was as in the case of the yeast and mould counts. The plates were incubated in an inverted position at 37ºC for 24 h. The colonies were counted and expressed as number of colony forming units per milliliter (cfu/ ml) of sample.

3.2.7 Physical tests of *mahewu* samples

3.2.7.1 Determination of soluble solids

Total soluble sugar of *mahewu* samples was measured using a refractometer (Mart´ınez-Romero *et al.*, 2006). Soluble solids (SS) refers to molecules that are truly soluble in aqueous solution. A convenient approximation here is to assume that 1 °Brix readings by a refractometer equals to 1 g of sugar in *mahewu* sample. Five drops of *mahewu* sample was placed on the refractometer plate and covered with a plate and the light turned on (on the

centre side of the refractometer), the refractometer viewer, adjusted interface so that it lined up with the X-shape on the viewer screen. Readings for soluble solids values were recorded.

3.2.7.2 Determination of colour

Colour was determined using the Hunter Lab System (Mart´ınez-Romero *et al.*, 2006). Approximately 6 g of each sample was weighed and the beaker with the sample was placed in a LabScan XE Spectrophotometer with illuminant D65 as the light source. The *L**, *a** and *b** colour coordinate values were measured. The results were expressed as positive *L** (lightness), *a** (redness) and *b** (yellowness) colour space value.

3.3. Sensory analysis of *mahewu* samples

Sensory evaluation of samples A, B and C was carried out to determine their organoleptic characteristics in terms of their appearance, colour, taste, sourness and overall acceptance. A nine point hedonic scale, varying from dislike extremely (score 1) to like extremely (score 9) was used according to Mbata *et al.*, (2009). A total of 50 panelists from the University of Venda community, including university students and staff participated in the study. Error due do bias was reduced by not including on the panel those persons who were directly involved with the preparation of samples during the sensory evaluation of *mahewu*. Samples were also coded so that the panelists could not identify them, as the code itself should introduce no bias.

3.4. Statistical analysis

Analysis of variance (ANOVA) was performed on the sets of data generated using the general linear model procedure (GLM) of a statistica software for windows7 (Tilsa, Okldama, USA 2003). The purpose of the statistical analysis in this study was to determine the differences or no differences among samples

A, B and C of *mahewu*. The statistical significance of the results was determined at a probability level of $P < 0.05$.

3.5. Potential outcome

The knowledge obtained from this research could help both home-and commercially- made *mahewu* producers to extend the shelf life of their products. Supplementing *mahewu* with AVP could make the product more nutritious for people in parts of sub-Saharan Africa and contribute to health benefits and also to food security.

CHAPTER 4: RESULTS

4.1 Physicochemical properties of *mahewu* samples

The changes in pH, titratable acidity (TA) and total soluble solids (TSS) during the storage of *mahewu* samples are presented in Table 8.

Table 8. Physicochemical properties of mahewu samples

Day	Sample	pH	TA (% lactic acid)	°Brix
0	A	3.9 ± 0.01 ^{a}	0.2 ± 0.01 ^{a}	4.7 ± 0.12 ^{a}
	B	4.0 ± 0.04 ^{a}	0.5 ± 0.05 ^{b}	4.7 ± 0.19 ^{a}
	C	4.0 ± 0.04 ^{a}	0.6 ± 0.05 ^{b}	5.4 ± 0.52 ^{b}
15	A	3.3 ± 0.01 ^{a}	0.8 ± 0.05 ^{a}	4.7 ± 0.04 ^{a}
	B	3.6 ± 0.09 ^{a}	0.8 ± 0.04 ^{a}	2.6 ± 0.03 ^{b}
	C	3.6 ± 0.00 ^{a}	0.8 ± 0.08 ^{a}	3.5 ± 0.04 ^{c}
30	A	3.0 ± 0.03 ^{a}	1.0 ± 0.06 ^{a}	4.7 ± 0.03 ^{a}
	B	3.4 ± 0.06 ^{b}	1.2 ± 0.05 ^{a}	3.2 ± 0.04 ^{b}
	C	3.2 ± 0.08 ^{b}	1.1 ± 0.04 ^{a}	2.4 ± 0.49 ^{c}
45	A	2.7 ± 0.17 ^{a}	1.3 ± 0.08 ^{a}	4.3 ± 0.13 ^{a}
	B	3.4 ± 0.29 ^{b}	1.3 ± 0.05 ^{a}	3.0 ± 0.2 ^{b}
	C	3.1 ± 0.01 ^{b}	1.4 ± 0.08 ^{a}	2.2 ± 0.21 ^{c}
60	A	2.4 ± 0.05 ^{a}	1.8 ± 0.07 ^{a}	4.2 ± 0.55 ^{a}
	B	2.9 ± 0.08 ^{a}	1.8 ± 0.06 ^{a}	3.0 ± 0.19 ^{b}
	C	2.8 ± 0.6 ^{a}	1.7 ± 0.05 ^{a}	2.0 ± 0.13 ^{c}

Mean ± Standard Deviation (SD). Mean values in the same column with different superscripts are significantly different from each other ($P < 0.05$). Sample A = Control, sample B = Home-made and sample C = Laboratory made *mahewu*.

There was a decrease in pH in all the three samples during the storage period and the pH ranged between pH 3.3 and pH 2.4 from day 15 to 60 days of storage. The pH of all the three samples kept on decreasing during the storage period. There were no significant difference amongst the samples in day 0. However, numerically, the control (Sample A) had the lowest pH (3.9) as compared to sample B (home-made) and sample C (laboratory made) (4.0) during day 0. The control also had the lowest pH during the entire storage period, even after day 60 of storage (2.4), whilst home-made and laboratory *mahewu* had pH 2.8 and pH 2.9, respectively. There was a significant difference amongst the pHs of the samples during day 30 and 45 of storage but there were no significant differences during day 60 of storage. The pH

value of a food is a direct function of the free hydrogen ions present in that food. Acids present in foods release these hydrogen ions, which give acid foods their distinct sour flavor. Thus, pH may be defined as a measure of free acidity. More precisely, pH is defined as the negative log of the hydrogen ion concentration (McGlynn, 2010). On the other hand, the TA of all the samples increased significantly ($P \leq 0.05$) throughout the storage period and it ranged between 0.2% and 1.8% lactic acid (v/v). TA is used as a guide to determine how acidic the product will taste. This determination measures the concentration of all available hydrogen ions present in the sample (McGlynn, 2010). A general increase in total acidity was recorded for all samples during day 15 (0.8) and it continued to increase until day 60 (1.8). The total acidity in the control sample rapidly increased until 60 days of storage. Acidity in the other two samples increased much slower during day 15 and 30, only rapidly rising after day 45 and 60 of storage. A general decrease in TSS were recorded in two samples (B and C) during day 15 of storage (2.6 and 3.5 °Brix), while sample A had the same value (4.7) °B during day 0 and 15 (Table 8). There was a significant difference amongst the samples during day 15 to day 60 with respect to TSS. A general decrease in TSS was recorded in all three samples during day 45 and 60, while sample B had an increase in TSS in day 30 (3.2). Sample A had the largest reduction in total solids over 45 days of storage (4.3) from 4.7 in day 30. Soluble solids are composed of polysaccharides that may be broken down enzymatically into free sugars which can be determined as Brix with refractive index measurements. These soluble solids are primarily sugars; sucrose, fructose, and glucose (Anthon *et al.*, 2011).

