The Microbial Succession in Indigenous Fermented Maize Products

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DEDICATED TO MY PARENTS

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LIST OF ABRREVIATIONS

ANOVA	Analysis of Variance
AIDS	Acquired Immune Deficiency Syndrome
AD	Ano Domino
a_w	Water activity
BC	Before Christ
ca	Approximately
cfu	Colony forming units
CO_2	Carbondioxide
DON	Deoxynivalenol
ETEC	Enterotoxigenic E. Coli
g	gram
GI	Gastrointestinal
h	hour (s)
kg	kilogram (s)
lb	pound (s)
LAB	Lactic Acid Bacteria
min	minute (s)
ml	millilitre
mg	milligram
mm	millimeter
NCSS	Number Cruncher Statistical Systems
nm	nanometer
^0C	degree Celsius
PF	power flour
rpm	revolutions per minute
s	second (s)
SEM	Scanning Electron Microscope
SC	Starter culture
µm	micrometer

Chapter 1

Literature Review

1.1. INTRODUCTION

Fermentation of food is one of the oldest methods of food preparation and preservation (Pederson 1971; Steinkraus *et al.*, 1983; Campbell-Platt, 1994). Fermented foods constitute a substantial part of the diet in many African countries and are considered an important means of preserving and introducing variety into the diet, which often consists of staple foods such as milk, cassava, fish and cereals (Steinkraus, 1995; Belton and Taylor, 2004). They have a role in social functions such as marriage, naming and rain making ceremonies, where they are served as inebriating drinks and weaning foods (Hounhouigan, 1994). 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 (Simango, 1997). Below is a trace of the use of fermentation by man through time as has been revealed by studies carried out by different researchers.

1.1.1. A historical account of indigenous fermented cereals

1.1.1.1. World perspective

Since the beginning of human civilization there has been an intimate companionship between the human being, his fare and the fermentative activities of microorganisms. These fermentative activities have been utilized in the production of fermented foods and beverages, which are defined as those products which have been subordinated to the effect of microorganisms or enzymes to cause desirable biochemical changes. The microorganisms responsible for the fermentation may be the microbiota indigenously present on the substrate, or they may be added as starter cultures (Harlender, 1992).

Since the dawn of civilization, methods for the fermentation of milks, meats, vegetables and cereals have been described. The earliest records appeared in the Fertile Crescent (Middle East) and date back to 6000BC. Of course the preparation of these fermented foods and beverages was in an artisan way and without any knowledge of the role of the microorganisms involved. However, by the middle of the 19th century, two events changed the way in which food fermentations were performed and the understanding of the process. Firstly, the industrial revolution resulted in the concentration of large masses of people in towns and cities. As a consequence, food had to be prepared in large quantities, requiring the industrialization of the manufacturing process. In the second place, the blossoming of Microbiology as a science in the 1850s formed the biological basis of fermentation, and the process was understood for the first time (Caplice and Fitzgerald, 1999). Ever since, the technologies for the industrial production of fermented products from milk, meat, fruits, vegetables and cereals are well developed and scientific work is actively carried out all over the world (Hirahara, 1998; Pagni, 1998).

1.1.1.2. African perspective

Fermented foods have a long history in Africa. However, the absence of a writing culture in most of Africa makes their origin 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 foods were made by the Arab travellers, mostly merchants and geographers (Odunfa and Oyewole, 1998). By this time, in the 8th to 16th centuries, the art of fermenting some foods had been perfected and being part of the culture of the people. Unfortunately the Arabs' world of knowledge was not extended to the forested regions of West and Central Africa hence there were hardly any records for these regions.

Perhaps the most documented of the fermented foods is sour milk. Fairly frequent references were made to it by the Arab authors (Farnworth, 2003). Sour

milk was consumed all over the Guinea Savannah of West Africa and Northern Africa by the Negro and Berber people of West Africa. Ibn Batoutah, an Arab traveller describing his journey in 1352 from Walata to the town of Mali recorded that the travellers arriving at the villages on the road were met by women selling various local products including sour milk. During his stay in the town of Mali (1392-1393), he was sent a hospitality gift by the local King, Mansa Sulayman '… a magnificent meal, according to Sudanic notions which included a gourd filled with sour milk. He also reported that people at Walata, (present day Sudan) consumed porridge made of millet and sour milk (Farnworth, 2003).

Next to sour milk in historical importance are alcoholic drinks. The earliest records traced back to Al-Idrisi in medieval times. He obtained his information from anonymous merchants and travellers and reported that the people of West Africa prepared an alcoholic drink from millet. This was prepared by adding boiling water to millet flour (presumably to gelatinize the starch constituent), filtering it and subjecting it to natural fermentation (Farnworth, 2003). This kind of beer was known throughout West Africa as *dolo*, *kimbi*, or *merissa*. Another alcoholic drink was mentioned by Al-Muhallabi before AD 996 and described by Golberry in the account of his travels in the land of the Bambuk (1785-1787) inhabited by the Mandingo group of Senegal (Farnworth, 2003). According to Golberry, the people prepared this alcoholic drink by putting millet into an earthen pot filled with water and kept it there until it turned sour. They then added honey and exposed it to the sun for 10 days. After the exposure, they filtered the contents through a sieve made of leaves, obtaining strong mead with a very pleasant flavour.

Various alcoholic drinks played an important part on various solemn occasions and were often offered as gifts. Al-Bakri reported in 1068 that the people of the ancient Ghana Empire brought offerings, including alcoholic drinks to their dead (Farnworth, 2003), since brewing was already a specialized art in Ghana. Oral history indicated that these brewers visited various places on festive occasions

and retailed beer in small calabashes. The brewing in Ghana was so specialized that the brewers who were in the King's service were buried with the King on his death in the belief that they would continue to give him such specialized service in the next World.

Early reports indicated that a more elaborate preparation in the medieval age, which is in line with the present-day brewing, was practiced by these traditional brewers. Millet or sorghum grain was malted by soaking it in water and placed in a pit for a short time to allow sprouting. The grains were then pounded to a thick meal and cooked in an earthen pot. After being filtered, a light sweet liquid was obtained which was then slowly fermented in calabashes. After 1-2 days, a mildly intoxicating drink was ready (Farnworth, 2003).

Sour porridges are quite common throughout Africa, south of the Sahara. The practice of soaking cereal grains to let them sour was long established. An Arab author, Al-Omari, between 1342 and 1349 reported that grains were wetted and pounded in mortars and soaked in water to make them sour (Farnworth, 2003). This resulted in more savoury flour hence adding flavour to the porridge prepared from it. A German traveller, Nachtigal, recorded that thirst was quenched in Bornu very largely by a drink made by allowing millet or sorghum to ferment in water for a short time. The supernatant water tasted sharp and sweet.

Dirar (1993) did extensive work on the origin of food fermentations in Sudan. It was evident that the practice had been developed over centuries. According to one of his sources, the writings of Strabo (7 BC), the Greek philosopher showed that the Ethiopians had been brewing merissa-like beer since 7 BC. Special utensils for making the staple kisra bread were discovered in archaeological sites which were traced back to 550 BC. Although wine from grapes largely displaced wine from dates in a certain era of Sudanese history, evidence from ancient inscriptions shows that ship loads of date wine, beer and other precious items were offered as gifts to the ruler of a powerful Sudanese kingdom of Yam in

recognition of his numerical military strength. The long-necked, narrow mouthed pottery styles reminiscent of the ancient practice of wine brewing were collected from excavation sites of Meroitic civilization of 690 BC to AD 560. This persisted till the Christian era about AD 560. Ancient drawings made in 1860 showed wine drinkers, horned and tailed men dancing and drawing wine from a typical wine jar.

1.1.1.3. Food fermentation types and special effects associated with it

Fermented foods are produced worldwide using various manufacturing techniques, raw materials and microorganisms. However, there are only four main fermentation processes namely, alcoholic, lactic acid, acetic acid and alkali fermentation (Soni and Sandhu, 1990). Alcoholic fermentation results in the production of ethanol, and yeasts are the predominant organisms (e.g. wines and beers). Lactic acid fermentation (e.g. fermented milks and cereals) is mainly carried out by lactic acid bacteria. A second group of bacteria of importance in food fermentations are the acetic acid producers from the *Acetobacter* species. *Acetobacter* convert alcohol to acetic acid in the presence of excess oxygen. Alkali fermentation often takes place during the fermentation of fish and seeds, popularly known as condiment (McKay and Baldwin, 1990).

The preparation of many indigenous or traditional fermented foods and beverages remains as a household art. They are produced in homes, villages and small scale industries. On the contrary, the preparation of others, such as soy sauce, has evolved to a bio-technological state and is carried out on a large commercial scale (Bol and de Vos, 1997). In the past, there was no verified data on the economic, nutritional, technical, and quality control implications of the indigenous fermented food. However, in the last 20 years, the numbers of books and articles that deal with indigenous fermented beverages and foods found around the whole World have rapidly increased (Steinkraus *et al*., 1993).

The beneficial effects associated with fermented foods and beverages are of special importance during the production of these products in developing countries. These effects include reduced loss of raw materials, reduced cooking time, improvement of protein quality and carbohydrate digestibility, improved bio-availability of micronutrients and elimination of toxic and anti-nutritional factors such as cyanogenic glycosides (Sanni, 1993; Iwuoha and Eke, 1996; Padmaja, 1995; Addo *et al.,* 1996; Amoa-Awua *et al.*, 1997; Svandberg and Lorri, 1997; Odunfa and Oyewole, 1998; Onilude *et al.,* 1999; Sindhu and Khertarpaul, 2001). In addition, the probiotic effects and the reduced level of pathogenic bacteria observed in fermented foods and beverages are especially important when it comes to developing countries where fermented foods have been reported to reduce the severity, duration and morbidity of diarrhoea (Mensah *et al.*, 1990; Mensah *et al.*, 1991; Nout, 1991; Mensah, 1997; Kimmons *et al.*, 1999).

Fermented foods have been noted for their superior nutritional value and digestibility compared to the unfermented counterpart. Fermentation of cereals such as maize, millet, sorghum and rice, results in improved protein quality, especially the level of available lysine (Hamad and Fields 1979; Padhye and Salunkhe, 1979). Fermentation also has the advantage of improving organoleptic properties by producing different flavours in different foods (Khetarpaul and Chauhan, 1993; Sarkar and Tamang, 1994; Steinkraus, 1994). Spoilage and pathogenic microorganisms are inhibited by the production of organic acids, hydrogen peroxide, antibiotic-like substances and the lowering of oxidation-reduction potential (Cooke *et al*., 1987; Nout *et al.*, 1989; Mensah *et al.*, 1991; Kingamko *et al.*, 1994; Lorri and Svanberg, 1994; Nout, 1994; Tanasupawat and Komagata, 1995). Lactic acid fermentation has also been described in sour milk, *sauerkraut*, and the Russian drink, *kwass.*

1.1.1.4. The microbiology of fermented foods

The microbiology of many of these products is quite complex and unexploited. In most of these products the fermentation is natural and involves mixed cultures of yeasts, bacteria and fungi. Some microorganisms may participate in parallel, while others act in a sequential manner with a changing dominant biota during the course of fermentation. The common fermenting bacteria are species of *Leuconostoc*, *Lactobacillus, Streptococcus, Pediococcus, Micrococcus* and *Bacillus.* The fungal genera are mainly representatives of *Aspergillus, Paecilomyces, Cladosporium, Fusarium, Penicillium* and *Trichothecium* whereas the most common fermenting yeast species is *Saccharomyces*, which contributes to alcoholic fermentation (Steinkraus, 1998). Yeasts have been reported to be involved in several different types of indigenous fermented foods and beverages (Zulu *et al.*, 1997; Amoa-Awua and Jacobsen, 1996; Halm and Olsen, 1996; Holzapfel, 1997; Hounhouigan *et al.*, 1999; Blanco *et al.*, 1999; Gadaga *et al.,* 2001). Despite their presence, the role of yeasts in these products is often poorly investigated. An overview of possible functions of yeasts in African indigenous fermented foods and beverages is given in Table 1. The most dominant yeast species associated with African indigenous fermented foods and beverages is *Saccharomyces cerevisiae* (Jespersen, 2003).

1.2. CEREALS AS FERMENTATION SUBSTRATES

Cereals are globally number one as food crops as well as substrates for fermentation. Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, corn or sorghum) are well known in many parts of the World. Some are utilized as colorants, spices, beverages and breakfasts or light meal foods, while a few of them are used as main foods in the diet.

In Africa, cereal grains such as maize, sorghum and millet are common substrates for producing a wide variety of fermented products. Cereal grains consist of an embryo (germ) and an endosperm enclosed by an epidermis and a seed coat (husk). Starch in the endosperm is found as granules of different sizes (Hoseney, 1992). The germ is basically a package of nutrients (amino acids, sugars, lipids, minerals, vitamins, and enzymes) as is shown in Table 2. The husk mainly comprised cellulose, pentosans, pectins and minerals (Nikolov, 1993). The grains are malted, milled and fermented to produce thin gruels and alcoholic beverages known by various names in different parts of Africa (Odunfa and Adeyele, 1995). The average chemical composition of the cereals is shown in Table 2.

Fermentation processes are enabled by technological measures that act on the metabolically resting grains and direct ecological factors controlling the activity of lactic acid bacteria and yeasts. Fermentable sugars originate from endogenous or added hydrolytic enzyme activities (Hammes *et al.*, 2005). The variation of the ecological parameters acting on the microbial association such as the nature of the cereal, temperature, size of inoculum, and length of propagation intervals, leads in each case to a characteristic species association, thus explaining the 46 LAB species and 13 yeast species that have been identified as sourdough specific (Hammes *et al*., 2005).

A multitude of fermented products prepared from cereals have been created in the history of human nutrition. In their production, the fermentation steps aim to achieve the following; conditioning for wet milling by steeping of maize (Johnson, 2000) and wild rice (Oelke and Boedicker, 2000), affecting sensory properties (aroma, taste, colour, texture), saccharification by use of *koji* (Yoshizawa, 1977) prior to alcoholic fermentation or producing sweetened rice (Wang and Hesseltine, 1970), preservation which relies mainly on acidification and or alcohol production (Hammes and Tichaczek, 1994), enhancing food safety by inhibition of pathogens , such as *Burkholderia gladioli* that had caused Bongkrek poisoning in products made from pre-soaked corn (Meng *et al*., 1988), improving the nutritive value by removing anti-nutritive compounds (such as phytate , enzyme inhibitors, polyphenols, tannins), and enhancing the bioavailability of components by affecting physio-chemical properties of starch and associations of fiber constituents with vitamins, minerals or proteins (Chavan and Kadam, 1989), removal of undesired compounds such as mycotoxins (FAO, 1999; Nakazato *et al*., 1990; Nout, 1994), endogenous toxins, cyanogenic compounds, flatulence producing carbohydrates, reducing energy required for cooking, achieving the condition of bake-ability as it is required for producing leavened rye bread (Hammes and Ganzle, 1998). A range of indigenous fermented foods prepared from cereals in different parts of the World are listed in Table 3. It can be observed from this table that most of these products are produced in Africa and Asia and a number of them utilize cereals in combination with legumes, thus improving the overall protein quality of the fermented product. Cereals are deficient in lysine, but rich in cysteine and methionine. Legumes, on the other hand, are rich in lysine but deficient in sulphur containing amino acids. Thus by combining cereal with legumes, the overall protein quality is improved (Campbell-Platt, 1994).

1.2.1. Cereal fermented, non-alcoholic beverages

These are fermented cereal products which on processing yield acidic, non-alcoholic 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.

1.2.1.1. *Mahewu*

Mahewu (*amahewu*) is an example of a non-alcoholic sour beverage made from corn meal, consumed in Africa and some Arabian Gulf countries (Chavan and Kadam, 1989). It is an adult type of food, although it is commonly used to wean children (Shahani *et al.*, 1983). 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 '*maphulo*' (Coetzee, 1982). The most commonly used term is *mahewu*. 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). The predominant microorganism in the spontaneous fermentation of African *mahewu* is *Lactococcus lactis* subsp. *lactis* (Steinkraus *et al.*, 1993).