4.2 Principal Component Analysis (PCA): pH, TA (% lactic acid) and TSS of *mahewu* samples

The principal component analysis for physicochemical properties of three *mahewu* samples was investigated by looking at the pH, TA and TSS (Figure 4). The main sample differences and similarities, and attributes relationships were explained by the first and second PC. Principal Component (PC) 1 is characterized mainly by brix and pH. Principal component (PC) 2 is characterized by lactic acid.

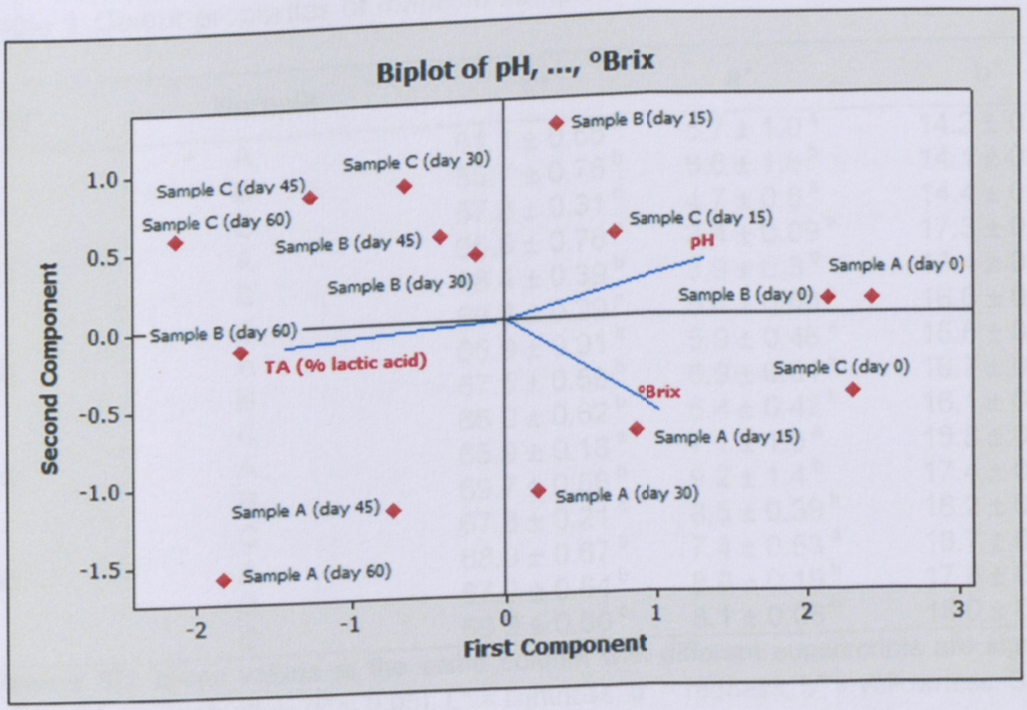


Figure 4. PCA Bi-plot indicating physicochemical properties of different *mahewu* samples.

There was correlation amongst sample C (day 0) and Sample A (day 15 and 30) with regard to the TSS. These results mean that samples C and A had high values of brix during the above-mentioned storage days. There was correlation amongst samples B (day 0 and 15), A (day 0) and C (day 15). These results show that these samples had high pH values in the above-mentioned storage days.

4.3 Colour properties of *mahewu* samples

The colour properties of the three *mahewu* samples were investigated by looking at three parameters: L*, a* and b* (Table 9). The L* (lightness) values of three *mahewu* samples were significantly different during day 0; where sample A had the highest L* value (61.1), followed by sample C (57.3) and sample B (55.7).

Table 9. Colour properties of *mahewu* samples

Day	Sample	L*	a*	b*
0	A	61.1 ± 0.55^{a}	5.7 ± 1.0^{a}	14.2 ± 0.81^{a}
	B	55.7 ± 0.76^{b}	8.6 ± 1.6^{b}	14.1 ± 0.01^{a}
	C	57.3 ± 0.31^{c}	4.7 ± 0.6^{a}	14.4 ± 0.23^{a}
15	A	66.8 ± 0.78^{a}	3.4 ± 0.09^{a}	17.3 ± 0.22^{a}
	B	68.4 ± 0.39^{b}	5.9 ± 0.3^{b}	17.4 ± 0.52^{b}
	C	66.8 ± 0.29^{a}	5.4 ± 0.86^{b}	16.6 ± 0.51^{b}
30	A	65.9 ± 0.91^{a}	5.9 ± 0.46^{a}	15.5 ± 0.29^{a}
	B	67.5 ± 0.59^{b}	6.9 ± 0.01^{b}	16.7 ± 0.18^{b}
	C	66.9 ± 0.62^{b}	6.4 ± 0.42^{b}	16.1 ± 0.59^{a}
45	A	65.9 ± 0.18^{a}	7.1 ± 1.0^{a}	18.8 ± 0.79^{a}
	B	69.7 ± 0.56^{b}	9.2 ± 1.4^{b}	17.4 ± 0.13^{b}
	C	67.8 ± 0.21^{c}	8.5 ± 0.39^{b}	18.2 ± 0.33^{a}
60	A	68.9 ± 0.67^{a}	7.4 ± 0.53^{a}	18.7 ± 0.27^{a}
	B	67.8 ± 0.64^{b}	8.9 ± 0.19^{b}	17.8 ± 0.16^{b}
	C	69.9 ± 0.50^{c}	8.1 ± 0.08^{b}	18.0 ± 0.03^{a}

Mean ± SD. Mean values in the same column with different superscripts are significantly different from each other ($P < 0.05$). L* = lightness; a* = redness; b* = yellowness. Sample A = Control, sample B = Home-made and sample C = Laboratory made *mahewu*.

There was an increase in the L* value for all the samples in day 15 with sample B having the highest L value of 68.4. Sample B was significantly different from samples A and C, whilst there was no significant difference between the two samples (A and C). There was a decrease in the L* value for sample A in day 30 and 45 (65.9) whilst the other two samples had an increase with sample B having the highest L value (67.5) in day 30 and 69.7 in day 45. There was an increase in the L* value for samples A (68.9) and C (69.9) in day 60 of storage, while sample B had a decrease in L* value (67.8). There was a significant difference amongst the samples in day 45 and 60. This result shows that the addition of AVP did not have any effect on the lightness of the samples because there was no significant difference during

the storage period of the samples. In terms of the a* values (redness) when it is positive and green when it is negative, there was no significant difference between sample B and C from day 15 to day 60 of storage while sample A was significantly different from the two samples during the same storage days. However, both samples (B and C) had a decrease in redness during day 60 as compared to day 45 (7.1) while sample A had an increase in day 60 (7.4). This result shows that the addition of AVP had an effect on the redness of the two samples (B and C) because they were significantly different ($P < 0.05$) from sample A which is a control even though all samples had positive a* values which indicate the presence of the red colour in all *mahewu* samples. The positive b* values indicate the yellowness while the negative values means the sample is blue. There was no significant difference in the b* values of samples B and C during day 15 while sample B was significantly different from sample A and C from day 30 until day 60 of storage ($P < 0.05$). Again this results shows that the addition of AVP did not have any effect on the yellowness of the samples since sample C was not significant different from sample A, which is a control during the storage period even though all samples had positive values.

4.4 Microbiological properties of *mahewu* samples

The microbial quality of three *mahewu* samples was investigated by looking at four parameters, coliform bacteria, lactic acid bacteria, mould and yeast, most of which are associated with microbial quality deterioration (Table 10). There was an increase in numbers of coliform bacteria, lactic acid bacteria, yeast and moulds during the storage period of 60 days. Sample B had the highest coliform counts from day 0 when compared to samples A and C which had no coliform detected during day 0, however all samples recorded the highest number of coliforms count during day 45 and 60 because the coliforms were too numerous to count.

Table 10. Microbiological content of *mahewu* samples

Day	Sample	Coliforms	LAB	Mould	Yeast
0	A	ND	3.0086	2.4771	4.8513
	B	2.9823	3.2434	1.4322	2.7075
	C	ND	3.2542	2.4771	2.3222
15	A	1.0000	7.9395	1.3222	7.0414
	B	5.0086	7.8062	1.0000	4.9912
	C	1.1304	7.7559	1.0000	4.5315
30	A	4.7482	>3.4771	>3.4771	9.1761
	B	>2.1761	>3.4771	>3.4771	9.1139
	C	5.9731	>3.4771	>3.4771	7.8633
45	A	>2.1761	>3.4771	>3.4771	>3.4771
	B	>2.1761	>3.4771	>3.4771	>3.4771
	C	>2.1761	>3.4771	>3.4771	>3.4771
60	A	>2.1761	>3.4771	>3.4771	>3.4771
	B	>2.1761	>3.4771	>3.4771	>3.4771
	C	>2.1761	>3.4771	>3.4771	>3.4771

Average bacterial counts $\log_{10}$ (cfu/ml); ND = Not Detected; Sample A = Control, sample B = Home-made and sample C = Laboratory made *mahewu*.

The highest LAB counts were obtained after 15 days in all samples, that is, LAB counts increased from 3.0086 in day 0 to 7.9395 $\log_{10}$ cfu/ml, 3.2434 to 7.8062 $\log_{10}$ cfu/ml and 3.2542 to 7.7559 $\log_{10}$ cfu/ml for samples A, B and C, respectively. The LAB counts continued to increase on day 30, 45 and 60 in all the three samples. Pattison *et al.,* (1998) reported high numbers of LAB in commercial sorghum beer and stated it indicated the ability of the bacteria to survive and grow in an acidic environment. The low pH of the beer reportedly inhibits or kills pathogenic or most anaerobic endospore-forming bacteria, thus improving the safety of the product (Haggblade and Holzapfel, 1989).

4.5 Sensory acceptability of *mahewu* samples

4.5.1 Appearance acceptability of *mahewu* samples

The home-made *mahewu* (sample B) had the lowest acceptance rating of 5.7 in terms of appearance (Table 11). The sample that had the highest mean of 7.3 in this sensory attribute was the control, sample A (Table 11).

Table 11. Mean scores for sensory acceptability of *mahewu* samples

Samples	Appearance	Colour	Taste	Sourness	Overall acceptability
Sample A	7.3 ± 1.6^{a}	7.2 ± 1.6^{a}	6.0 ± 1.6^{a}	6.0 ± 1.9^{a}	5.5 ± 2.3^{a}
Sample B	5.7 ± 2.1^{b}	5.9 ± 1.7^{b}	3.3 ± 2.1^{b}	3.3 ± 2.2^{b}	3.5 ± 2.3^{b}
Sample C	6.0 ± 2.2^{b}	6.3 ± 1.6^{b}	3.2 ± 1.6^{b}	3.7 ± 2.3^{b}	3.7 ± 2.3^{b}

Mean ± SD. Mean values in the same column with different superscripts (a-b) are significantly different from each other ($P<0.05$). Sample A = Control, sample B = Home-made and sample C = Laboratory made *mahewu*

The appearance acceptability of sample A was significantly different from sample B and C. There was no significant difference between sample B and C. The frequency of hedonic responses for appearance were more concentrated between scores 7 and 8 which represented the control sample while the hedonic responses for the other two samples were concentrated between scores 5 and 6 (Figure 5). For sample B, the frequency of responses were more concentrated at score 5 and this also confirmed the results of its appearance where this sample had the lowest mean. The appearance of sample A was acceptable just as the appearance of sample B and C. These results show that the panelist preferred sample B the least in terms of appearance as compared to all the other *mahewu* samples (A and C).

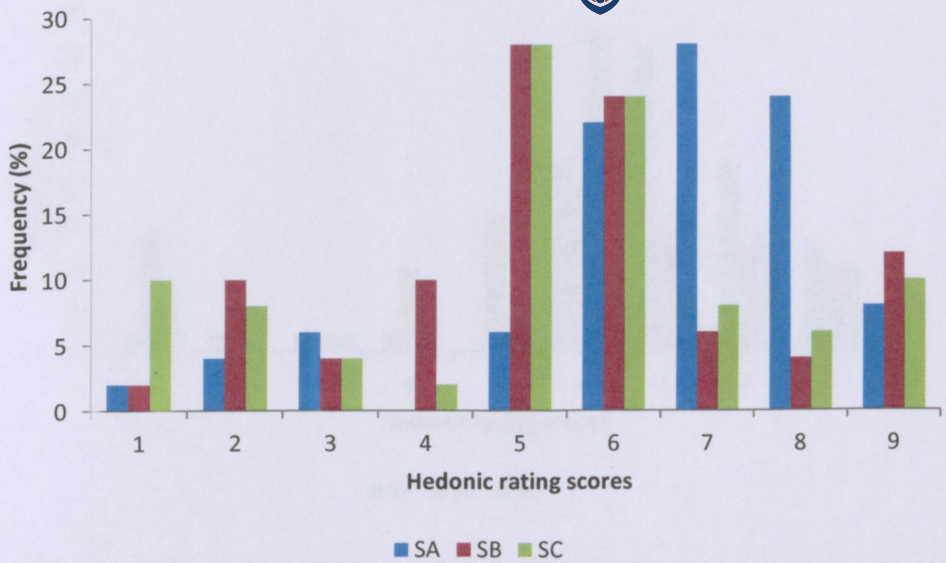


Figure 5. Frequency of hedonic rating scores of *mahewu* samples for appearance (1=dislike extremely; 9=like extremely); SA = Control, SB = Home-made *mahewu*, and SC = Laboratory-made *mahewu*

4.5.2 Colour acceptability of *mahewu* samples

The sample that had the lowest mean of 5.9 in terms of colour acceptability (Table 11) was sample B, which was home-made mahewu. Sample A had the highest mean score of 7.2 followed by sample B which had a mean score of 6.3 (Figure 7). There was no significant differences between two samples (B and C) with AVP at 95% level ($P < 0.05$), while sample A was significantly different from both samples. The frequency of responses in terms of how respondents rated the colour acceptability of sample A and C were concentrated between scores 6 and 7 (Figure 6). However, the frequency of responses for sample B were more concentrated between between scores 5 and 6. Frequency results obtained for the three samples in terms of how the respondents rated their colour acceptability also confirm the results for colour where sample B was the least preferred whereas sample A was the most preferred.