1.2.1.2. *Ogi*

This is a sour gruel obtained as a result of the submerged fermentation of some cereals. The common cereal used in West Africa is maize in the southern part, while sorghum and millet are used in the north where it is drier. *Ogi* is normally prepared as a water suspension and cooked before consumption. The cooked

product is usually a gel of variable degree of stiffness. The fluid or semi-solid cooked *ogi* is called by different names such as '*eko*', '*akamu*' or '*kafa*', in different localities, while the stiff gel is called '*agidi*' in Nigeria. *Agidi* is prepared by cooking, wrapping in leaves, and then allowed to set to form a stiff jelly. *Ogi* is an important indigenous, traditional weaning food common in the whole of West Africa. It is consumed as a breakfast meal by many and it serves as the food of choice for the sick in many cases. The predominant microorganism in the fermentation responsible for the production of lactic acid is *L. plantarum. Corynebacterium* hydrolyses the corn starch to form organic acids while *Saccharomyces cerevisiae* and *Candida mycoderma* contribute to the flavour development.

1.2.1.3. *Uji*

This sour cereal gruel is from East Africa. The basic cereal used for *uji* production is maize, but mixtures of maize and sorghum or millet in the proportion of 4:1 are also used (Mbugua, 1984). The raw cereal is finely ground and slurred with water in a concentration of 30 % (w/v) and allowed to ferment for 2-5 days at room (25 ^{0}C) temperature. The product is diluted to about 8-10 % solids and boiled. It can then be further diluted and sweetened with sugar before consumption. *Uji* production is an acidic fermentation process during which the pH of the slurry is reduced to 3.5-4.0 in 40 h (Mbugua, 1982). Spontaneous *uji* fermentation is characterized by the sequential growth of the dominant microorganisms, initiated by the growth of coliforms and later succeeded by the growth of lactic acid bacteria. Early acid production at high concentrations by the lactic acid bacteria rapidly restricts coliform activity, thereby eliminating the problems of off-flavours and flavour instability. *Lactobacillus plantarum* is mainly responsible for souring of uji, although early activity of hetero-fermentative strains of *L. fermentum, L. cellolbiosus* and *L. buchneri* during the fermentation is evident (Mbugua, 1981). Sucrose is the major fermentable sugar in the *uji* flours.

1.2.2. Cereal fermented alcoholic beverages

1.2.2.1. *Umqombothi*

Umqombothi is popular among the black South African population. It is pink, opaque, has a yoghurt-like flavour, a thin consistency, and is effervescent and alcoholic (3 %) (Bleiberg, 1979). It is consumed in the active state of fermentation and therefore has a short shelf life of 2-3 days (Novellie, 1966; Quin, 1959; Hesseltine, 1979; Deacon, 1980; Coetzee, 1982). Although it is produced commercially on large scale using new techniques, the old traditional way of making *umqombothi* still exists. In the townships, women still brew *umqombothi* either for social gatherings or for sale using the age-old techniques and village art methods. Maize and sorghum, used in combination, are the most common cereals used in South Africa to make *umqombothi*. Lactic acid bacteria and yeasts are thought to be the predominant microorganisms during this fermentation.

1.2.2.2. *Busaa*

Busaa is a Kenyan opaque maize beer. It is similar to the *malwa* beer in Uganda and the *kaffir* beer in South Africa. In fact, there are numerous opaque beers in several African countries, each with a local name. The nutritional value of opaque maize beer is considered superior to that of clear lager beer due to higher content of crude protein, thiamine and riboflavin. *Busaa* is commonly prepared from maize endosperm grits and finger-millet malt, *Eleusine coracana.* Its preparation is similar to that of *kaffir* beer (Farnworth, 2003).

On average, when *busaa* is ready for consumption, it contains 0.5-1 % lactic acid and 2-4 volume % ethanol (Nout, 1980a, b). At the maize souring stage, the microorganisms involved are lactic acid bacteria and a few yeasts, mainly

representatives of *L. helveticus, L. salivarius, Pediococcus damnosus, P. partulus*, *Candida krusei* and *S. cerevisiae.* The main fermentation is also governed by a mixture of yeasts and lactic acid bacteria including *C. krusei* and *L. casei* var*. rhamnosus*.

Although the production techniques of *kaffir* beer and *busaa* are similar, there are some fundamental differences. The corn souring in *busaa* is done before the malt is added. Furthermore, souring in *kaffir* beer is done at about 50 ^{O}C, thereby providing a selectively favourable temperature for the lactobacilli – perhaps that is why yeasts are excluded. In *kaffir* beer, the wort resulting from saccharification of maize by sorghum malt is boiled for 2 h before inoculation with a quantity of previously manufactured beer. The boiling eliminates the lactic acid bacteria from the souring stage. *busaa* beer fermentation is spontaneous and uncontrolled, having much in common with *pito* production.

1.3. THE NATURE OF CEREAL FERMENTATIONS

The stored grains of cereals are metabolically in a resting state, which is primarily controlled by low water activity ($a_w \leq 0.6$, 14 % moisture). In this state the constituents are not available for microorganisms, and the endogenous enzymes are inactive. Fermentation processes will be enabled under the influence of technological measures including the addition of water, comminution by milling, and controlled management of microorganisms and enzyme activities (Hammes *et al*., 2005). It is especially the addition of water that affects the ecological factors dramatically. After the water activity increases by water absorption, a reduction of the redox potential takes place by respiration, as well as a drop of pH by respiration and fermentation, whereupon substrates become available from (i) endogenous hydrolytic activities (e.g. amylolysis, proteolysis and lipolysis) and (ii) physiological activities of deliberately added or contaminating microorganisms. These events cause a continuous change of the ecological state in the cereal matrix, for example, in sourdough (Hammes and Ganzle, 1998).

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 at a defined quality. These variables include the following (Hammes and Ganzle, 1998);

- The type of cereal determining the fermentable substrates, nutrients, growth factors, minerals, buffering capacity, and efficacy of growth inhibiting principles.
- The water content
- The degree and amount of comminution of the grains. That is, before or after soaking or fermentation.
- The duration and temperature of fermentation

- The components added to the fermenting substrate, such as, sugar, salt, hops and oxygen.
- The source of amylolytic activities that are required to gain fermentable sugars from starch or even other polysaccharides.

Among these variables, the type of cereal plays a key role. It affects the amount and quality of carbohydrates as primary fermentation substrates, nitrogen sources, and growth factors such as vitamins, minerals, buffering capacity, and the efficacy of growth inhibitors. With regard to fermentable carbohydrates, microorganisms are initially well supplied. The concentrations of free total sugars in cereal grains range between 0.5 and 3 %. Sucrose is the major compound (Shelton and Lee, 2000), representing >50 %. Especially through the activities of β-amylase present in the endosperm, the maltose generation in dough proceeds efficiently after the addition of water to the flour. The endogenous hydrolytic activities further contribute to the supply of free sugars (Hammes *et al*., 2005).

Similarly, peptides and amino acids become available through proteolytic activities. As shown by Prieto *et al.* (1990), the content of total free fatty amino acids increases by 64 % in the course of 15 minutes mixing of an, unfermented wheat dough.

The mineral content of grains is generally sufficient for microbial growth but differs in the various fractions obtained after milling (Betschart, 1988). It is strongly decreased in the white flour and increased in the germ and bran fractions. For example, manganese as an important growth factor of LAB occurs in whole wheat, white flour, wheat germ, and wheat bran at concentrations of 4.6, 0.7, 13.7, and 6.4-11.9 mg/100g, respectively (Hammes *et al*., 2005). The minerals of the grain are not readily available for microorganisms as they are complexed with phytate. However, at pH values of <5.5 the endogenous grain phytase hydrolyses phytate and minerals are released from the complex (Hammes *et al*., 2005). Therefore, a limitation in minerals may occur only at

starting a spontaneous fermentation. In processes as exemplified by sourdough propagation, the addition of sourdough to the bread dough lowers the pH and, thus, ensures that the phytase activity is sufficient and no need for physiological microbial activity exists (Fretzdorff and Brummer, 1993; Tangkongchitr *et al.,* 1982). The concentration of phytate in the various cereals ranges between 0.2 and 1.35 %, and again is strongly enhanced in the bran fraction. As phytate develops a high buffering capacity, the degree of flour extraction affects the metabolic activity of LAB in substrates such as dough. Therein lies the formation of titratable acids which correlates with the phytate content.

Inhibitors in cereals exert a selective effect on microbial growth. Known compounds are purothions and complexing compounds that interfere with the hydrolytic activities of the organisms or the availability of growth factors (Wrigley and Bietz, 1988). Little is known to what extent these factors determine the development of a specific fermentation association, which can be shown to become established, for example, in sourdoughs prepared from different types or fractions of cereals.

The addition of water to cereals usually ensures optimum water activity for fermenting microorganisms. The “driest” fermenting substrates are traditional sourdoughs, which are commonly adjusted to dough yields [(mass (water) + mass (flour))/mass (flour) X 100] ranging between 160 and 220, corresponding to a_w values of 0.965 and 0.980, respectively (Hammes *et al*., 2005). Clearly, the lower value is already in the stress range for LAB, and optimum values are approached with increasing dough yields.

The type of bacterial flora developed in each of the fermented food depends on the water activity, pH, salt concentration, temperature and composition of the food matrix (Blandino *et al*., 2003). Most fermented foods, including the major products that are common in the western World, as well as 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 towards the safety, nutritional value, shelf life and acceptability of a wide range of cereal based foods (Oyewole, 1997). In many of these processes, cereal grains, after cleaning, are soaked in water for a few days during which a succession of naturally occurring microorganisms will result in a population dominated by LAB. In such fermentations, endogenous grain amylases generate fermentable sugars that serve as a source of fermentable energy for the lactic acid bacteria (Blandino *et al.,* 2003). Fermentation is just one step in the process of fermented food preparation. Other operations such as size reduction, salting or heating also affect the final product properties (Nout and Motarjemi, 1997). According to Aguirre and Collins (1993), 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 4). According to the pathways by which hexoses are metabolized they are divided into two groups: homofermentative and heterofermentative. Homofermentative microorganisms such as *Pediococcus, Streptococcus, Lactococcus* and some Lactobacilli produce lactic acid as the major or sole end product of glucose fermentation. Heterofermenters such as *Wiesella* and *Leuconostoc* and some Lactobacilli produce equimolar amounts of lactate, CO_2 and ethanol from glucose (Aguirre and Collins, 1993; Tamime and O'Connor, 1995).

The preservative role of lactic acid fermentation technology has been confirmed in some cereal products. The antibiosis mediated by LAB has been attributed to the production of acids, hydrogen peroxide and antibiotics. The production of organic acids reduces the pH to below 4.0 making it difficult for some spoilage organisms that are present in cereals to survive (Daly, 1991; Oyewole, 1997). The antimicrobial effect is believed to result from the action of the acids in the bacterial cytoplasmic membrane, which interferes with the maintenance of the membrane potential and inhibits the active transport. Apart from their ability to produce organic acids, LAB possess the ability to produce hydrogen peroxide

through the oxidation of reduced nicotin-amide adenine dinucleotide (NADH) by flavin nucleotides, which react rapidly with oxygen. As LAB lack true catalase to break down the hydrogen peroxide generated, it can accumulate and be inhibitory to some microorganisms (Caplice and Fitzgerald, 1999). On the other hand, tannin levels may be reduced as a result of lactic acid fermentation, leading to increased absorption of iron, except in some high tannin cereals, where little or no improvement in iron availability has been observed (Nout and Motarjemi, 1997). Another advantage of lactic acid fermentation is that fermented products involving LAB have viricidal (Esser *et al*., 1983) and anti-tumor effects (Oberman and Libudzisz, 1996; Seo *et al*., 1996).

1.4. BIOCHEMICAL CHANGES DURING CEREAL FERMENTATION

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 determined antinutrients (phytic acid, tannins and polyphenols) and the coarse nature of the grains (Chavan and Kadam, 1989).

A number of methods have been employed with the aim of ameliorating the nutritional qualities of cereals. These include genetic improvement and amino acid supplementation with protein concentrates or other protein rich sources such as grain legumes or defatted oil seed meals of cereals. Additionally, several processing technologies which include cooking, sprouting, milling and fermentation, have been put into practice to improve the nutritional properties of cereals, although probably the best one is fermentation (Mattila-Sandholm, 1998). 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 polyvalent 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 fold (Chavan and Kadam, 1989; Gillooly *et al.*, 1984; Haard *et al.*, 1999; Khetarpaul and Chauhan, 1990; Nout and Motarjemi, 1997; Stewart and Gatechew, 1962).

Fermentation also leads to a general improvement in the shelf life, texture, taste and aroma of the final product. During cereal fermentations several volatile

compounds are formed, which contribute to a complex blend of flavours in the products (Chavan and Kadam, 1989). The presence of aromas representative of diacetyl acetic acid and butyric acid make fermented cereal based products more appetizing (Table 5).

1.5. POTENTIAL INFECTIVE AND TOXIC MICROBIOLOGICAL HAZARDS

The traditional fermentation of cereal products is widely practiced in Africa and other developing regions and usually involves a spontaneous development of different lactic acid producing bacteria. The final bacteriological status of the product however, is influenced in part by the raw materials and process method (Steinkraus, 1983). This natural lactic fermentation process is considered to be an effective method of preserving these foods, thus providing the population with a safe, nutritious food supply (Smith and Palumbo, 1983).

From a historical perspective it is not difficult to understand how such preservation processes could have arisen in primitive societies driven by the need to optimize the use of scarce food resources. An attestation of the intrinsic value of the approach reveals the extent to which the operating principles underlying solid-state food fermentations have been discovered and developed in many regions of the World. While the microbiological changes involved are generally highly reproducible and robust, nevertheless the use of natural substrates with their associated microbiota, together with the potential for operator 'mistakes' or alterations in processing procedures, can introduce some degree of variation in the microbiological quality of the final product (Farnworth, 2003).

Not all products carry the same degree of risk. For many, there appear to be little if any risk, but for some the results can be disastrous, as in the classical case of *tempe bongkrek* poisoning which has caused many deaths over the years (van Veen, 1967; Steinkraus, 1983). Fortunately, such dramatic consequences of process failure are rare with indigenous fermented foods. However, the extent to which other less acute microbiological problems might occur is difficult to assess because of the difficulties of establishing cause and effect with some types of

food-related illnesses and the general lack of good epidemiological surveillance data.

In addition to potential problems caused by pathogenic and toxigenic bacteria, it has long been realized that certain moulds and their toxic metabolic products (mycotoxins) can pose a threat in fermented food products. Although mould strains used in these processes have generally been found to be non-toxigenic, unknown mould contaminants which might develop during the fermentation always enhance the possibility that mycotoxins can be produced. Moreover, since the raw materials used are good substrates for mould growth and mycotoxin formation, these relatively stable toxic compounds may be present in commodities before the fermentation, and persist into the final product (Farnworth, 2003).

1.5.1. Spoilage of popular fermented foods

A number of spoilage bacteria and or opportunistic food borne microbial pathogens are encountered during common solid-state fermentations of food. Lactic fermented foods provide protection against food borne illnesses, and children consuming lactic fermented products on a regular basis (e.g. *togwa*, a commonly used weaning food in Tanzania with a final pH < 4) show a significantly lower number of diarrhoeal episodes than non-users (Lorri and Svanberg, 1994). While fermented foods, the mainstay of many developing countries, have long been perceived as safe for consumption, a number of researchers have investigated the fate of many prevalent food borne bacterial pathogens during the production of such foods (Farnworth, 2003).

The growth and survival of different enteropathogenic microorganisms like *Bacillus cereus, Campylobacter jejuni,* and enterotoxigenic *Escherichia coli* were studied during the fermentation of cereal gruels (e.g. *togwa*) prepared from low tannin (white) and high tannin (red) sorghum varieties by Kingamkono *et al.,*

(1994). The authors used fermented gruel (starter culture of *Lactococcus lactis* and *Candida krusei*, SC) which was recycled daily or stored for 7, 14 or 28 days, germinated cereal flour (power flour, PF) or a combination of PF and SC (PF + SC) as starters. After 24 h, the pH of all the gruels with added starter was ≤ 4 whereas the pH in the indigenously fermented control gruels without starter cultures were ≥ 5.2.