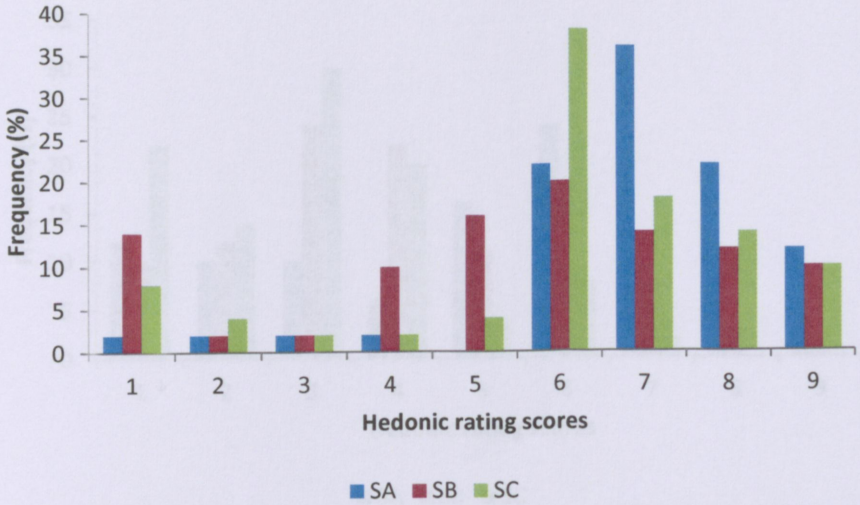


Figure 6. Frequency of hedonic rating scores of *mahewu* samples for colour (1=dislike extremely; 9=like extremely); SA = Control, SB = Home-made *mahewu*, SC = Laboratory-made *mahewu*.

4.5.3 Taste acceptability of *mahewu* samples

Sample C, which is a laboratory made had the lowest mean score of 3 (Table 11). In terms of the taste acceptability of the different *mahewu* samples, sample A had the highest mean of 6.0. The second highest mean score of 3.3 was for sample B. There was no significant difference between the two samples (B and C), which had AVP ($P < 0.05$), while sample A was significantly different from both samples. The frequency of responses in terms of taste acceptability for sample A were more concentrated between the scores 6 and 7 (Figure 7), while the frequency of the responses for sample B were more concentrated between the scores 3 and 4. In the case of sample C, the frequency of respondents were more concentrated in the scores 1, 3 and 4 (Figure 7). These results show that a large percentage of the respondents preferred the taste of sample A as compared to samples B and C.

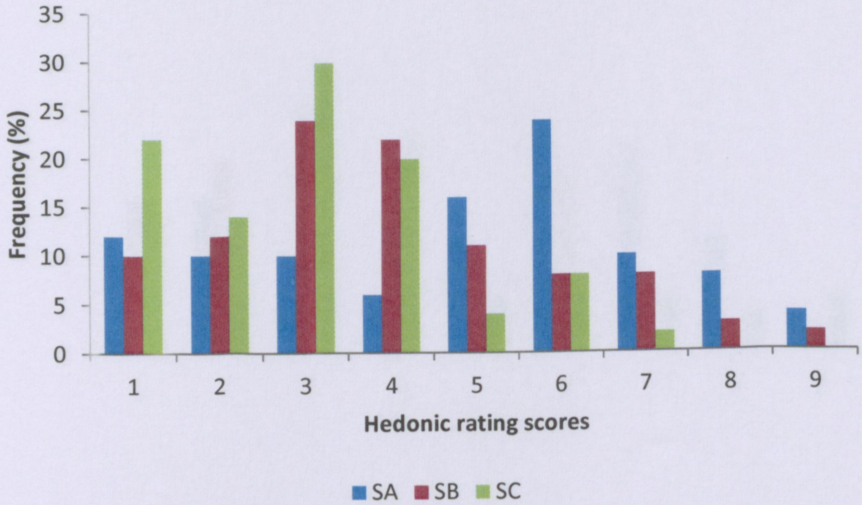


Figure 7. Frequency of hedonic rating scores of mahewu samples for taste (1=dislike extremely; 9=like extremely); SA = Control, SB = Home-made *mahewu*, SC = Laboratory-made *mahewu*

4.5.4 Sourness acceptability of *mahewu* samples

In terms of sourness acceptability of the different *mahewu* samples, the sample with the lowest mean score of 3.3 was B. As was the case with taste, sample A again had the highest mean of 6.0 (Table 11). There was no significant difference between the two samples (B and C) which had AVP ($P < 0.05$), while sample A again was significantly different from both samples. The frequency results of the respondents for sourness were more concentrated between the scores 6 and 7 for sample A, 2 and 3 for sample B and 3 and 4 for sample C (Figure 8). This showed that a high percentage of the respondents still preferred the sourness of sample A while sample B was the least preferred.

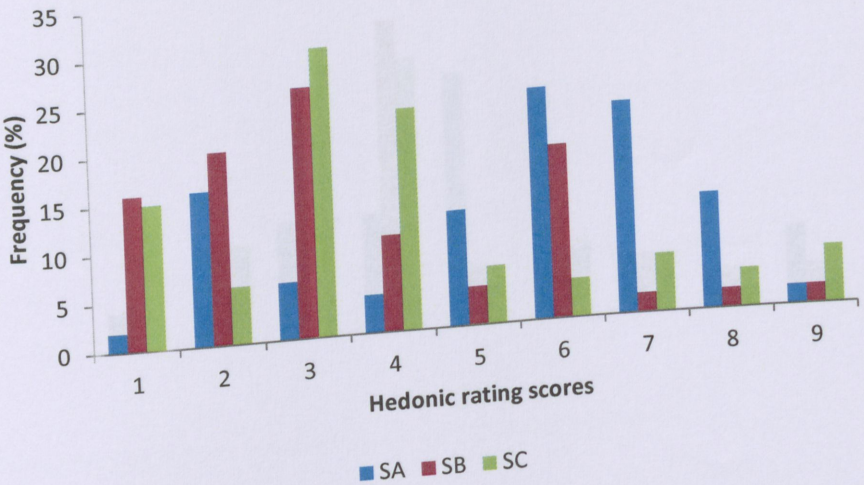


Figure 8. Frequency of hedonic rating scores of mahewu samples for sourness (1=dislike extremely; 9=like extremely); SA = Control, SB = Home-made *mahewu*, SC = Laboratory-made *mahewu*.