In the control gruels, the enteropathogens remained at the inoculation level or increased in number, while these organisms were inhibited within 24 to 48 h in the fermented gruels (PF + SC, SC) in the order, *Bacillus, Campylobacter, Escherichia, Salmonella* and finally *Shigella.* While the use of starter cultures rendered *togwa* free of these enteropathogens after 48 h, an indigenous fermentation was not sufficient to inhibit their development and proliferation when these organisms were present as contaminants (Kingamkono *et al.,*1994). The slower inhibition of ETEC, *Salmonella typhimurium* and *Shigella flexneri* (gram-negative bacteria) than *Bacillus cereus* (Gram-positive bacterium) was attributed to the fact that Gram-negative bacteria had a cell wall which required breaching before cell death (Andersson, 1986) and partly to the acid tolerance response, an inducible pH-homeostatic function protecting the cells from acid stress (Foster and Hall, 1991).

While the contrasting behaviour of *Campylobacter jejuni* compared with other Gram-negative bacteria may be attributed in part to the fact that *Campylobacter* is microaerophilic, other factors may also have an effect (Kingamkono *et al.,* 1994). The enhanced pH decrease in gruels inoculated with PF + SC that had been recycled daily, was explained by the fact that the starter cultures produced through daily recycling, undergo selection of microorganisms that grow best in acid and near-anaerobic conditions, making them dominant in the media (Nout *et al*., 1987).

1.5.2. Factors increasing susceptibility

The study and control of established and new emerging pathogens in solid state fermented foods must always be examined under the conditions which exist in the environment where these products are prepared, fermented and consumed. Atopic people (particularly children) may suffer bouts of food related infections or poisonings depending on a number of inter-related factors co-existing. These factors include, preparation of foods under unhygienic conditions, poor personal hygiene, contaminated water supply, the presence of enteropathogens in raw materials or fermented foods as contaminants, the type and virulence of the enteropathogens present, the immune status of the individual being compromised (either from an earlier food or waterborne infection / poisoning and or due to the atopic individual suffering from AIDS or underdeveloped (i.e. children), consumption of inadequately fermented foods (i.e. possibly an indigenous fermentation process without the involvement of a LAB starter culture)(Farnworth, 2003).

It is of paramount importance that these fermented products be prepared under good sanitary and hygienic conditions. In developing countries, most food borne illnesses are of a bacterial nature. This is due to poverty, low level of education, poor sanitation and hygiene practices, poor methods of food preservation and keeping, unavailability of potable drinking water and absence or scarcity of health facilities (Nigatu and Gashe, 1994). Sources of food contamination are numerous, like nightsoil, polluted water, flies, pests, domestic animals, unclean utensils and pots, dirty hands, and a polluted environment caused by lack of sanitation, domestic animal droppings, dust and dirt, etc. (Motarjemi *et al*., 1993). In order to ensure that solid-state fermented foods such as *tempe, tef*, (which are the mainstay of many developing communities) are microbiologically safe, food handlers, particularly mothers, must be educated about the above dangers.

1.5.3. The role of fungal toxins (mycotoxins) in fermented foods

Provision for a safe food supply, including fermented foods and beverages is a fundamental requirement of any modern society. Mycotoxins are secondary metabolites of certain filamentous fungi that can cause toxicity to humans and animals when low concentrations are ingested or inhaled (Smith *et al*., 1995). In nature, such toxins are primarily derived from agricultural crops such as cereals and oil seeds and products derived from them and from animal derived foods such as milk. Mycotoxins can enter the human dietary system by indirect or direct contamination. Indirect contamination can occur when an ingredient of a food or beverage fermentation (e.g. cereals or legumes) has previously been contaminated with toxin-producing moulds and although the mould may be killed or removed during processing, mycotoxins will often remain in the final product (Miller and Trenholm, 1994; Smith *et al.,* 1994). Direct contamination can occur in two ways by the fermentation process that may involve a fungus essential for the process but also capable of producing myctotoxins and if the process or final product become infected with a toxigenic mould with subsequent toxin formation. Thus, almost all fermented foods and beverages have the potential to be affected by toxigenic moulds at some stage during their production, processing, transport or storage.

Mycotoxins are a structurally diverse group of mainly small molecular weight compounds produced mostly by five genera of fungi, *viz*: *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps* (Smith and Moss, 1985). Mycotoxins can elicit a wide spectrum of toxicological effects (a mycotoxicosis) which have been extensively studied in various animal species and increasingly confirmed in humans (Smith *et al.,* 1995). While over 300 mycotoxins have been characterized under laboratory conditions, only about 20 mycotoxins have been shown to occur naturally in agricultural raw materials at significant levels and frequency to be considered to represent a food safety concern.

The principal and most regularly documented mycotoxins produced by the five genera are *Aspergillus* toxins: aflatoxins B_1, G_1 and M_1, ochratoxin A, sterigmatocystin and cyclopiazonic acid; *Penicillium* toxins: cyclopiazonic acid, citrinin and patulin; *Fusarium* toxins: deoxynivalenol, nivalenol, zearalenone, T-2 toxin, diacetoxyscirpenol, moniliform and fumonisins; *Alternaria* toxins: tenuazonic acid, alternariol and alternariol methyl ether; *Claviceps* toxins: ergot alkaloids (Steyn, 1995).

Consumption of fermented foods and beverages heavily contaminated with mycotoxins is not likely to occur to any extent in most advanced societies because of the existence of strict food regulations. However, the widespread presence of fumonisins in maize especially in humid, developing countries could be a notable exception (Sydenham *et al*., 1994). High quality agricultural practices with improved storage and transportation facilities have much reduced toxigenic mould growth in raw agricultural materials destined for the human food chain. Where poor agricultural practices and warm, hot climates prevail, such as in many developing countries, higher endemic levels of mycotoxins must be anticipated in the raw food materials such as maize, rice and peanuts.

1.6. RECENT ADVANCES IN THE MALTING AND BREWING INDUSTRY

1.6.1. Lactic acid starter cultures in malting

Barley and malt sometimes give problems for the brewer. A heavy *Fusarium* contamination of malting barley may lead to the formation of deoxynivalenol (DON) and other mycotoxins (Haikara, 1983; Flannigan *et al*., 1985; Schwarz *et al*., 1995; Munar and Sebree, 1997). As a water soluble compound DON is washed out during steeping of barley, but during germination DON is produced by *Fusarium* fungi. DON is not removed or destroyed during the brewing process. *Fusarium* contamination may also lead to the so-called gushing of beer, which means quick uncontrolled spontaneous over-foaming immediately when opening the bottle or can (Amaha and Kitabatake, 1981). *Fusarium graminearum, Fusarium culmorum* and *Fusarium poe* are active gushing inducers (Haikara, 1983; Niessen *et al.*, 1992; Vaag *et al*., 1993; Schwarz *et al*., 1996; Munar and Sebree, 1997). The production of mycotoxins may parallel the production of components responsible for gushing.

Strict control of incoming barley lots is, of course, vitally important. However, sometimes in some areas there is simply not enough high quality barley available. Microflora management in such a way that harmful microorganisms are discouraged and neutral or beneficial organisms are favoured, could minimize the risk caused by microbial contamination of barley. A novel method is to use lactic acid bacteria or *Geotrichum candidum* as starter cultures in malting to reduce the fungal contamination and to improve the malt quality (Boivin and Malanda, 1993; Haikara *et al*., 1993; Haikara and Laitila, 1995; Boivin and Malanda, 1997a, b). Addition of starter cultures ensures high quality of malt regardless of the natural variation of the microbiota of barley.

The effect of lactic acid starter culture on the malting and brewing processes is based on the microbicidic compounds produced and also on their other characteristics such as enzyme activities (Linko *et al*., 1998). Certain *Lactobacillus plantarum* and *Pediococcus pentosaceus* strains are especially efficient when added to the steeping waters of barley at the level of about 10^7 cells g^{-1}. The whole cultures are needed for the restriction of harmful microorganisms, because the effect of lactic acid bacteria is essentially based on the microbicidic compounds present in the medium (Haikara *et al.*, 1993). The addition of starter cultures in the early stage of malting is important due to the intensive growth of *Fusarium* during the first hours of steeping. The effect of lactic acid bacteria depends on the composition of the biota and on the contamination level of the barley. However, numerous laboratory and pilot experiments using barley crops from different years as well as industrial scale trials have confirmed the fungicidal effect of starter cultures (Linko *et al*., 1998).

Lactic acid starter cultures also restrict the growth of harmful Gram-negative and –positive bacteria, which compete in the grain tissue for the dissolved oxygen and may retard mash filtration (Haikara and Home, 1991; Doran and Briggs, 1993). A marked reduction in aerobic bacterial biota has been observed throughout the malting process when starter cultures have been applied (Haikara *et al*., 1993; Haikara and Laitila, 1995). *Pseudomonas* species are especially sensitive.

1.6.2. New cereal based probiotic foods

Despite the antimicrobial effects of the lactic acid bacteria from cereal based fermented foods, the use of these microorganisms and their fermented products for the production of new probiotic foods is also a new trend. The term “probiotic” refers to a product containing mono or mixed cultures of live microorganisms, which when ingested will improve the health status and or affect beneficially the host by improving its microbial balance (Salovaara, 1996). Most of the probiotics

strains are isolated from the human gut and belong to the group of lactic acid bacteria, of which, *Lactobacillus* species are the most important (Table 6).
There are some new cereal based fermented foods that are considered as probiotic products, for example, *yosa* (Wood, 1997). Other traditional cereal based fermented foods have been modified to aid the control of some diseases. An improved *ogi* named *dogik* has been developed using a lactic acid starter with antimicrobial activities against some diarrhoeagenic bacteria (Okagbue, 1995).

1.6.3. Future use of *S. cerevisiae* as a starter culture.

The advantages obtained by fermentation of foods are many, especially in developing countries. Also the socio-economic and cultural effects of the production of traditionally fermented foods are important. Despite this and the fact that indigenous fermented foods and beverages form a great part of the diet in many African countries, by far the majority of these products are still produced by spontaneous fermentation at household or semi-industrial scale. Many of these fermentations are not described in detail and the losses due to inadequate process equipment and uncontrolled fermentation are likely to be very significant. In order to maintain and sustain the production of indigenous fermented foods in Africa, up-scaling of production plants is required, as is the change from spontaneous to controlled fermentation and the introduction of purified indigenous starter cultures.

The fact that isolates of *S. cerevisiae* from African fermented products have properties different from those of well recognized starter cultures (van der Aa Kuhle *et al.*, 2001; Hayford and Jespersen, 1999) demonstrates that starter cultures for indigenous fermented foods and beverages should be isolated from products that they are supposed to be used for, and selected according to technological properties required for the actual type of product. Moreover, antimicrobial, probiotic and pathogenic properties as well as genetic stability should be taken into consideration (Holzapfel, 1997). Reported examples where

strains of *S. cerevisiae* have been isolated from indigenous fermented foods and beverages and thereafter been successfully used as starter cultures are Ghanaian fermented maize dough for '*kenkey*' and '*koko'* production (Halm *et al.*, 1996), Ghanaian fermented sorghum beer (Sefa-Dedeh *et al.*, 1999), Zambian '*munkoyo'* maize beverage (Zulu *et al.*, 1997), and Nigerian '*ogi'* based on maize (Teniola and Odunfa, 2001).

1.6.4. Future of fermented foods

In the future, fermented foods will become even more important in our diet and the maintenance of health, as we identify different microorganisms that can be used in the production of probiotic foods (Farnworth, 2003). Probiotic foods will be made that target specific age groups that have specific metabolic requirements (newborns, adolescents, elderly), people in specific disease states (irritable bowel syndrome, Crohn's disease, intestinal cancers) or those who have had their immunoflora compromised (irradiation patients, intestinal surgery patients, people who have received antibiotics). These advances will occur as we understand more about the role of the intestinal bacteria play in human health and as we are able to identify the mechanisms involved in the interaction between food bacteria passing through the GI tract and the host intestinal bacteria.

Fermented foods are consumed in every country of the World and there is growing scientific evidence that many fermented foods are good for health or contain ingredients that are good for health. Foods that improve or change the intestinal microbiota are of particular interest because of our increased knowledge of the role the intestinal microbiota plays in health and disease resistance. In the future, more fermented foods with health promoting properties will become available on the market, with many directed towards consumers with very specific health and metabolic needs.

1.7. CONCLUSION

Fermented foods are of great significance because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavours, aromas and textures which enrich the human diet. Fermented foods have been with us since man's first existence on earth. They will remain important in the future as they are the source of alcoholic foods / beverages, vinegar, pickled vegetables, sausages, cheeses, yogurts, vegetable protein amino acid/ peptide sauces and pastes with meat-like flavours, leavened and sour-dough breads. The affluent western World cans and freezes much of its foods but the developing World must rely upon fermentation and dehydration to preserve its foods at costs within the budgets of the average consumer.

Despite the conventional foods and beverages largely produced from cereals in the western World (breads, pastas and beers), there is a wide variety of products produced World-wide that have not received the scientific attention that they deserve. These products are often fermented, and have an improved shelf life and nutritional properties in comparison with the raw materials used. The biota responsible for the fermentation is in many cases indigenous and includes strains of lactic acid bacteria, yeasts and fungi. Individual or mixed cereals sometimes mixed with other pulses are used, and the final texture of the product can vary according to the processing and fermentation conditions. Similar fermentation procedures are used nowadays to develop new foods with enhanced health properties, which is a trend likely to continue in the future.

In order to maintain and sustain African indigenous fermented foods and beverages, improved control of fermentations and product characteristics is strongly recommended, including the use of purified starter cultures with appropriate technological properties.

The numbers of African fermented indigenous foods and beverages are many and their production is often not described in detail. Depending on country and even local region, various local names may be given to the same product or to products that are basically similar but are produced with slight variations. Be that as it may, advances in food technology, microbiology, and nutrition will give us even more fermented foods to eat and more reasons to eat them in future.

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Table. 1. Possible functions of yeasts in African indigenous fermented foods and beverages (Blandino *et al.,* 2003).

- Fermentation of carbohydrates (formation of alcohols, etc.)
- Production of aroma compounds (esters, alcohols, organic acids, carbonyls, etc.)
- Stimulation of lactic acid bacteria providing essential metabolites
- Inhibition of mycotoxin-producing moulds (nutrient competition, toxic compounds, etc.)
- Degradation of mycotoxins
- Degradation of cyanogenic glycosides (linamarase activity)
- Production of tissue degrading enzymes (cellulases and pectinases)
- Probiotic properties

Table 2; Average chemical composition of feeding stuff (Oyenga, 1968; Oyewole and Akingbaba, 1993; BOSTID, 1996).

				[vitamins]			
Food	Starch (%)	Protein (%)	Fat (%)	Ascorbic acid (mg/ml)	Thiamine (mg/ml)	Riboflavin (mg/ml)	Niacin (mg/ml)
Cassava	94.1	2.6	0.5	3.5	0.04	0.02	0.6
Maize	82.6	10.2	4.1	11.4	0.5	0.08	2.0
Guinea corn	76.2	14.2	3.2	29.1	-	-	-
Millet	83.2	8.0	5.0	0	0.3	0.04	1.8
Acha	78.3	7.5	1.2		0.47	0.10	1.9

Table 3; Common indigenous cereal and cereal-legume based fermented foods and beverages (Adams, 1998; Chavan and Kadam, 1989; Harlander, 1992; Sankaran, 1998; Soni and Sandhu, 1990)

Product	Substrates	Microorganisms	Nature of use	Regions
Burukutu	Sorghum	*Saccharomyces cerevisiae, S. chavelieri, Leuconostoc mesenteroides, T.candida, Acetobacter*	Alcoholic beverage of vinegar-like flavour	Nigeria, Benin, Ghana
Chikokivana	Maize and Millet	*Saccharomyces cerevisiae*	Alcoholic Beverage	Zimbabwe
Dhokla	Rice or wheat and Bengal gram	*Leuconostoc mesenteroides, Streptococcus faecalis, Torulopsis candida, T. pullulans*	Steamed cake for breakfast or snack food	Northern India
Doro	Finger millet malt	*Yeasts and bacteria*	Colloidal thick alcoholic drink	Zimbabwe
Injera	Sorghum, tef, maize or wheat	*Candida guilliermondii*	Bread-like staple	Ethiopia
Kaffir beer	Kaffir corn	*Yeasts, LAB*	Alcoholic drink	South Africa
Kishk	Wheat and milk	*Lactobacillus plantarum, L. brevis, L.casei, Bacillus subtilis and* yeasts	Solid, dried balls, dispersed rapidly in water	Egypt, Syria, Arabian countries
Kwunu-Zaki	Millet	LAB, yeasts	Paste used as breakfast dish	Nigeria

Table 4; Genera of lactic acid bacteria involved in cereal fermentations (McKay and Baldwin, 1990; Oberman and Libudzisz, 1996; Suskovic *et al.,* 1997).

Genera of LAB	**Cell form**	**Catalase**	**Gram (±)**
Lactobacillus	Rods (Bacilli; coccobacilli)	-	+
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	-	+
Vagococcus	Cocci	-	+
Aerococcus	Cocci	-	+
Weisella	Cocci	-	+

Table 5; Major Volatile and Nonvolatile constituents of beer (Reed and Nagodamithana, 1991)

Constituents	Concentration (ppm)	Flavor threshold (ppm)	Aroma or Taste
Alcohols			
Ethyl alcohol (%)	2.5-3.8 (%)	1.5-2.0 (%)	Harsh, sweet
2-phenyl ethanol	25-30	45-50	Rosy, sweet
2,3-butanediol	10-128	400-500	Sweet aroma and taste
Isoamyl alcohol	50-60	50-60	Fusely, throat catching
Active amyl alcohol	8-15	50-60	Fusely , pungent
n-propanol	10-15	50-60	Slightly pungent, harsh
Isobutanol	9-12	80-100	Fusely, penetrating
Esters			
Ethyl acetate	20-25	50-60	Fruity, solventy
Isoamyl acetate	2-15	2-3	Fruity, banana
Phenyl ethyl acetate	0.5-1.2	3.0	Honey, sweet, fruity
Aldehydes			
Acetaldehyde	5-10	12-15	Pungent, green house
2-norenal	0.0005	0.0003-0.0005	Cardboardy
2-hexanal	4.5	0.5-0.7	Spicy, cinnamonlike
5-OH-methyl furfural	0.1-3.0	1000	Caramel, sweet
Diketones			
Diacetyl	0.05-0.10	0.10-0.15	Buttery
2,3-pentanedione	0.01-0.03	0.09	Butterscotch
Acetoin	1-4	50	Buttery, smooth
Sulfur compounds			
Dimethyl sulfide (ppb)	5-100	35-60	Cabbage, malty
Diethyl disulfide (ppb)	0-10		
H_2S (ppb)	0-5	5	Rotten egg
3-methyl butane thiol (ppb)	2	1-3	Sun-struck

Table 6; Some of the common strains currently used in probiotic foods. (Blandino *et al.,* 2003)

Bifidobacterium spp.	*Lactobacillus spp.*	Others
B. adolescentis	*L. fermentum*	*Propionibacterium freudenreichii*
B. brevis	*L. bifidus*	*Saccharomyces cerevisiae*
B. Lactis	*L.bulgaricus*	*Pediococcus acidilactici*

Chapter 2

Isolation and characterization of the microbiota associated with *umqombothi*, a South African fermented beverage

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ABSTRACT

The changes in microbial composition of traditionally fermented *umqombothi* were studied over 48 h. Three different types of samples were compared, namely; Sample A (home-made), Sample B (made in the laboratory), and Sample C (made in the townships). Sample C is commercially produced. Sub-samples of the fermented beer were taken at 0, 24, 30 and 48 h fermentation time and lactic acid bacteria (LAB), yeasts, moulds and enterobacteriaceae were enumerated. The pH of the fermenting product was also monitored. It was observed that yeasts and lactic acid bacteria were the predominant microorganisms during the whole fermentation period in all the samples. The LAB counts were 8.56, 7.96, and 7.82 log cfu / ml for samples A, B and C respectively, after 48 h. The yeast counts were 6.52, 7.1, 6.42 log cfu / ml, for A, B and C respectively. There were no significant differences ($P<0.05$) in the final LAB and yeast counts between the samples from the three sites. Enterobacteriaceae, which were initially high, were reduced to non detectable levels after 48 h. The isolated yeasts were identified and *Candida ethanolica, C. haemuloni, C. sorbophila, Dekkera anomala, Dekkera bruxellensis, Saccharomycopsis capsularis* and *Saccharomyces cerevisiae* were found to be the predominant yeasts isolated throughout the fermentation. This study showed several strains of yeast and lactic acid bacteria growing symbiotically during the spontaneous fermentation of *umqombothi*.

Keywords: fermented beverage, *umqombothi*, lactic acid bacteria, yeast

2.1. INTRODUCTION

There are many different forms of beer produced and consumed in Africa, most of which are different from the “clear” lager beers from the Western World. African traditional beers are made from malted sorghum, millet or maize, and it is much thicker than clear beer and resembles a thin gruel with a distinctive sour yoghurt-like refreshing taste (Hesseltine, 1979). These beers are opaque and generally pinkish-brown in colour due to the large quantity of solid particles and yeasts suspended in solution (Novellie, 1968). Alcohol contents of indigenous beers normally vary between 2 to 3 % although it may be as high 8 % (Richards, 1939; White, 1947; Bryant, 1948; Schwartz, 1956; Aucamp *et al*., 1961; Netting, 1964; Novellie, 1968; Botswana Government, 1976; Bleiberg, 1979). In this study the alcohol content of the *umqombothi* was found to be 2.6 %. There are different forms of indigenous beer arising from the numerous possible combinations of malt and starch. Consequently a wide variety of recipes exists across the African continent and the beers are known by many local names like ‘*pito’* in Nigeria, ‘*bouza’* in Ethiopia, ‘*gwalo’* in Congo, ‘*munkoyo* in Zambia, *doro*/*uthwala* in Zimbabwe and ‘*amgba’* in Cameroon (Van der Walt, 1956; Rosenthal, 1961; Ekundayo, 1969; Hesseltine, 1979; Vogel and Graham, 1979; Hulse *et al*., 1980; Zulu *et al*., 1997; Zvauya *et al.*, 1997). This type of beer made from sorghum and maize is called *umqombothi* in the Shangaan language in South Africa. The south Sothos call it ‘*joala’*, *Vendas*, ‘*mela’*, Tswanas, ‘*bobule’*, Zulus and Xhosas ‘*tshwala’* and Ndebeles, ‘*utshwala’*.

Umqombothi is popular among the black South African population. It is cream colored after sieving, is opaque, has a yoghurt-like flavour, a thin consistency and is effervescent. As was observed for similar African fermented alcoholic beverages (Wood, 1998), *umqombothi* fermentation is spontaneous and uncontrolled therefore acetic acid bacteria usually succeed the alcoholic fermentation giving unacceptable vinegar flavours. Hence these beverages have a short shelf life and are often consumed in an actively fermenting stage (Wood,

1998). Although it is produced commercially on large scale using new techniques, the old traditional way of making *umqombothi* still exists. In the townships, women still brew *umqombothi* either for social gatherings or for sale using the age-old techniques and village art methods.

Considerable work has been done on the microbiology of spontaneously fermented cereal based products. Yeasts and lactic acid bacteria (LAB) have been found to be the predominant flora (Fields *et al.*, 1981; Odunfa and Adeyele, 1985; Okafor and Uzuegbu, 1987; Oyewole and Odunfa, 1990; Halm *et al*., 1993; Hounhouigan *et al*., 1993). The microorganisms exhibit great stability and can dominate a wide variety of other microorganisms (Wood, 1985). By their metabolic activities the fermenting microorganisms produce acids which inhibit and eliminate unwanted microorganisms. They also produce aromatic compounds which give flavour to the food (Banigo and Muller, 1972; Kaminsky *et al.*, 1979; Plahar and Leung, 1982). However, the microorganisms involved in the traditional fermentation of *umqombothi* have not been studied. This study therefore aims to investigate the microbial changes during the fermentation of *umqombothi* and to assess the stability of the microbiota.

Keywords: *umqombothi*, fermented beverages, traditional food

2.2. MATERIALS AND METHODS

2.2.1. Source of raw materials

Sorghum malt (1kg) and maize flour (1kg) used to make beer in the laboratory were bought from the local market. 20 ml samples of fermented beer from a previous fermentation (*umqombothi*) were obtained from the township.

2.2.2. Collection of fermented beer samples

The microbial changes taking place during the spontaneous fermentation of *umqombothi* were observed in samples taken from a private household, prepared in the laboratory, and that taken from the townships. These were labelled A, B and C, respectively. The sample obtained from the townships had been commercially produced. Sub-samples (50 ml) were taken immediately after soaking, before cooking, after the addition of malt (this happens after the mixture has been cooked, then left to cool/stand for approximately 6 h) and at the end of the fermentation. That is, at the 0, 24, 30 and 48 h mark respectively. Sampling was done using a sterile beaker and transferred to a sterile sampling bottle and transported to the laboratory in a cooler box.

2.2.3. Preparation of *umqombothi* in the laboratory

The production process is illustrated in Fig. 1. Briefly, maize flour (1 kg) was inoculated with a hand-full of sorghum malt and soaked in water for 24 h. The mixture was then cooked into a soft porridge and allowed to stand for 6 h to cool to room temperature. Additional sorghum malt (0.25 kg) was then added to the porridge and thoroughly stirred. This was then inoculated with a small portion of o *umqombothi* (20 ml) from a previous batch. The mixture was then left to ferment for 18 h and then strained through a sieve with a pore size of ca. 0.5 mm. The chaff was sun-dried and set aside for use as inoculum in later productions. Nine batches of *umqombothi* were prepared this way using the same batch of materials.

2.2.4. Chemical analysis

The pH of each of the *umqombothi* samples was measured at room temperature using a CyberScan 500 (Eutech Instruments, Singapore) pH meter.
Each sample (5 ml) was then centrifuged at 11,000 rpm for 5 minutes in a J2-21 centrifuge (Beckman, USA). The supernatant obtained was used for lactic acid determination by high performance liquid chromatography (Waters Lambda-Max 480). Sulphuric acid (0.01 N) was used as the mobile phase with a flow rate of 0.5 ml/min and lactic acid was detected at 210 nm (Bouzas *et al*., 1991).

Supernatant obtained from each of the final product samples was also used for ethanol content determination by gas chromatography (Gas Chromatograph Series II, Hewlett Packard) (Guzel-Seydim *et al*., 2000).

2.2.5. Microbiological analysis

Portions of the different beer samples (10 ml) were serially diluted using sterile Peptone Physiological Salt solution (Oxoid, Basingstoke, UK) and aliquots (1 ml) of each of the six dilutions were then inoculated on plates in duplicate. Total aerobic mesophilic counts were obtained by spread plating on Plate Count Agar (PCA) (Oxoid, Basingstoke, UK) and incubating at 25 ^{o}C for 48 h. Lactic acid bacterial (LAB) counts were obtained by spread plating on de Man Rogosa and Sharpe (MRS) agar (Biolab, Midrand, RSA) and incubating at 25 ^{o}C for 48 h. Yeast and mould were determined on Potato Dextrose Agar (PDA) (Detroit, Michigan, USA) containing 0.01 % chloramphenicol, and incubating for 4 days at 25 ^{o}C. Enterobacteriaceae were enumerated by spread plating on MacConkey agar (Biolab, Gauteng, RSA) and incubating at 37 ^{o}C for 24 h.

2.2.6. Identification of yeasts

Yeast colonies with different colonial morphology were randomly picked from selected PDA plates and purified by further streaking onto PDA plates. The pure cultures were sub-cultured onto Malt Extract agar (Wickerham, 1951) slants and stored at 4 ^{o}C. Strains were identified to the species level according to the conventional identification method of Kreger-van Rij (1984), Barnett *et al*. (1990) and Kurtzman and Fell (1998). Each isolate was inoculated into six fermentation media, 35 carbon source assimilation media, vitamin free medium, 0.01 % and 0.1 % cyclohexamide. Assimilation of nitrogen compounds was performed by means of the auxonographic method (Lodder and Kreger-van Rij, 1952).

Additional tests performed included growth at 37 ^{o}C, growth in 50 % D-glucose medium, hydrolysis of urea, starch hydrolysis, production of acetic acid, the Diazonium Blue test (Van der Walt and Hopsu-Havu, 1976). Ascospore formation was examined on McClary's acetate agar, potato glucose agar, Gorodkowa agar, corn meal agar and malt extract agar (Kreger-van Rij, 1984). The formation of pseudomycelium and true mycelium was examined on corn meal agar according to the Dalmau plate technique (Wickerham, 1951).

Confirmation of the yeast identity was done using sequence analysis of the D1/D2 domain using primer pairs NL-1(5'-GCATATCAATAAGCGGAGGAAAAG) and NL-4(5'-GGTCCGTGTTTCAAGACGG) (Kurtzman and Robert, 1998). Sequencing reactions were carried out using the ABI Prism^{TM} Big Dye Terminator^{TM} v3.1 cycle sequencing ready reaction kit. Data was collected on an ABI Prism 377 DNA sequencer (Applied Biosystems, USA) and analysed using sequencing analysis V3.3 and sequences assembled using Auto-assembler V1.4.0.

2.2.7. Statistical analysis

Results from the three sites were compared using the analysis of variance (ANOVA) procedure (NCSS, 2004).

2.3. RESULTS AND DISCUSSION

2.3.1. Chemical analysis

Fig. 2 shows the changes in pH of the *umqombothi*. The pH dropped from 5.71 to 3.56, 6.01 to 3.26, and 5.45 to 3.06 for samples from A (house), B (laboratory) and C (township) respectively during the first 24 h of fermentation. After cooking, the pH of the values slightly increased to 5.10, 4.05 and 3.85, for samples A, B and C, respectively. This could have been due to addition of water during cooking.

The pH dropped significantly ($P< 0.05$) in all products after 48 h of fermentation with the township-made *umqombothi* attaining the lowest pH of 3.52.The home-made product had a pH of 3.54, while the laboratory made product had a pH of 3.58 after fermentation. The pH of the samples was, however, not significantly different ($P< 0.05$). During fermentation lactic acid bacteria produce lactic acid (Jay *et al*., 2005). It is this lactic acid that leads to the observed decrease in pH due to increased counts of LAB and their subsequent production of lactic acid as one of the end products of metabolism of the sugars in the samples (Jay *et al*., 2005). The observed increase in pH after cooking could be due to the fact that lactic acid is a weak and therefore unstable acid, addition of water as is done during cooking decreases the acidity of the fermentation. This is due to dissociation of the acid in water (Barret, 2003). Similar trends were observed during the fermentation of *kenkey*, an indigenous fermented maize food from Ghana (Nout *et al*., 1996).

The ethanol content of the final product samples was measured and determined to be an average of 2.6 %. This is similar to related products such as *pito*, *kaffir beer* and *busaa* (Wood, 1998). Traditional fermentation processes from different locations may feature process variables that may give different levels of ethanol from the one obtained in this study.

2.3.2. Microbiological analysis

2.3.2.1. Lactic acid bacteria

Fig. 3 shows the changes in LAB counts in *umqombothi* samples during the fermentation period. In the home prepared sample, the LAB counts increased by about 3 log cycles, from 5.53 to 8.56 log cfu / ml in 48 h. The same trend was observed for the laboratory prepared sample. However the sample obtained from the townships had a higher initial count of LAB of 6.28 log cfu / ml, which increased to 7.82 log cfu / ml after 48 h. The final LAB counts in the three cases were not significantly different ($P<0.05$) from each other. The higher initial LAB count in the township sample could be attributed to the fact that the maize used in the production of the beer is soaked in a fermentation pot that had been used for previous fermentations. The observed trends are similar to those reported during the production of *masvusvu, mangisi, mawe* and *kenkey* (Halm *et al*., 1993; Hounhouigan *et al.*, 1994; Zvauya *et al*., 1997).