4.5.5 Overall acceptability of *mahewu* samples

In terms of the overall acceptability of all the three *mahewu* samples, sample B had the lowest mean score of 3.5, followed by sample C with 3.7 mean score (Table 11). Sample A had the highest mean score of 5.5. There was no significant difference between the two samples (B and C) wich had AVP ($P < 0.05$), while sample A was significantly different from both samples. The frequency of responses were more concentrated between scores 5 and 6 for sample A (Figure 9), 3 and 4 for sample B and C. The overall acceptability of samples B and C was similar. This similarity between the overall acceptability of *mahewu* samples containing AVP is also confirmed by the results obtained for the mean scores of the samples in terms of their overall acceptability.

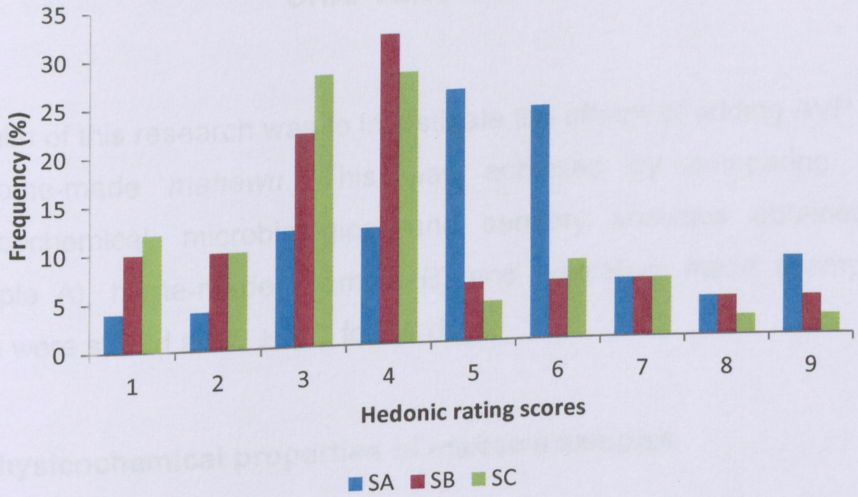


Figure 9. Frequency of hedonic rating scores of *mahewu* samples for overall acceptability (1=dislike extremely; 9=like extremely); SA = Control, SB = Home-made *mahewu*, and SC = Laboratory-made *mahewu*

CHAPTER FIVE. DISCUSSION

The aim of this research was to investigate the effects of adding AVP on the shelf-life of home-made *mahewu*. This was achieved by comparing the results of physicochemical, microbiological and sensory analyses obtained from control (Sample A), home-made (Sample B) and laboratory made (Sample C) *mahewu* which were stored at 36 ± 5ºC for 60 days.

5.1 Physicochemical properties of *mahewu* samples

The results from this study showed that there was a significant decrease in pH during the storage period of all *mahewu* samples with a corresponding increase in titratable acidity even those samples with AVP. The decrease in pH in all samples might have been due to competition amongst the various organisms present in the fermented *mahewu* samples such that they inhibited the fermentation process until one organism became dominant and out-competed the others by a process of natural selection and succession (Ramaite, 2004). This could be also as a result of the ability of these organisms to produce a high quantity of organic acids and the ability to live within the acidic environment (Fowoyo and Ogunbanwo, 2010). The influence of pH on various characteristics has been well studied; the optimum pH established for growth and product formation is around 6.0 (Bibal *et al.*, 1988; Parente *et al.*, 1994; Akerberg *et al.*, 1998). The low pH obtained during the storage period is important since most bacteria including the pathogenic organisms do not survive in low pH environment and this imparts microbial safety as well as increasing the shelf life of the final product (Halm *et al.*, 1993). The undissociated forms of the acetic and lactic acids at low pH exhibit inhibitory activities against a wide range of pathogens such as such as *Shigella, Salmonella* and *E. coli* (Holzapfel, 2002; Mensah, 1997). Fermented maize gruel and high-tannin sorghum gruel at pH 3.8 inhibited *E. coli*, *Campylobacter jejuni*, *Shigella flexneri*, *Salmonella typhimurium* and *Staphylococcus aureus* (Holzapfel, 1997).The results show that there was no significance difference ($P < 0.05$) in TA during the storage periods (15, 30, 45 and 60 days) amongst the three samples. This implies that the addition of AVP did not have any effect on the acidity of the two samples. Food acidity is important parameters in

foods, besides affecting the flavour, food acidity affects the ability of microorganisms to grow in a food. Microorganisms prefer minimal acidity and are prevented from growing when the acid level gets high enough (Nummer, 2008). The early increase in TA is important to avoid proliferation of undesirable organisms resulting in poor fermentation. The early increase could be as a result of acid production by the fermentative organisms such as lactic acid bacteria breaking down sugars to produce, among other secondary fermentation products, lactic acid, hence, the sour taste which makes *mahewu* popular among the indigenous population of South Africa (Adesokan *et al.*, 2008; Sanni 1993; Oyewole, 1997). The organic acids released (e.g. lactic, acetic, propionic and butyric acids), as by-products during lactic acid fermentation, have been found to lower the pH to levels of 3 to 4 with a TA of about 0.6% (as lactic acid) (Holzapfel, 2002; Edema and Sanni, 2008; Mensah, 1997). There was correlation between samples B (30, 45 and 60 days) and C (30, 45 and 60 days) (figure 4). This implies that these two samples had high TA during the above mentioned storage days. There was a negative correlation between TA and pH while there was a positive correlation between brix and pH. The negative correlation between pH and TA is expected since it is known that the increase in TA results in the decrease of pH in fermenting cereal grains (Hounhouigan, 1994; Efiuvwevwere, 1995). The progressive fall in pH and increase in TA (Table 8) and negative correlation between the two parameters that occurred during storage days is characteristic of fermenting cereal grains (Efiuvwevwere and Akona, 1995; Hounhouigan *et al.*, 1994; Achi, 1990; Odunfa and Adeleye, 1985). The results are similar to those reported by other researchers working with *mahewu* and similar products (Simango and Rukure, 1992; Jespersen *et al.*, 1994; Gotcheva *et al.*, 2001; Adesokan et al., 2011). Low pH and increased TA of the samples were also due to organic acids (Akinrele, 1970). This increase in acidity in all samples could also be a result of hydrolysis of some complex organic molecules. Besides carbohydrates, the three main sources of acid in cereals have been identified as lipids, phytin and protein with lipids being hydrolysed to fatty acids, phytin to acid phosphates and proteins to free amino acids (Inyang and Idoko, 2006). Thus, the increase in acidity accompanying fermentation during storage could be an indication of extent of hydrolysis of these complex molecules (Adeyemo *et al.*, 1992), and therefore of the digestibility of traditional non-alcoholic fermented beverages and products. An acidity

of 0.4 to 0.45% lactic acid with a pH of about 3.5 is generally acceptable in *mahewu*. Generally acidity increased as fermentation advanced (Akpinar-Bayizit *et al.*, 2007).

Mahewu contains carbohydrates which include reducing sugars such as glucose and fructose. These contributed to the TSS of *mahewu*. The TSS also contributed to the increase in fermentation which led to the decrease in pH and increase in acidity.The largest reduction in the TSS of the control samples during day 45 was probably due to a high bacterial load in the control sample which meant fast utilization of available solids (Kutyauripo *et al.*, 2009). As lactic acid bacteria proliferated, acidity increased, presumably due to fermentation of sugars (Jay *et al.*, 2005). The rate of sugar utilization was therefore, higher in the control than the other two samples. Not all dissolved solids were available for utilization by the microorganisms as shown by the leveling off of the solids during day 60 in the three products. The levelling off may be due to inhibition of metabolic activity by the increasing fermentation process (Zvauya *et al.*, 1996). The addition of sugar during home-made *mahewu* production is strongly recommended because it will add taste to the final product maybe by reducing the bitterness caused by the aloin.

Generally, all the three samples had high values of lightness ranging from Hunter L 55.7 during day 0 to 69.9 during day 60. The increase in the lightness could be due to the fermentation process during the storage period. Fermentation reduces bulk by reducing the viscosity of the cereal gruel making it lighter. During cereal fermentation, microbial activity hydrolyses starch granules, resulting in the cereal gruel becoming lighter (Onofiok and Nnanyelugo, 1998). The redness of the samples can be attributed to the use of sorghum flour during the production process. Traditionally red sorghum varieties are grown in South Africa, as the red colour is desired by the sorghum beer industry and this has resulted in large quantities of red sorghum bran as a by-product, compared to white sorghum being produced (Da Silva, 2003). Pericarp colours include white (colourless), lemon-yellow or red (Rooney and Miller, 1982). The characteristic colour of red sorghum is due to anthocyanin and anthocyanidin pigments present in the pericarp (Hahn *et al.*, 1984). According to Kambal and Bate-Smith (1976), flavonoid compounds are responsible for the pericarp colour of sorghum grains. Tannin-containing sorghum varieties give pink coloured starches due to adsorption and retention of tannins by the starch (Beta

et al., 2001). The high values of redness and yellowness in samples B and C during day 30 and 45 may be due to aloin in the AVP. It is a yellow-brown compound estimated at levels from 0.1 to 0.66 % of dry leaf present in cells adjacent to the rind of the leaf gel (Patel and Patel, 2013). Aloin is an anthraquinone glycoside, meaning that its anthraquinone skeleton has been modified by the addition of a sugar molecule. Anthraquinones are a common family of naturally occurring yellow, orange, and red pigments of which many have cathartic properties, attributes shared by aloin (Grun and Franz, 1981).

5.2 Microbiological properties of mahewu samples

The composition of the micropopulation as well as viable counts obtained in this study showed a succession of coliform bacteria, lactic acid bacteria, mould and yeasts respectively (Table 10). Coliforms were used as an indicator of the general hygiene of the production process and packaging material. Sample B (home-made *mahewu*) recorded a high count of coliforms from day 0, suggesting that good hygienic was not practised during the production process. The presence of coliforms in sample B during day 0 might be due to dirty equipment or poor hygienic handling and cross contamination during processing. Microorganisms could have originated above all from the flours, utensils and possibly from the tap water used for mixing during its traditional preparation (Kutyauripo *et al.*, 2009). When water is added to flour, the micro-population in the flour begins to grow and metabolize. This process is the basis of the preparation of cereal gruels which are common weaning foods in developing countries (*Mbata et al.*, 2009 and Akinrele, 1970). The fermentation is spontaneous and uncontrolled. Previous studies suggested that microorganisms are associated with cereal grains and their products and that the bacterial inoculum for natural fermentation process is derived from the grains (Odunfa and Adeyele, 1985). M*ahewu* is a naturally fermented beverage from maize flour; no starter culture is added. The earthenware pots used as the fermenting vessels have been found to be better containers of traditional fermented foods than the stainless steel pots (Feresu, 1992). This is because the micropores in their walls harbor microorganisms from previous fermentations which act as inocula for the next fermentation (Zvauya *et al.*, 1997). However, the high number of coliform counts in all the three samples during

day 45 and 60 of storage could be due to contamination of product and packaging materials during the storage period. The results of this study show that as fermentation progressed, the LAB population increased and thus aiding the rapid fermentation of the *mahewu* samples. This agrees with the work of Oyeyiola (1990). The increase in the production of lactic acid with time has been attributed to lowered pH, which permits the growth of LAB to the detriment of the competing organisms (Kandler and Weiss, 1986). LAB cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, aroma compounds, bacteriocins, exopolysaccharides and several enzymes are of importance (Fowoyo and Ogunbanwo, 2010). Studies on LAB isolated from fermented foods in Africa have demonstrated an antimicrobial activity of these LABs towards pathogenic bacteria (Lei and Jacobsen, 2004). The inhibition mechanism of LAB is attributed to low pH as a result of presence of lactic acid (among other metabolite acid products) and antibiotic substances produced by these microorganisms (Mbugua and Njenga, 1991; Lei and Jacobsen, 2004; Parvez *et al.*, 2006; Vasiljevic and Shah, 2008; Reid, 2008; Kalui *et al.*, 2009). The microbial inhibitory effects have been studied and documented in: traditionally fermented milk against *Listeria monocytogenes*, *mahewu* and sour porridge against *Aeromonas* sp., *Campylobacter jejuni*, *Salmonella* sp., and *mukumbi* against *Salmonella enteritidis, Shigella sonnei* and *Shigella flexneri* (Pswarai, 2013). These observations suggest that traditional fermented foods may contain probiotic bacteria that potentially could be used in diarrhoea management and/or for other health benefits. The ability to produce a high quantity of lactic acid is grossly dependent on the ability of the producer organisms to utilize carbon and nitrogen source of the medium (Suma *et al.*, 1999). The increased counts of LAB during the storage period of *mahewu* samples might also be due to the ability of the LAB isolates to predominate and suppress the growth of other undesirable microorganisms. The dominance of lactic acid bacteria in the traditional fermentation of maize based products was reported by some researchers. *Lactobacillus fermentum* and *Lactobacillus plantarum* have been reported to be the most commonly associated lactic acid bacteria species with spontaneous lactic acid fermentations of cereal products (Kunene *et al.*, 2000; Lei and Jacobsen, 2004). Schweigart and Fellingham (1963) reported that LAB are the main fermenting microorganisms during the production of *mahewu*, and recommended the use of *Lactobacillus delbruecki, L. bulgaricus, L.acidophilus and*