Lactic acid bacteria (LAB) counts were observed to have slightly decreased after the cooking stage. This may be due to the increased temperatures during cooking that may have killed off some of the bacterial cells. The township sample C showed a lesser decrease than samples A and B and this may have arisen from slight process variations. As mentioned earlier, the final LAB counts for all samples were not significantly different ($P<0.05$).

2.3.2.2. Yeasts

After 48 h fermentation, yeast counts increased from 5.09, 5.09 and 5.25 to 6.52, 7.1 and 6.42 log cfu / ml for samples A, B and C respectively (Fig. 4). This suggests an important role for the yeasts in this product. During the initial stages of fermentation starches are broken down to sugars and it is these sugars that are then fermented by yeasts resulting in ethanol production (Wood, 1998). In addition, yeasts produce a wide range of flavour compounds such as organic

acids, esters, alcohols, aldehydes, lactones and terpenes (Janssens, 1992), that may be important in the final characteristics of the product. *S. cerevisiae* is the predominant yeast in many African indigenous fermented foods and beverages where it is mainly occurs as a result of spontaneous fermentation. Its major functions include the production of alcohols and other aroma compounds, especially esters and organic acids, but other effects such as stimulation of lactic acid, improvement of nutritional value, probiotic activity, inhibition of mycotoxin-producing moulds, and production of tissue degrading enzymes may also be seen (Jespersen, 2003).

2.3.2.3. Moulds

After soaking the sorghum and maize mixture, the moulds decreased from about log 1 to non-detectable levels (Data not shown). Halm *et al.,* (1996), reported that *S.cerevisiae* and *C. krusei* were observed to have an inhibitory effect on the growth of mycotoxin-producing moulds such as *Penicillium citrinum*, *Aspergillus flavus* and *Aspergillus parasiticus.* The inhibitory effects of the yeast were shown to be mainly due to substrate competition, but inhibition of spore germination might also occur due to the production of high concentrations of organic acids.

2.3.2.4. Enterobacteriaceae

Enterobacteriaceae counts decreased markedly in all cases (Fig. 5). There was an average decrease from, 4.88 to 2.09 log cfu / ml for samples from site A, 4.41 to 0.26 log cfu / ml for samples from site B and 3.32 log cfu / ml to non-detectable levels for samples from C. The home made sample (A) showed an increase in the counts after cooking. This may be attributed to contamination being introduced by the handler at this particular stage of the production process and this might explain why a similar trend was not observed for the other two samples at this same process period.

Mensah (1997) found that the growth of *Shigella flexneri* and enterotoxigenic *Escherichia coli* in fermented maize dough was inhibited compared to growth in unfermented dough. The mechanisms behind the growth inhibition were unclear but likely to involve the decreased pH of the fermented dough and the production of antimicrobial compounds. This trend is what is thought to have occurred in the *umqombothi* as similar observations were made to Mensah (1997), as the enterobacteriaceae counts had markedly decreased by the end of the 48 h fermentation period.

The presence of coliforms in fermented foods especially above log 2 cfu / ml is a cause for concern and it also indicates the likely contamination of the fermented product by other enteric pathogens such as *Salmonella* and pathogenic *Escherichia coli*. In addition, coliforms may influence other characteristics, such as the sensory attributes, of the fermented product. For example, Mbugua (1982) reported that coliforms resulted in the production of off-flavours and flavour instability in Kenyan *uji*, a fermented maize-based product.

2.3.2.5. Microbial interaction

At the end of the 48 h, LAB and yeast numbers had stabilized. The growth of both the yeast and LAB together suggests a lack of antagonism and possibly even a symbiotic relationship between them. Such cases of yeast-LAB interaction have been reported in other products such as *kefir* and *koumis* (Roostita and Fleet, 1996). *Candida krusei* and *Saccharomyces cerevisiae* together with LAB were found to be the predominant microorganisms during the fermentation of *busaa*, a Kenyan opaque maize-millet beer (Nout, 1980). *Saccharomyces* yeasts have been found to stimulate the growth of other microorganisms, including lactic acid bacteria, by providing essential metabolites such as pyruvate, amino acids and vitamins. On the other hand, *S.cerevisiae* has been reported to utilize certain metabolites as carbon sources (Gadaga, *et al.,* 2001; Leroi *et al.,* 1993). However, the mechanisms have not been described in

detail. Similar associations have been found in other traditional fermented foods such as *ogi-baba*, *ogi*, *mawe* and *kenkey* (Odunfa and Adeyele; 1985; Adegoka and Babalola, 1988; Halm *et al.*, 1993; Hounhouigan *et al*., 1993). Inhibition of *Enterobacteriaceae* and moulds can be attributed to the low pH, high acid and presence of alcohol. However, there is need for further investigation of the basis for interaction between the different categories of microorganisms in this product, in order to be able to simulate the characteristics of this product using starter cultures.

2.3.2.6. Yeast identification

Eleven species of yeast that belonged to the genera *Debaryomyces, Dekkera, Candida, Rhodotorula, Saccharomyces and Octosporomyces* were isolated and identified. *Candida ethanolica, C. haemuloni, C. sorbophilila, Dekkera anomala, Dekkera bruxellensis Saccharomysis capsularis* and *Saccharomyces cerevisiae* were the predominant yeast species isolated throughout the production. They survived the various stages of the production process and became established in the beer as part of the microbial ecology. Some of the yeasts isolated in this study are similar to those found in other traditional fermented foods such as *merrisa*, *ogi*, *fufu*, *khamir*, *takju boza* and *busaa* (Dirar, 1978; Hancioglu and Karapiner, 1997; Lee, 1997; Gassem, 1999). Sorghum malt and maize have also been shown to be contaminated by such yeast species (Van der Walt, 1956; Novellie, 1968). *Saccharomyces cerevisiae* is commonly used in the production of fermented alcoholic beverages (Beauchat, 1987). However, *Debaryomyces hansenii, Rhodotorula minuta* and *Octosporomyces octosporus* are not commonly used in beer fermentation but may occur in spontaneously fermented foods as contaminants from the air, hands or utensils used.

2.4. CONCLUSION

This study has shown that the initial microbiota of *umqombothi* is made up of lactic acid bacteria (LAB), yeasts, moulds and enterobacteriaceae. Associations of lactic acid bacteria (LAB) and yeast were found to be responsible for fermentation of *umqombothi*. It has also been established that no matter where *umqombothi* is produced, hygienic practices being observed, the growth pattern of the microbiota is basically the same since there was no significant differences among the products obtained from the three different sites. Yeast genera associated with the production of spontaneously fermented *umqombothi* include *Candida, Debaryomyces, Dekkera, Octosporus, Rhodotorula and Saccharomyces.* In order to maintain and sustain African indigenous fermented foods and beverages, improved control of fermentations and product characteristics is strongly recommended, including the use of purified starter cultures with appropriate technological properties.

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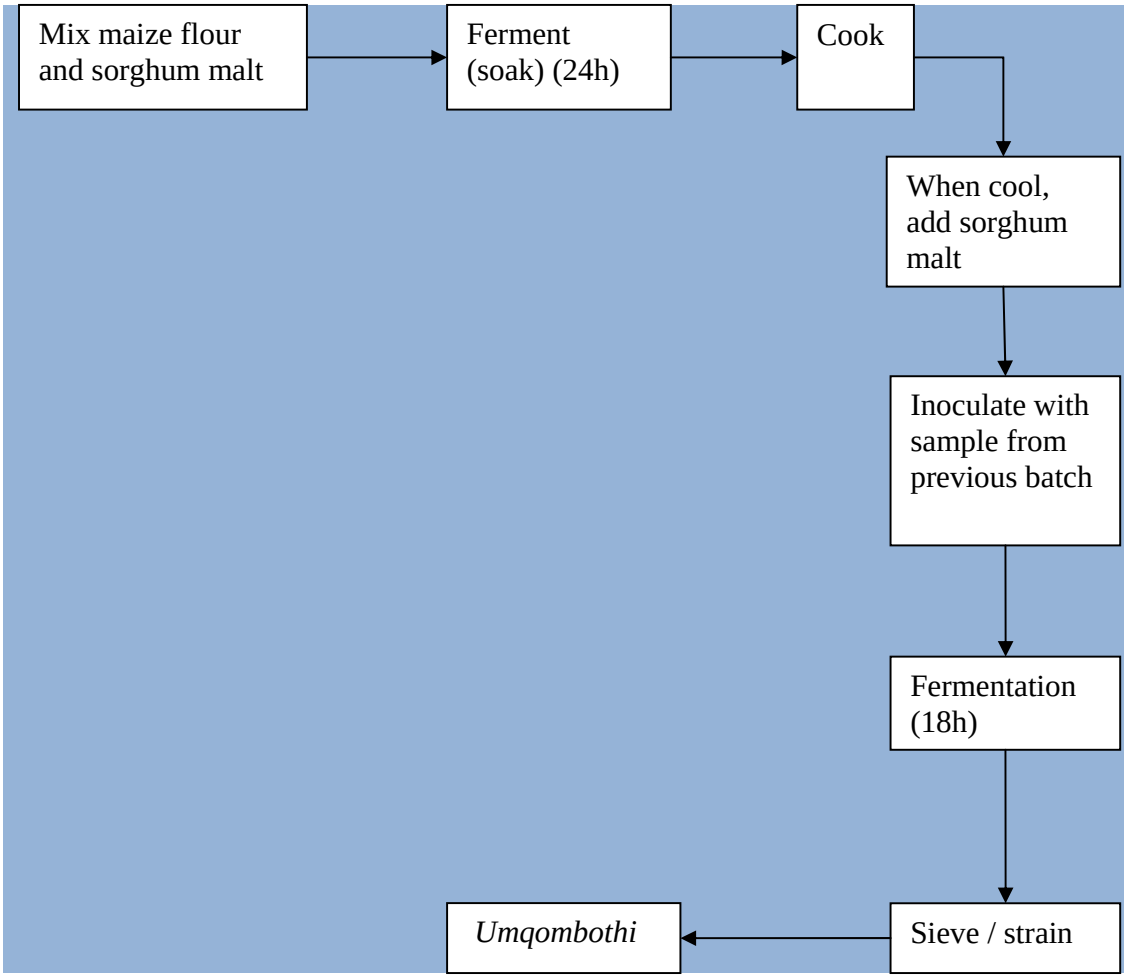

Fig. 1: Flow diagram for the production of *umqombothi*

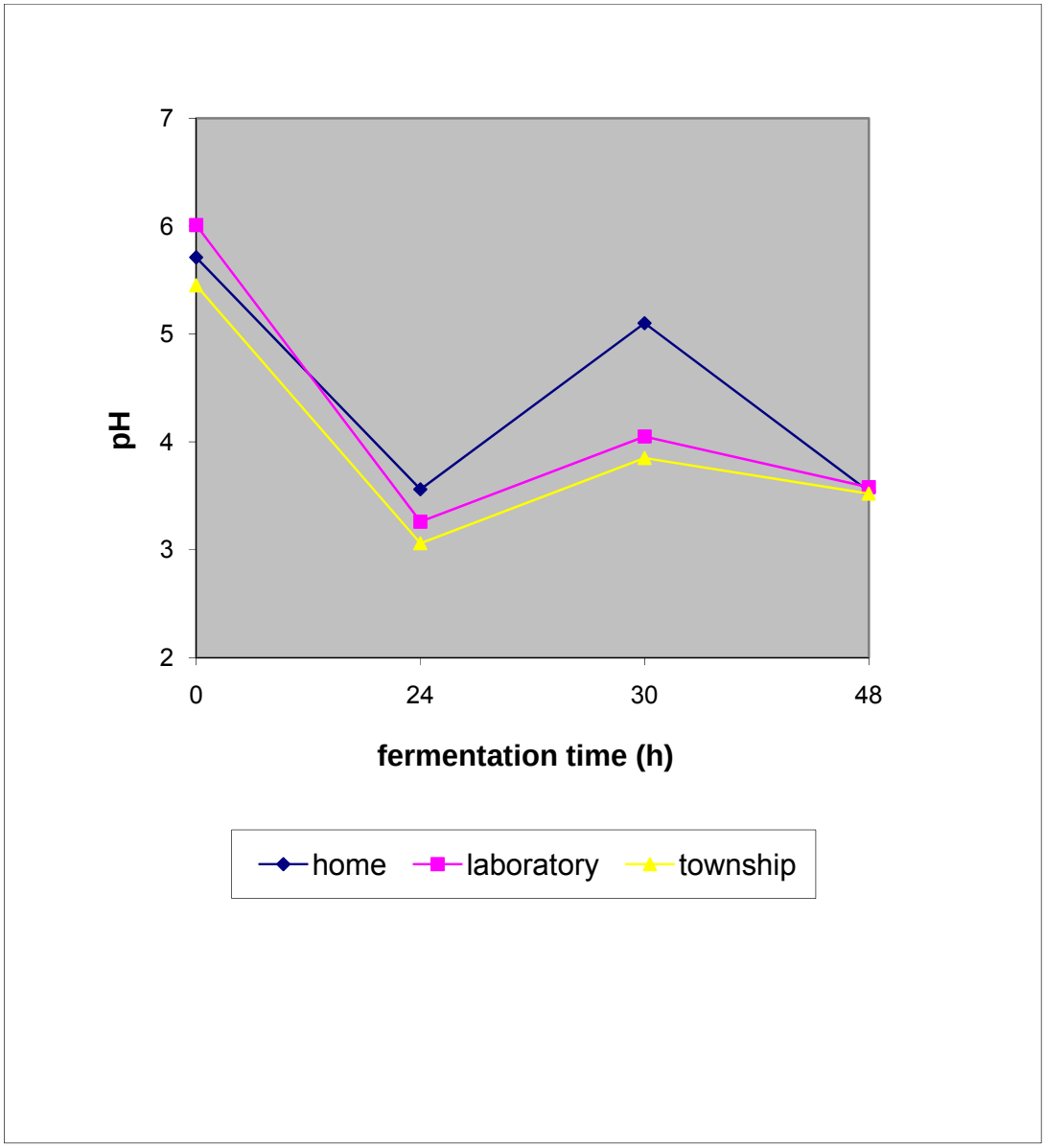

Fig. 2: Changes in the pH of home, laboratory, and township samples during *umqombothi* fermentation. Home (sample A), Laboratory (sample B), Township (sample C)

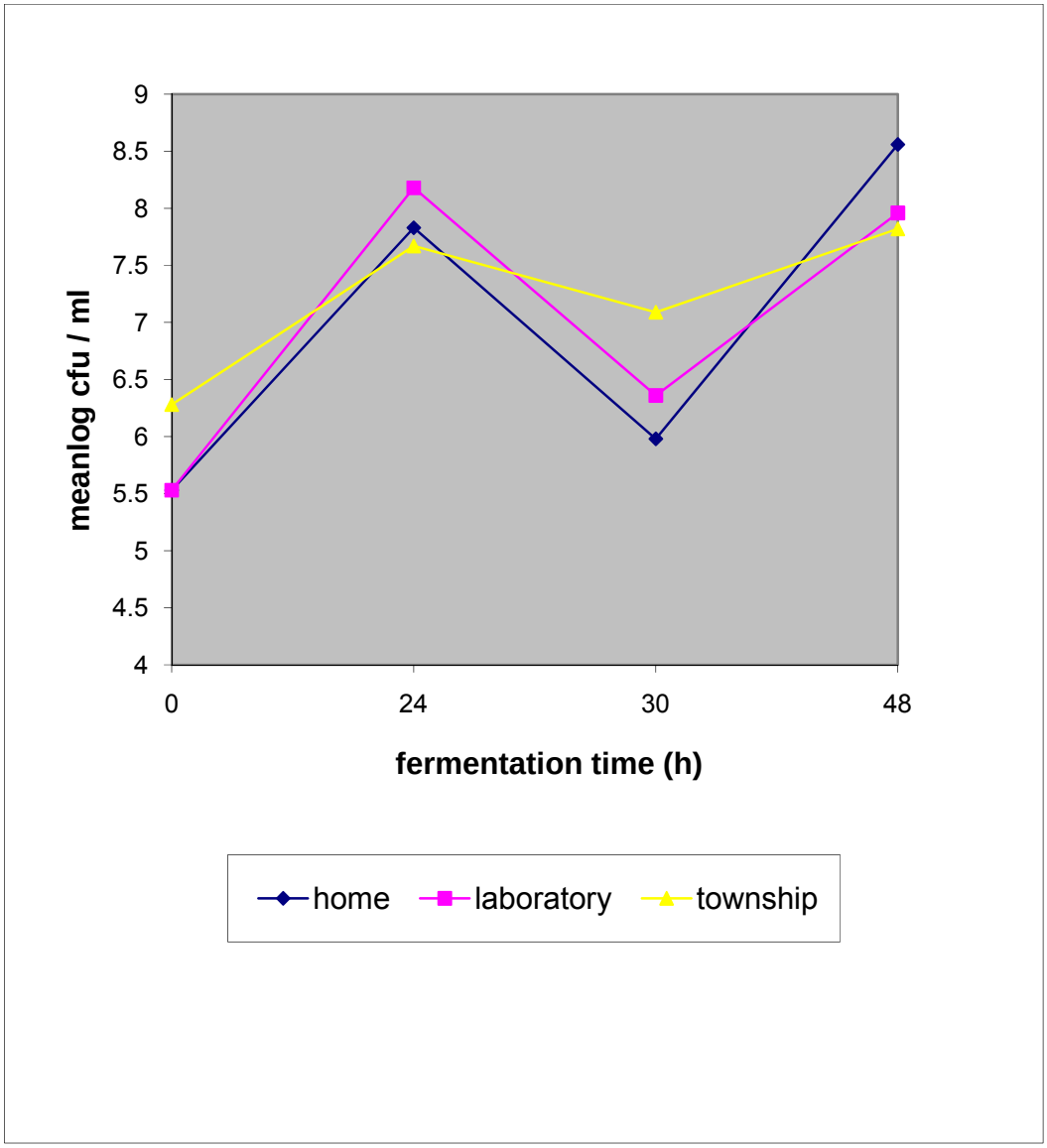

Fig. 3: Changes in LAB counts for the home, laboratory and township samples during *umqombothi* fermentation. Home (sample A), Laboratory (sample B), Township (sample C)

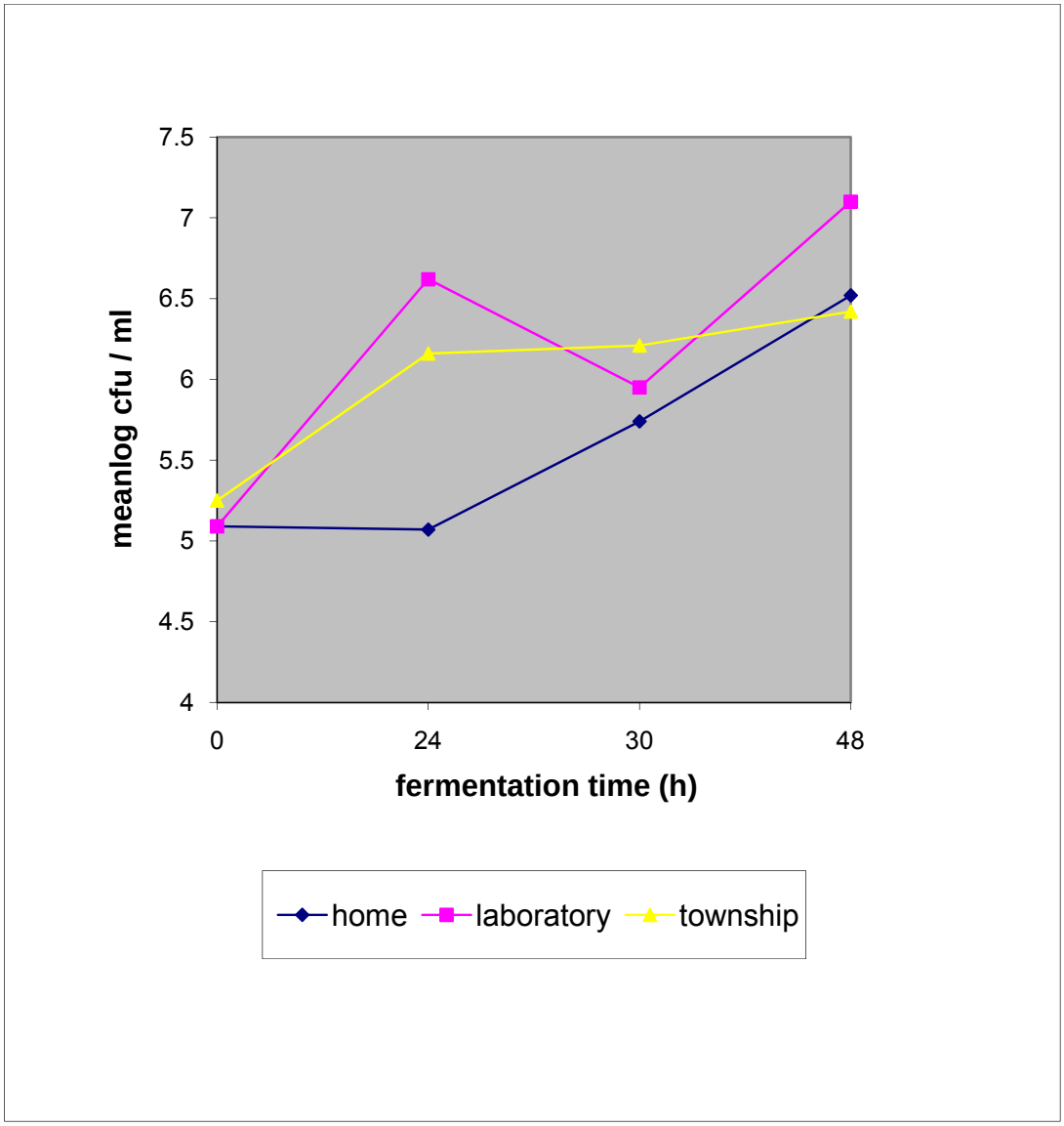

Fig. 4: Changes in yeast counts for the home, laboratory and, township samples during *umqombothi* fermentation. Home (sample A), Laboratory (sample B), Township (sample C)

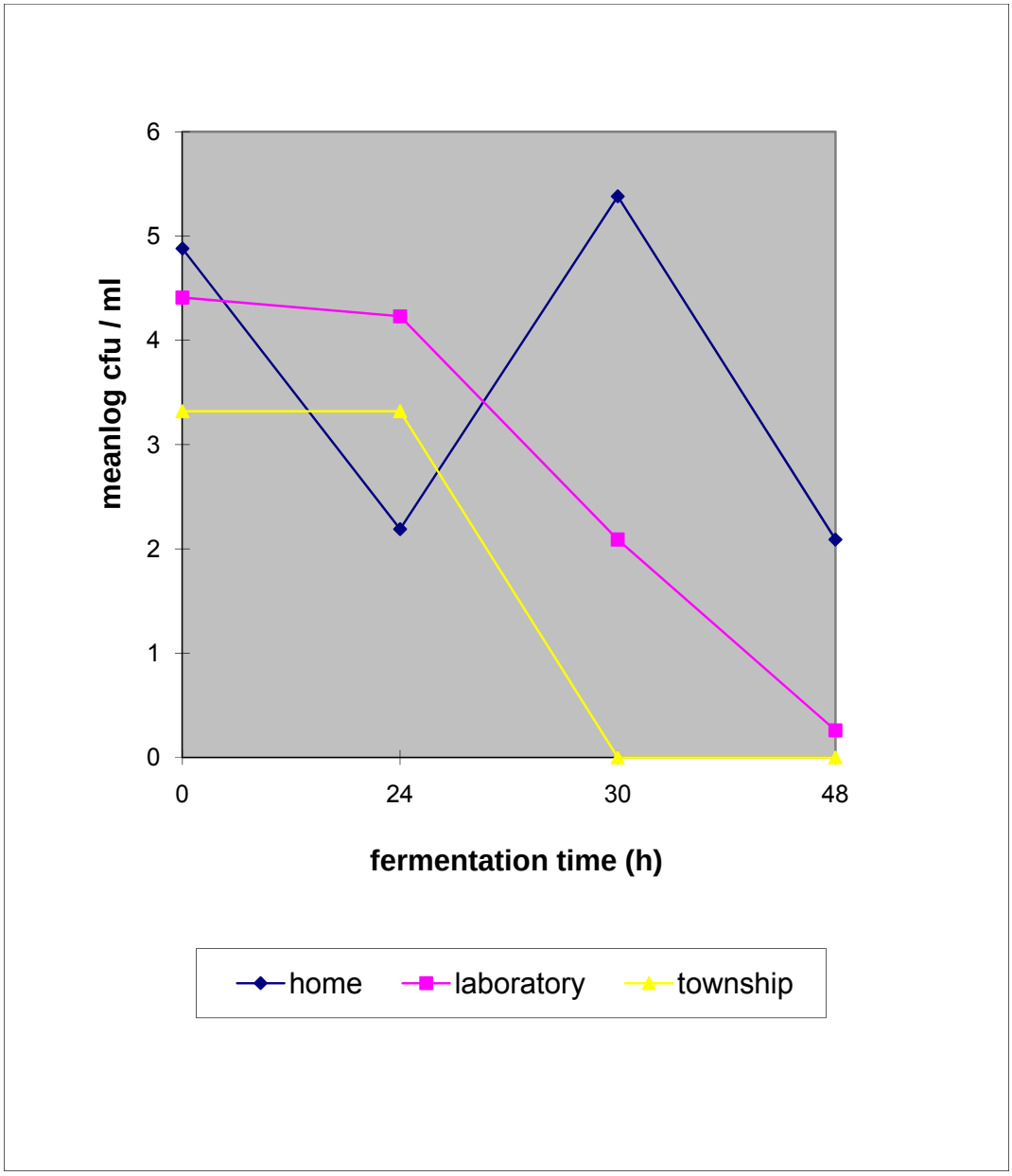

Fig. 5: Changes in enterobacteriaceae counts for the home, laboratory, and township samples during *umqombothi* fermentation. Home (sample A), Laboratory (sample B), Township (sample C)

Chapter 3

Microbiological ecology of *Mahewu*, a traditionally fermented non-alcoholic beverage

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ABSTRACT

The microbiological changes taking place in laboratory produced *mahewu* (A) and that produced in a private home (B) were investigated during the production process. Lactic acid bacteria (LAB) counts increased from 3.09 to 7.19 log cfu / ml and 3.25 to 6.28 log cfu / ml for A and B, respectively after 3 days. There was no significant difference between LAB counts and total aerobic mesophilic counts ($P<0.05$). Yeast counts increased from 1.49 to 5.81 log cfu / ml and 3.47 to 5.58 log cfu / ml for A and B, respectively. The yeasts were isolated and identified. The predominant strains were *Candida haemuloni, Candida sorbophila, Debaryomyces hansenii, Saccharomyces capsularis and Saccharomyces cerevisiae*. Yeast growth was shown to have an inhibitory effect on mould growth. Mould counts decreased from 2.2 log cfu / ml to below detection level after the third day of fermentation. Enterobacteriaceae counts initially increased from 0.22 to 6.64 log cfu / ml thereafter decreasing to non detectable levels by the end of the fermentation period. The pH decreased from 6.09 to 3.8 and 6.24 to 3.83 for samples taken from A and B, respectively. Lactic acid bacteria (LAB) and yeasts were found to be the predominant microorganisms in *mahewu* which is similar to results found in the literature on similar indigenous cereal-based fermented foods.

Keywords: fermented beverage, *mahewu***,** yeasts, lactic acid bacteria

3.1. INTRODUCTION

Fermentation of food is one of the oldest methods of food preparation and preservation (Pederson, 1971; Steinkraus, *et al.*, 1983; Campbell-Platt, 1994). Fermented beverages constitute a major part of the diet of traditional African rural homes. They have a role in social functions such as marriage, naming and rain making ceremonies, where they are served as inebriating drinks and weaning foods (Hounhouigan, 1994). Fermented foods have been noted for their superior nutritional value and digestibility compared to the unfermented counterpart. Fermentation of cereals such as maize, millet, sorghum and rice, results in improved protein quality, especially the level of available lysine (Hamad and Fields, 1979; Padhye and Salunkhe, 1979). Fermentation also has the advantage of improving organoleptic properties by producing different flavours in different foods (Khetarpaul and Chauhan, 1993; Sarkar and Tamang, 1994; Steinkraus, 1994). Spoilage and pathogenic microorganisms are inhibited by the production of organic acids, hydrogen peroxide, antibiotic-like substances and the lowering of oxidation-reduction potential (Cooke *et al*., 1987; Nout *et al*,. 1989; Mensah *et al*,. 1991; Kingamko *et al*., 1994; Lorri and Svanberg, 1994; Nout, 1994; Tanasupawat and Komagata, 1995).

Mahewu is the name of a maize based non-alcoholic beverage produced and consumed by many people in Southern Africa (Holzapfel, 1989; 1991). 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 '*maphulo'* (Coetzee, 1982). The most commonly used term is *mahewu*. The product is prepared by mashing left over pap (pap is a name used in South Africa in reference to cooked maize meal or porridge) into a slurry that is then fermented overnight (Gadaga *et al*., 1999). It may also be prepared by cooking thin maize porridge containing 8-10 % solids and fermenting it at room temperature. Sorghum, millet malt or wheat flour can be added and provides the inoculum (Holzapfel, 1989). *Mahewu* is popular

during the hot summer months due to its refreshing sour taste and thirst quenching ability. According to Wehmeyer (1962) about 4000 kJ of energy is produced from one liter of *mahewu*. However, like many cereal products, it is deficient in essential amino acids (Dryer and Schweigart, 1961). The protein content and biological value can be improved by the addition of skim milk powder, whey protein, soy flour, food yeast or fishmeal flour (Schweigart *et al.*, 1960). Earlier studies have shown that the microorganisms responsible for the fermentation of mahewu are *Lactococcus lactis subsp. lactis* (Hesseltine, 1979; Steinkraus, 1996), and yeasts (Holzapfel, 1989), and should therefore play an important part in the fermentation process. This product has also been shown to have antibacterial effect (Simango *et al.*, 1991; 1992). Lactic acid bacteria have been shown to have an inhibitory effect on aflatoxin production by *Aspergillus sp*. (Maing *et al.*, 1973). Some bacteria also have the ability to remove some mycotoxins from food commodities, such as apple juice (Doyle *et al.*, 1982). However, not much work has been done on the detoxification properties of yeasts. The aims of this study therefore are to monitor the microbial changes occurring during the production of *mahewu.*

3.2. MATERIALS AND METHODS

3.2.1. Production of *mahewu*

Maize meal (40g) was soaked in water for 24 h and then cooked into porridge with 8-10 % solids. The porridge was cooled and allowed to ferment at temperatures between 25 – 30 ^{o}C for 3 days. This was the basic procedure followed for preparation of *mahewu* in the laboratory (Sample A) and that prepared at home (Sample B).

3.2.2. Sampling for microbiological analysis

Nine batches of *mahewu,* from the same batch of maize meal, were made using the method described above. Aliquots (40ml) of the home-made and laboratory-made *mahewu* were taken after 0, 1, 2 and 3 days of fermentation and used for microbiological analysis. Sampling was done using a sterile beaker, and then transferred to a sterile sampling bottle. Thereafter the samples were transported to the laboratory in a cooler box.