Streptococcus lactis as starter cultures for the production of *mahewu*. Halm *et al.*, (1993) reported obligately heterofermentative lactic acid bacteria as the predominant organisms of fermented maize dough for *kenkey* production. Sanni *et al.* (1999) also identified *L. plantarum, L. brevis, L. casei, Pediococcus, pentosaseus and Pediococcus acidilactici* as the organisms that were isolated from spontaneously fermented sour maize meal during the production of sour maize bread using starter culture. Oyeyiola (1990) identified *L. plantarum, P. acidilactic* and *L. fermentum* as associated microorganisms of fermented maize grains for *masa* production. Akinrele (1970) also observed that when the pH of fermenting maize falls below 4, it gives rise to conditions favourable for subsequent souring of the product by *Lactobacillus plantarum, Aerobacter cloacae, Corynebacterium* and yeast. The decrease in pH and increase in LAB counts followed the same trend as reported for other natural fermented foods (Sulma *et al.*, 1991; Choi *et al.*, 1994). The growth of LAB is greatly influenced by temperature and it has only been studied in few cases. Most lactic acid bacteria work best at temperatures of 18 to 22°C. Akerberg *et al.* (1998) found out that 33.5°C was the temperature at which *L. lactis* produced the biggest quantity of lactic acid from glucose. The effect of temperature on LAB isolates indicates that at temperatures of 10 and 15°C all the isolates grow well with higher viable counts at 15°C as compared to 10°C while there was a decline in the viable count of LAB at 45°C (Fowoyo and Ogunbanwo, 2010).

LAB involved in the production of African fermented foods are being investigated widely for their probiotic potential (Kalui *et al.*, 2010; Lei and Jacobsen, 2004; Helland *et al.*, 2004). The beneficial effects of food with added live microbes (probiotics) on human health are being increasingly promoted by health professionals (Vasudha and Mishra, 2013). These foods are cheap, accessible and acceptable to the African indigenous people (Lei and Jacobsen, 2004). Probiotic strains have been reported to have health benefits for a range of health disorders, spanning from intestinal to non-intestinal disorders (Gawkowski and Chikindas, 2013). For the use of LAB as probiotics, some desirable characteristics such as low cost, maintaining its viability during the processing and storage and resistance to the physicochemical processing must be considered (Song *et al.*, 2012).

There was a decrease in mould growth in all the three samples on day 15. Sample A had a decrease of 1.3222 from 2.4771 $\log_{10}$ cfu/ml on day 0, whilst samples B and C had a decrease of 1.0000 from 1.4322 and 2.4771 $\log_{10}$ cfu/ml, respectively and this could be attributed to the observed decrease in pH (Table 8). Bacteria have been shown to suppress the growth of moulds by the production of acids (El- Gendy *et al.*, 1980). Jespersen *et al.* (1994) reported that 5.0000 $\log_{10}$ cfu / g of mould count, found in raw maize, are reduced to less than 2.0000 $\log_{10}$ cfu / g within 24 h of fermentation. The highest numbers of yeasts were recorded on day 15 for sample A (7.0414 from 4.8513 $\log_{10}$ cfu/ml in day 0) while samples B and C recorded high numbers of yeasts in day 30 9.1139 and 7.8633 $\log_{10}$ cfu/ml respectively. This is in agreement with observations in similar products (Akinrele 1970; Nyako and Obiri-Danso 1992; Hounhouigan *et al.*, 1992; Halm *et al.*, 1993; Nche *et al.*, 1994). The yeasts were too numerous to count in day 45 and 60 of storage. Moulds are commonly present as contaminants in cereals and do not appear to play any significant important role in the fermentation. The yeast counts increased in all the three samples during the storage period. The increase in the yeast numbers during storage was attributed to the decrease in the pH that creates conditions ideal for yeast growth (Serna-Saldivar and Rooney, 1995). According to Mugula *et al.* (2003), the presence of yeasts stimulates the growth of bacteria and may also provide growth factors such as vitamins and nitrogen compounds. The coexistence and free proliferation of LAB and yeasts, as was observed in this study, is a common occurrence in food and beverage fermentations (Gobbetti *et al.*, 1994, Nout, 1991; Wood, 1981). The association of LAB and yeasts during fermentation may also contribute metabolites, which could give desirable taste and flavour to foods (Ramaite, 2004). The production of acids and other antimicrobial components in the cereal gruel during fermentation may also improve microbial safety of the resulting fermented product. The major product of fermentation is lactic acid; a compound with a high commercial value, with applications in the food, cosmetic, medical, and pharmaceutical industries (Boonmee *et al.*, 2003). Previous workers have found several yeast species in spontaneous lactic fermenting cereals, including species of *Saccharomyces* and *Candida* (Jespersen *et al.*, 1994).

The control sample (A) was significantly different from Samples B (home-made *mahewu* and C (laboratory made *mahewu*) in terms of appearance, colour, taste, sourness and overall acceptability. Sample A was the most preferred in terms of the above mentioned quality attributes. This implies that the addition of AVP had an effect on the organoleptic characteristics of two *mahewu* (samples B and C). The two new products (samples B and C) had a 39% (mean score 3.5) and 41% (mean score 3.7) overall acceptability rating compared to 60% (mean score 5.5) acceptability in the control sample (Table 11). The unacceptable taste, sourness and overall acceptability recorded in samples B and C warrant rejection of the new *mahewu* products. The comments made by most of the panel members indicated that samples B and C had a bitter taste and this is the characteristics of *Aloe vera* plant. The bitterness is caused by aloin (glycoside group) compounds which are found in most parts of the skin of *Aloe vera* plants (Bozzi et al., 2006). It is used in the alcoholic beverages due to its bitter principle (Patel *et al.*, 2012). The availability of aloin in *Aloe vera* plants was reported by some researchers. Singh *et al.*(2010) noted that aloin in *Aloe vera* is a bitter component which is applied to the skin as first aid for burns, laxative, anti-obesity preparation and pharmaceutical formulations. Adhusan (2008) reported that aloin also can act as antioxidant compounds, but can lead negative effect for health in an excessive amount. Lullmann *et al.* (2005) noted that aloin was included in anthraquinon compound which is strong in the *aloe vera* plants. Ramachandra and Rao (2011), found that heating at 30-80°C can decrease aloin compound due to the destruction of a parenkim tissue on the extracted material. Gulia *et al.* (2009) noted that the decrease of aloin was due to thermal processing at 50-80°C from 10.6 ppm to 1.7 ppm and also aloin has not the nature of heat resistant and will be hydrolyzed. A method for reducing aloin should be investigated during the production of AVP. This will help to reduce the bitterness of the product and make the product acceptable in the market.