3.2.3. Microbiological analysis

A portion (10ml) of each of the *mahewu* samples was serially diluted using Peptone physiological salt solution (Oxoid, Basingstoke, UK). Appropriate dilutions (1ml) were made and 0.1ml of the dilutions then spread plated in duplicate on Plate Count Agar (Oxoid, Basingstoke, UK) and incubated at 25 ^{o}C for 48 h for the enumeration of aerobic mesophilic bacteria. Lactic Acid Bacteria were enumerated by spread plating on MRS agar (Biolab, Gauteng, RSA) and incubating at 25 ^{o}C for 48 h. Yeasts and moulds were enumerated on spread plates of Potato Dextrose Agar (Detroit, Michigan, USA) (containing 0.01 % w/w chloramphenicol) and incubating at 25 ^{o}C for 96 h. Enterobacteriaceae were

enumerated on MacConkey agar (Biolab, Gauteng, RSA) after incubating for 96 h at 37 ^{o}C.

3.2.4. Isolation and identification of yeasts

Yeast isolation and identification were performed by following morphological, physiological and cultural characterizations. Representative colonies from each PDA plate were picked and purified by streaking on fresh PDA plates. The pure cultures were stored on Malt Extract agar slants (Biolab, Gauteng, RSA) at 4 ^{o}C. Strains were then identified to the species level according to the conventional identification method outlined by Kreger-van Rij (1984), Barnett *et al*. (1990) and Kurtzman and Fell (1998). Briefly, each isolate was inoculated into six fermentation media (glucose, fructose, lactose, maltose, raffinose, galactose), 35 carbon assimilation media, vitamin free medium, 0.01 % and 0.1 % cyclohexamide. Assimilation of nitrogen compounds was performed by means of an auxonographic method (Lodder and Kreger-van Rij, 1952).

Additional tests performed included growth at 37 ^{o}C, growth in 50 % D-glucose medium, hydrolysis of urea, starch hydrolysis, acetic acid formation, and staining of 4-week old cultures with Diazonium blue B salt reagent (Van der Walt and Hopsu-Havu, 1976). Ascospore formation was examined on McClary's acetate agar, potato glucose agar, Gorodkowa agar, corn meal agar and malt extract agar (Kreger-van Rij, 1984). The formation of pseudomycelium and true mycelium was examined on corn meal agar according to the Dalmau plate technique (Wickerham, 1951).

Confirmation of the yeast identity was done using sequence analysis of the D1/D2 domain using primer pairs NL-1(5'-GCATATCAATAAGCGGAGGAAAAG) and NL-4(5'-GGTCCGTGTTTCAAGACGG) (Kurtzman and Robert, 1998). Sequencing reactions were carried out using the ABI Prism^{TM} Big Dye

Terminator™ v3.1 cycle sequencing ready reaction kit. Data was collected on an ABI Prism 377 DNA sequencer (Applied Biosystems, USA) and analysed using sequencing analysis V3.3 and sequences assembled using Auto-assembler V1.4.0.

3.2.5. Chemical analysis

The pH of each sample was measured at room temperature using a CyberScan 500 pH meter (Eutech Instruments, Singapore). A portion of each sample (5 ml) was centrifuged at 11,000 rpm for 5 min in a Beckman J2-21centrifuge (Beckman, USA). The supernatant was collected and used for lactic acid determination by liquid chromatography (Waters Lambda-Max 480) with a high performance exclusion column (Bio-Rad Aminex APX-87H) and 0.01 N sulphuric acid as the mobile phase. The flow rate was 0.5 ml/min and detection was by a UV detector at 210 nm (Bouzas *et al*., 1991).

3.2.6. Statistical analysis

Samples were compared using the analysis of variance (ANOVA) procedure (NCSS, 2004).

3.3. RESULTS AND DISCUSSION

3.3.1. Chemical analysis

The pH of the laboratory-made (A) and the home-made (B) products decreased from 6.09 to 3.8 and from 6.24 to 3.83 respectively after three days of fermentation (Fig. 1). As lactic acid bacteria proliferated, acidity increased, presumably due to fermentation of sugars (Jay *et al*., 2005). The acidity that developed, as illustrated in Fig. 2, favoured the growth of yeasts which subsequently multiplied rapidly as shown in Fig. 3, (Bvochora *et al*., 1999). The results are similar to those reported by other researchers working with *mahewu* and similar products (Simango *et al.,* 1992; Jespersen *et al*., 1994; Gotcheva *et al*., 2001). The reduction in pH is desirable because the growth of undesirable spoilage and pathogenic microorganisms is minimised at low pH (Halm *et al.*, 1993).

Addition of water during cooking after 24 h of soaking leads to an increase in the pH of the sample as shown in Fig. 1. This is because water, as the solvent in this case, acts as the base in the acid-base reaction that results in the dissociation of the lactic acid (Barret, 2003), hence the observed increase in pH at this stage of the fermentation process.

3.3.2. Microbial analysis

3.3.2.1. Lactic acid bacteria (LAB)

Fig. 3 shows the microbial composition of *mahewu*. Lactic acid bacteria and the yeasts were the predominant microorganisms in the *mahewu* at the end of the fermentation. Similar to trends in other fermented products, total aerobic mesophilic counts were not significantly different from that of the LAB in both samples ($P<0.05$) (Hounhouigan, 1994). The highest LAB counts were obtained after fermenting the porridge for a day, that is, LAB counts increased from 3.09 to

7.56 log cfu / ml, and 3.25 to 7.85 log cfu /ml for samples A and B, respectively. Pattison *et al.,* (1998) reported high numbers of LAB in commercial sorghum beer and that this 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, 1998).

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*. 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.

3.3.2.2. Yeasts

The highest numbers of yeasts were recorded on the last day of fermentation (Fig. 3). Yeast counts had increased significantly by the end of production ($P<0.05$). At the end of fermentation, there was no significant difference ($P<0.05$) between the numbers of yeasts obtained from samples A and B, which had increased from 1.49 to 5.81 log cfu / ml, and 3.47 to 5.58 log cfu / ml respectively. This is in agreement with observations in similar products (Akinrele 1970; Nyarko and Obiri-Danso 1992; Hounhouigan *et al*., 1992; Halm *et al*., 1993; Nche *et al*., 1994). The high numbers of yeasts observed in this study suggest that these microorganisms would make an important contribution to the organoleptic and structural quality of the drink (Janssens *et al*., 1992). Yeasts grow in association with bacteria during food fermentation and their by-products, such as alcohols, aldehydes, esters, and lactones, add pleasant flavour to the food (Janssens *et al.,* 1992).

3.3.2.3. Moulds and Enterobacteriaceae

Mould counts decreased from log 2.2 cfu / ml to non-detectable levels after a day of soaking (Fig. 3) and this could be attributed to the observed decrease in pH (Fig. 1). 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 10^5 cfu / g of mould counts, found in raw maize, are reduced to less than 10^2 cfu / g within 24 h of fermentation.

Similarly, enterobacteriaceae numbers decreased to non-detectable levels after 3 days of fermentation (Fig. 3). Mensah (1997) found the growth of *Shigella flexneri* and enterotoxigenic *Escherichia coli* in fermented maize dough was inhibited compared to growth in unfermented dough. The mechanisms behind the growth inhibition were unclear but likely to involve the decreased pH of the fermented dough and the production of antimicrobial compounds.

3.3.3. Microbial succession during the production of *mahewu*

During this fermentation to produce *mahewu*, there was an increase in numbers of aerobic mesophilic bacteria, lactic acid bacteria and yeasts as shown (Fig. 3). Predominant microorganisms involved in this production were lactic acid bacteria and yeasts. This is similar to results reported by Bvochora *et al.* (1999) during the fermentation to produce *mahewu* from sorghum. The association between the yeasts and LAB are important in the production of fermented foods (Wood and Hodge, 1985). Yeasts and LAB coexist in symbiotic association where the yeasts thrive on the lactic acid produced by the bacteria resulting in the bacteria living longer because the yeasts prevent the accumulation of a toxic concentration of lactic acid (Wood, 1981). A similar association is thought to be important in *mahewu* as shown by the microbial succession in Fig. 3. *Saccharomyces* yeasts have been found to stimulate the growth of other microorganisms, including lactic acid bacteria, by providing essential metabolites such as pyruvate, amino acids

and vitamins (Jespersen, 2003). On the other hand, *S. cerevisiae* has been reported to utilize certain bacterial metabolites as carbon sources (Gadaga *et al*., 2001; Leroi and Pidoux, 1993). Associations of lactic acid bacteria (LAB) and yeast were found to be responsible for *boza* fermentation (Gotcheva *et al.*, 2001), which agreed with similar results for other indigenous cereal based fermented foods such as Kenyan *busaa*, kaffir beer, Nigerian *ogi*, *pito*, *sekete* and *busa* (Adegoke *et al*., 1995; Beuchat, 1995; Pederson, 1979; Sanni, 1993; Steinkraus, 1977).

Christian (1970) analyzed fermented maize dough at the advanced stage and found that a mixture of LAB and yeasts predominated the microbiota. *Candida krusei* and *Saccharomyces cerevisiae* were found together with LAB during the fermentation of busaa, a Kenyan opaque maize-millet beer (Nout 1980). Odunfa and Adeyele (1985) found *Lactobacillus spp.* and *Lactococcus lactis* together with *C. krusei* and *Debaryomyces hansenii* during the fermentation of *ogi-baba*, a West African fermented sorghum gruel. Adegoka *et al.* (1988) found *S. cerevisiae, Lactobacillus fermentum* together with *L. brevis* in *ogi*, while Akinrele (1970) found that *Corynebacterium, Saccharomyces cerevisiae and L. plantarum* were dominant in *ogi*. Halm *et al.* (1993) found obligate heterofermentative lactobacilli closely related to *L. fermentum* and *L. reuteri* in association with yeasts dominated by *Candida spp.* and *Saccharomyces cerevisiae* in fermented maize dough from Ghana. These same bacteria and yeasts were found by Hounhouigan *et al*. (1993) in *mewe*, a fermented maize dough from Benin.

3.3.4. Yeast identification

The range of yeast species isolated from both production sites was similar, and the predominant ones were *Candida haemuloni, C. sorbophila, Debaryomyces hansenii, Saccharomyces capsularis, Saccharomyces cerevisiae*. *Candida* strains occurred in higher numbers. *Candida spp., Debaryomyces hansenii* and *Saccharomyces spp.* especially*, Saccharomyces cerevisiae* strains, are

commonly associated with fermented maize products (Christian, 1970; Nout, 1980; Adegoka and Babalola, 1988; Halm *et al*., 1993; Hounhouigan *et al*., 1993).

3.3.5. Effect of growth of yeasts on the survival of *Aspergillus parasiticus*

The growth effect of yeasts isolated from *mahewu* on the growth of *Aspergillus parasiticus* is shown in Table1. *S. cerevisiae* was the only yeast species among those identified in *mahewu* that was antagonistic towards growth of *Aspergillus parasiticus.* Jespersen *et al*., (2003) reported that *S.cerevisiae* together with *C. krusei* had an inhibitory effect on the growth of mycotoxin-producing moulds such as *Penicillium citrinum, Aspergillus flavus* and *Aspergillus parasiticus*. The inhibitory effects of the yeasts were mainly due to substrate competition, but inhibition of spore germination might also have occurred due to production of high concentrations of organic acids. In this study, the observed inhibition of *A. parasiticus* by *S.cerevisiae* may be due to similar factors.

3.4. CONCLUSION

Lactic acid bacteria (LAB) and yeasts have been shown in this study to be the main fermenting microorganisms during the production of *mahewu*. They form the microbial ecology of the drink. Species of the genera *Candida, Saccharomyces* and *Debaryomyces* are the yeasts that thrived in *mahewu* with LAB. It was observed that one of the yeast species, *S.cerevisiae,* when inoculated on PDA plates with aflatoxin producing *Aspergillus parasiticus* was inhibitory to its growth. Moulds and enterobacteriaceae were found in the initial stages but did not survive the environmental conditions that developed in the beverage during the later stages of production.

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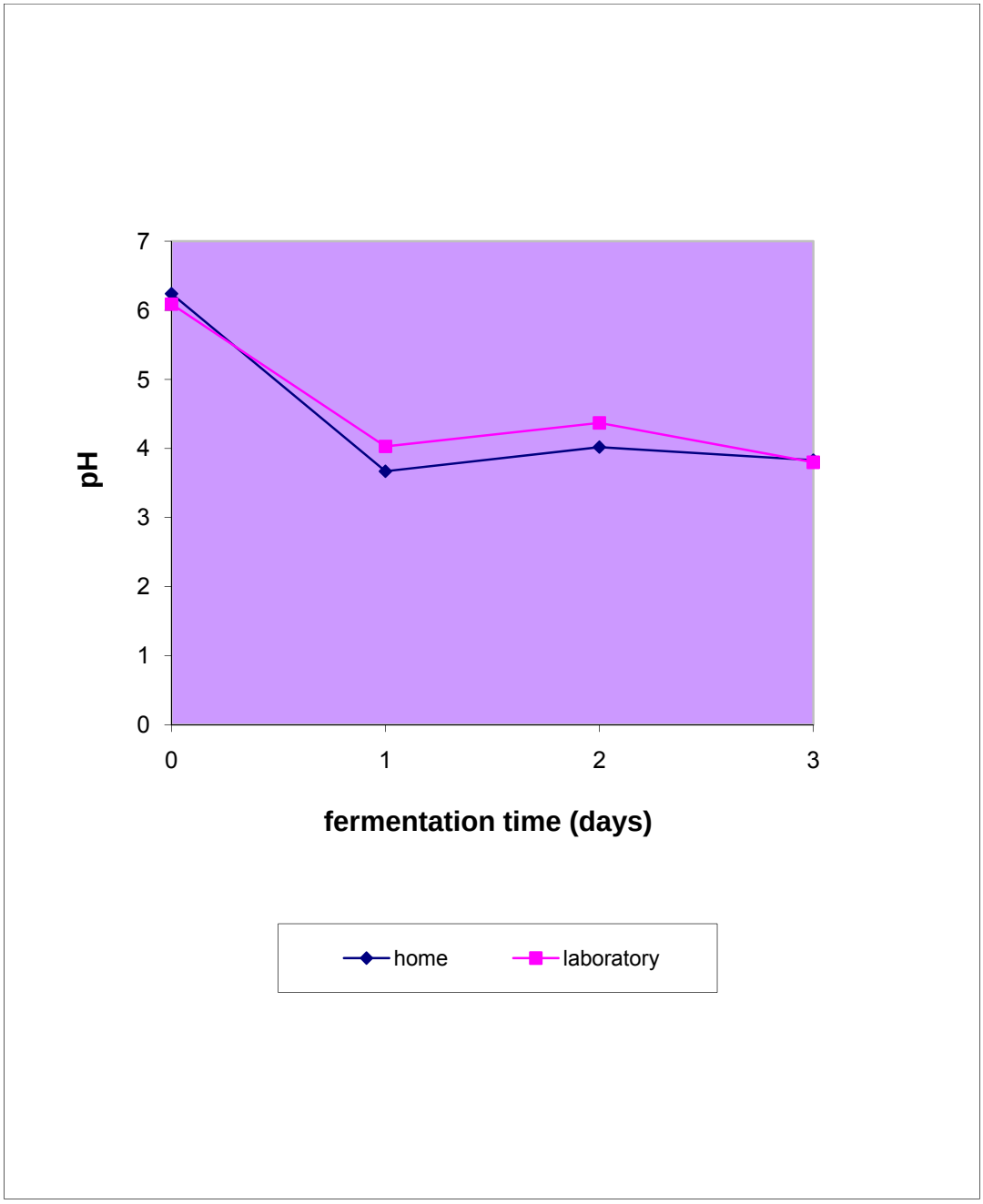

Fig. 1: Changes in the pH of samples A and B during *mahewu* fermentation. Sample A (laboratory), Sample B (home)