5.4 Extension of shelf life of *mahewu* samples

Growth of mould and yeast was used as indicator of spoilage and acceptability of the product. The most important microorganisms that can cause the spoilage of *mahewu* are yeasts belonging to the *Pichia* spp. *Acetobacter liquefaciens* have been found to be another major spoilage microorganism. It converts lactic acid into acetic acid leading to off-odours and also causes discolouration of the product (Holzapfel, 1989). Acceptability of the products decreased after 15 days in the control and after 30 days in samples B and C (Table 10). According to the National Department of Health,Guideline for microbial standards, (South Africa, 1972), fermented food products (including *mahewu*) should not exceed a coliform count of $\log_{10}$ <2.3010 cfu/ml and $\log_{10}$ <4.0000 cfu/ml for yeasts and moulds. The control sample (A) had a high yeast counts ($\log_{10}$ 7.0414 cfu/ml) on day 15 meaning the product was no longer acceptable for human consumption while samples B ($\log_{10}$ 9.1139 and C ($\log_{10}$ 7.8633) had high counts on day 30 meaning that the two products were out of detection limit until day 60 of storage. The control (sample A) deteriorated faster than samples B and C. The results of this study show that addition of AVP can extend the shelf-life of home-made *mahewu* up to 15 days at 36 ± 5°C. The addition of AVP greatly reduces the production of organic acids, with the possibility of reducing undesirable souring. However, the temperature and other conditions of *mahewu* storage (temperature and water quality) need to be carefully controlled if meaningful shelf-life is to be attained. Coliform count was used as an indicator of the hygiene associated with the products. Good hygiene should be practised during home preparation of *mahewu* to prevent the growth of coliforms as was the evidence in this study. Most contamination probably comes from the raw materials that do not go through any rigorous microbiological analysis and treatment before being used.

5.3 Sensory properties of *mahewu* samples

The control sample (A) was significantly different from Samples B (home-made *mahewu* and C (laboratory made *mahewu*) in terms of appearance, colour, taste, sourness and overall acceptability. Sample A was the most preferred in terms of the above mentioned quality attributes. This implies that the addition of AVP had an effect on the organoleptic characteristics of two *mahewu* (samples B and C). The two new products (samples B and C) had a 39% (mean score 3.5) and 41% (mean score 3.7) overall acceptability rating compared to 60% (mean score 5.5) acceptability in the control sample (Table 11). The unacceptable taste, sourness and overall acceptability recorded in samples B and C warrant rejection of the new *mahewu* products. The comments made by most of the panel members indicated that samples B and C had a bitter taste and this is the characteristics of *Aloe vera* plant. The bitterness is caused by aloin (glycoside group) compounds which are found in most parts of the skin of *Aloe vera* plants (Bozzi et al., 2006). It is used in the alcoholic beverages due to its bitter principle (Patel *et al.*, 2012). The availability of aloin in *Aloe vera* plants was reported by some researchers. Singh *et al.*(2010) noted that aloin in *Aloe vera* is a bitter component which is applied to the skin as first aid for burns, laxative, anti-obesity preparation and pharmaceutical formulations. Adhusan (2008) reported that aloin also can act as antioxidant compounds, but can lead negative effect for health in an excessive amount. Lullmann *et al.* (2005) noted that aloin was included in anthraquinon compound which is strong in the *aloe vera* plants. Ramachandra and Rao (2011), found that heating at 30-80°C can decrease aloin compound due to the destruction of a parenkim tissue on the extracted material. Gulia *et al.* (2009) noted that the decrease of aloin was due to thermal processing at 50-80°C from 10.6 ppm to 1.7 ppm and also aloin has not the nature of heat resistant and will be hydrolyzed. A method for reducing aloin should be investigated during the production of AVP. This will help to reduce the bitterness of the product and make the product acceptable in the market.

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CHAPTER 6. CONCLUSION AND RECOMMENDATIONS

The results of this study showed that the addition of *Aloe vera* powder (AVP) had an effect on the shelf-life of home-made *mahewu*. It increased the shelf-life of home-made *mahewu* which is normally less than four days to fifteen days. Spoilage of samples B (home-made) and C (laboratory made *mahewu*, AVP added) products as evidenced by high counts of moulds and yeasts and appearance of roppiness was observed at 30 days and this may be due to the proliferation of yeast in the products which is favoured by the acidic environment created by lactic acid bacteria. The addition of AVP did not have any effects on pH, titratable acidity (TA), total soluble solids (TSS) and colour (L* and a*) during 60 days of storage. There was a decrease in pH and TSS in all the three samples, whilst there was an increase in TA. As lactic acid bacteria proliferated, acidity increased, presumably due to the fermentation of sugars. The acidity that developed favoured the growth of yeasts which subsequently multiplied rapidly at day 30 of storage. TTA (expressed as percent lactic acid) increased throughout the storage period, resulting in the gradual fall in pH. However, the challenge is the acceptability of the products since the sensory results show that most of the panelists did not like the taste and sourness of the two samples (B and C). If addition of AVP is to be adopted as a way of producing home-made *mahewu* with extended shelf life, it would be necessary to carry out further studies with different concentrations of AVP and to determine acceptability of the product in the market. It would also be advantageous to identify the types and microbial load in raw materials and those remaining in the product, so as to effect targeted preservation measures such as using hurdle technology. This is the first research on the effect of AVP in the production of home-made *mahewu*; hence extensive research is still needed in the optimization of the process variables, such as cooking temperature and time including the storage conditions that favour extended shelf-life. The aim of these studies would be to improve the fermentation processes in terms of reduced process time while still maintaining and /or improving the properties of the final fermented product with the addition of AVP. In order to maintain and sustain African indigenous fermented foods and beverages, improved control of fermentations and product characteristics is strongly recommended because there will undoubtedly be a need in the future to produce these foods in circumstances where quality and safety can be guaranteed. Nutritional analysis of

the product should also be investigated especially the antioxidants vitamins and minerals. This will help in determining whether the addition of AVP improves the nutritional properties of home-made *mahewu*.

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APPENDIX

Appendix I. Score sheet of acceptance test of *mahewu* samples

You have received three samples of *mahewu* (Sample A, B and C). Place an X in the box that best describes how much you like or dislike each quality attribute. Rinse your mouth with water in between tasting.

Sample ☐

How much do you like or dislike the look/appearance of this *mahewu*?

9	Like extremely	
8	Like very much	
7	Like moderate	
6	Like slightly	
5	Neither like nor dislike	
4	Dislike slightly	
3	Dislike moderately	
2	Dislike very much	
1	Dislike extremely	

Any comments?

How much do you like or dislike the colour of this *mahewu*?

9	Like extremely	
8	Like very much	
7	Like moderate	
6	Like slightly	
5	Neither like nor dislike	
4	Dislike slightly	
3	Dislike moderately	
2	Dislike very much	
1	Dislike extremely	

Any comments?

How much do you like or dislike the taste of this *mahewu*?

9	Like extremely	
8	Like very much	
7	Like moderate	
6	Like slightly	
5	Neither like nor dislike	
4	Dislike slightly	
3	Dislike moderately	
2	Dislike very much	
1	Dislike extremely	

Any comments?

How much do you like or dislike the sourness of this *mahewu*?

9	Like extremely	
8	Like very much	
7	Like moderate	
6	Like slightly	
5	Neither like nor dislike	
4	Dislike slightly	
3	Dislike moderately	
2	Dislike very much	
1	Dislike extremely	

Any comments?

How much do you like or dislike this *mahewu*?

9	Like extremely	
8	Like very much	
7	Like moderate	
6	Like slightly	
5	Neither like nor dislike	
4	Dislike slightly	
3	Dislike moderately	
2	Dislike very much	
1	Dislike extremely	

Any comments?

Is there any specific that you really liked about this *mahewu*?

.....

Is there any specific that you really disliked about this *mahewu*?

.....