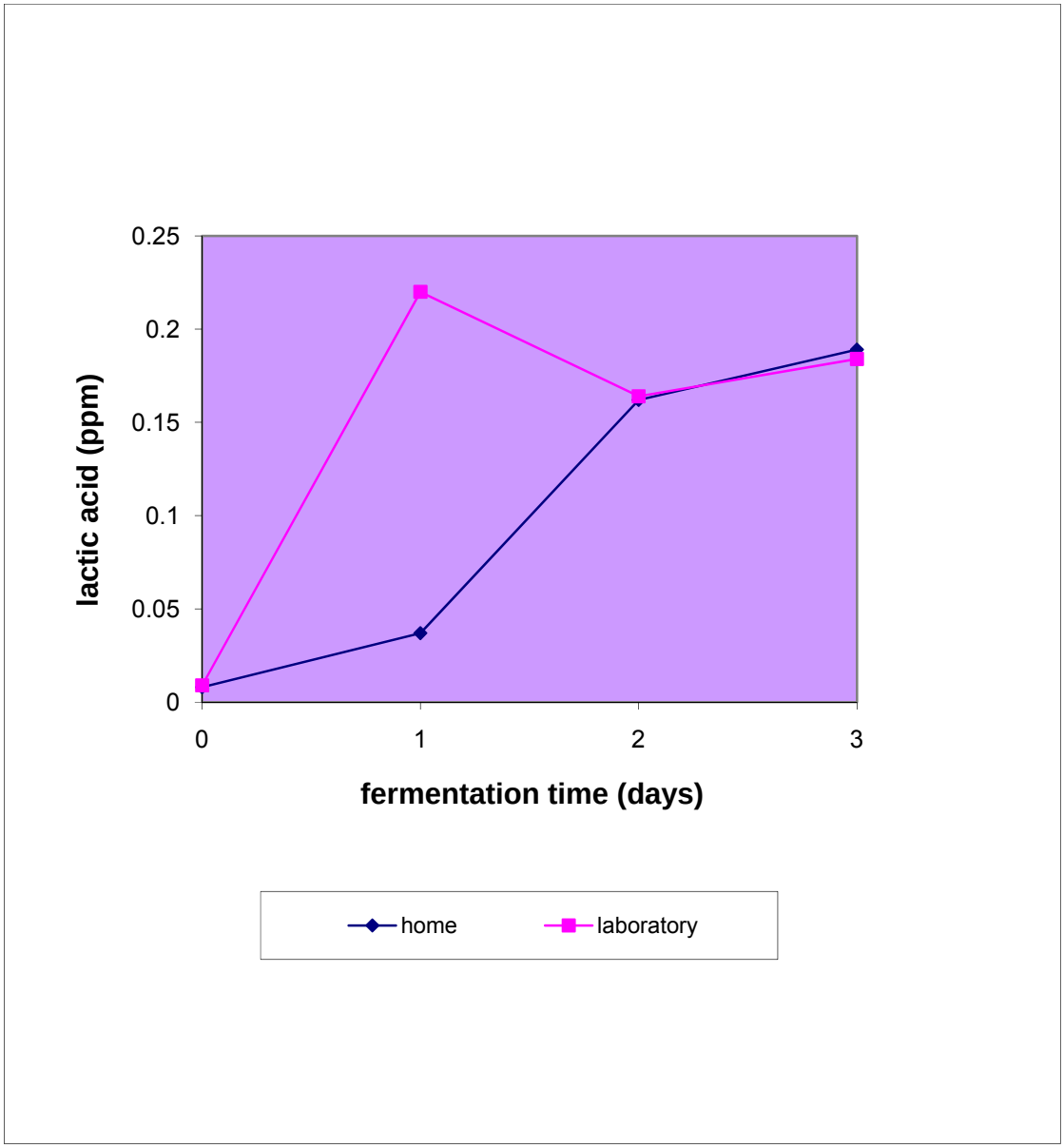

Fig. 2: Changes in the lactic acid concentration of samples A and B during *mahewu* fermentation. Sample A (laboratory), Sample B (home)

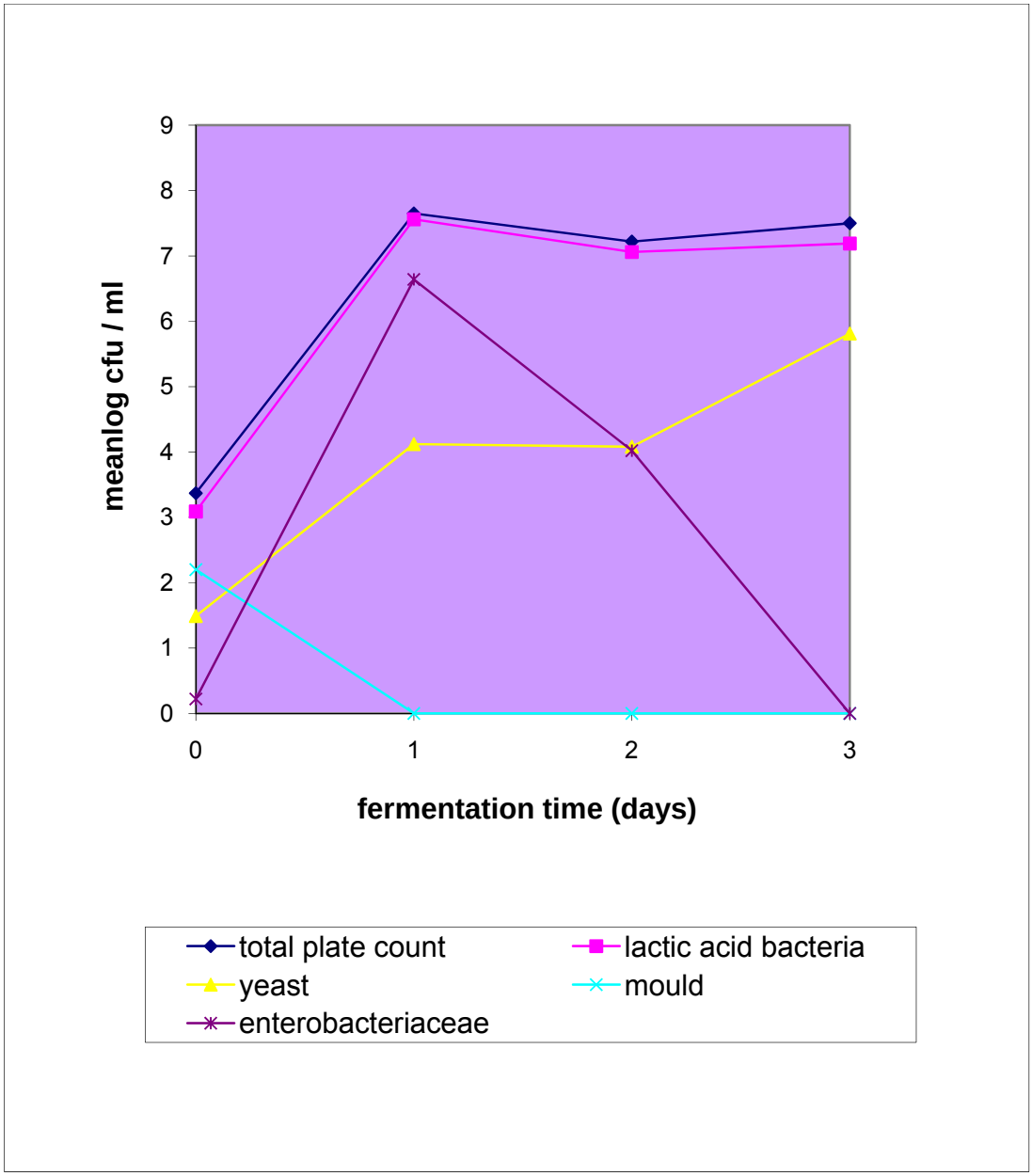

Fig. 3: Microbial interactions during *mahewu* fermentation

Table 1; Effect of yeasts isolated from *mahewu* on growth of *Aspergillus parasiticus.*

Yeast	Effect
Candida haemuloni	-
Candida sorbophila	-
Debaryomyces hansenii	-
Saccharomyces capsularis	-
Saccharomyces cerevisiae	+

Chapter 4

Scanning electron microscopy of the biofilm of an earthen ware pot used for cereal fermentation

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ABSTRACT

Local beer (*umqombothi*) is made in many South African townships by putting maize flour, sorghum malt and water into a pot and fermenting it in a warm spot for a few days, occasionally stirring and adding more liquids to it. As is the custom, specific pots are used for several repeats of fermenting these beverages separately. It is assumed that a biofilm remains as inoculate after each session.

Pieces were broken off the sides of such an earthen ware pot that had been used on several occasions for the cereal fermentation of a local beverage. These pieces were then immersed into another fermentation pot for the duration of a *umqombothi* fermentation after which the biofilm on the interior surfaces of the pot pieces was analysed using scanning electron microscopy. Standard scanning electron microscope preparation techniques were applied on clay pot shards covered with the fresh *umqombothi*. A problem encountered was the size and porous nature of the shards that hampered dehydration and critical point drying. Fairly large pieces had to be used so as not to disturb the biofilm when breaking larger shards smaller.

The *umqombothi* residue had a dense appearance and formed a thick crust on the shards. A high concentration of yeast cells were found on the *umqombothi* shards and a comparatively lower concentration of bacteria were observed. The thick crust of *umqombothi* residue remained attached to the shard representing the biofilm of the beverages and will most probably be a sufficient and viable inoculate for starting the next fermentation and brewing processes.

The purpose of this study was to find out whether biofilm formation takes place within such pots and if so, to find out as well which microorganisms are involved. The images obtained confirmed that biofilm formation occurs and that yeasts and bacteria were the dominant, if not the only microorganisms, within this particular environment. The proximity observed between the yeasts and bacterial cells has

added weight to the suggestion of a symbiotic relationship existing between yeasts and bacteria in indigenous cereal fermentations.

Keywords; Scanning electron microscopy, biofilm, yeasts, bacteria, symbiotic relationship

4.1. INTRODUCTION

In order that we may gain greater insight into the ecology of the microorganisms that exists in biofilms, it is necessary not only to be able to isolate them by traditional culture methods but also to have some understanding of the way in which these individual microorganisms interact in situ in their environment. Microscopical examination of entire biofilms enables us to increase our understanding of the spatial organisation that occurs within them and on the surfaces supporting their development (Lawrence *et al*., 1991). The information that may be gained from various microscopy techniques includes quantification (Denyer and Lynn., 1987); for example, measurement of the depth of the depth of biofilm (Bakke and Olsson., 1986), also labelling of cell surface components and the identification of specific species within the biofilm consortia (Rogers and Keevil, 1992). A distinction between viable and non-viable cells using vital stains can also be achieved (Rodriguez *et al*., 1992). The ability to visualize the biofilm and the supporting surface simultaneously can demonstrate not only the heterogeneity which exists within biofilms but also the biodeterioration of the material supporting the microbial growth (Surman *et al.,* 1996).

Bacterial biofilms are generally described as surface associated bacterial community forming microcolonies surrounded by a matrix of exopolymers (Costerton and Lewandowski, 1995). Microbial biofilms can exist as aggregates more or less confluent, single layer mat or three-dimensional architecture with channels allowing liquid and gas flow and dispersion of nutrients and waste components (Stoodley *et al*., 2002). Such structures can develop on many abiotic and biotic surfaces (Lasa *et al*., 2005; Chavant *et al*., 2007).

Microorganisms grow on surfaces enclosed in biofilms. The biofilm traps nutrients for growth of the microbial population and helps prevent detachment of cells on surfaces present in flowing systems. Biofilms typically contain many layers, and microscopic examination of the microorganisms in each layer can be

done using scanning laser confocal microscopy (Madigan *et al*., 2003). Surfaces are important microbial habitats because nutrients can adsorb to them. In the microenvironment of a surface, nutrient levels may be much higher than they are in the bulk solution. As a consequence, microbial numbers and activities are usually much greater on surfaces than in water (Madigan *et al*., 2003).

The biological materials to be examined are usually living specimens or sometimes one would like to examine a complete intact organism. Living tissue can't be examined in the electron microscope due to the high vacuum in the microscope. The vacuum is needed to allow the movement of the electrons in a beam. The result of the vacuum is that the water in biological material evaporates, damaging the specimen. The electron beam (as light source) can also damage the specimen by radiation and heating. To examine a specimen in the microscope, the specimen must be preserved as close as possible to its original living state as possible. The material must be prepared in such a way that it can be dried and mounted, or if required, sectioned. The preparation of the specimen is known as fixation and the 'drying' as dehydration. Scanning electron microscopy allows the visualisation of complex structures at high magnification and thus is the method of choice in the current study.

4.1.1. The scanning electron microscope

4.1.1.1. The electron gun and lens system

The microscope is designed so that the 'light' source (electron gun) is at the top of the lens column. The lens system consists of two condenser lenses focussing the electron beam into a specific area in the lens aperture of the objective lens. The objective lens again focuses the electron beam on the specimen to obtain a focussed image. In the lens aperture of the objective lens is also a diaphragm to eliminate spherical aberration. Close to the objective lens are the necessary

stigmator lenses. Also close to the objective lens in the column is an additional set of smaller lenses known as the scanning coils. The diaphragm also has an effect on resolution; a larger lens aperture produces a higher resolution, using an electron beam with a small spot size.

To be able to use scanning electron microscope (SEM), the specimen is coated with a thin film of a heavy metal such as gold. An electron beam from the SEM is then directed down onto the specimen and scans back and forth across it. Electrons scattered by the metal are collected, and they activate a viewing screen to produce an image. In the SEM, even fairly large specimens can be observed, and the depth of field is extremely good. A wide range of magnifications can be obtained with the SEM, from as low as x15 up to about x100000 but only the surface of an object can be visualised. All electron microscopes are fitted with cameras to allow a photograph, called an electron micrograph, to be taken.

4.1.1.2. The Concept

Electron microscopes have far greater resolving power than do light microscopes, the limits of resolution being about 0.2 nm. Two major types of electron microscopy are performed; transmission electron microscopy, to observe internal cell structure down to the molecular level, and scanning electron microscopy, useful for three-dimensional imaging and for examining surfaces.

4.1.2. Fixation

The purpose of fixation is that the material to be examined must be preserved well. Fixation prevents any further chemical changes after the organism was killed and the necessary tissue removed. The original form and size of the

material must be preserved, and the material must be reinforced by the fixative to resist any additional preparation procedures.

4.1.3. Dehydration

The high vacuum of the electron microscope hampers the observation of water containing specimens, making dehydration essential. For SEM examination the material must also be dried. Drying the specimens through evaporation of the water destroys the ultrastructure of cells thus damaging the specimen. A special drying apparatus, namely the critical point dryer is used. The use of acetone, ethanol or freon is an important intermediate step during the dehydration of material, before drying with the critical point dryer.

4.2. MATERIALS AND METHODS

4.2.1. The source

A pot that had been used for several cereal fermentations was obtained from a local household. Four pieces were broken off the sides of this pot. Three of these pieces were then immersed into another pot for the duration of three consecutive *umqombothi* fermentations. The fourth piece was not immersed into the fermentation. After the duration of the fermentation, the three pieces were retrieved from the fermented pot and all four pieces were then prepared for analysis of their surfaces using scanning electron microscopy.

4.2.2. Preparation of specimens (pieces of pot)

The methods followed during specimen preparation were according to Glauert (1974).

4.2.2.1. Fixation

Aldehyde fixatives are used as primary fixatives, followed by an oxidative fixative as a secondary fixative. The specimens are immersed in the solutions, with the surface to be examined facing upwards. A 3 % gluterdialdehye solution was used in this case as the primary fixative and the fixation time was 3-5 h. Osmiumtetroxide (O_sO_4) was used as the secondary fixative. Fixation time was 1-2 h with a 0.5 % solution.

4.2.2.2. Dehydration

The specimens were then immersed in ethanol of 50, 70, and 95 %, concentration, for a period of 1.5 h at each concentration. The specimens were then immersed over night in 100 % ethanol and the final stage was a second immersion in 100 % ethanol the next day for 3 h.

4.2.2.3. Air drying

The ethanol was allowed to evaporate. This technique is only used when a dedicated dryer is not available therefore it is not recommended.

4.2.2.4. Freeze drying

Nitrogen frozen material is dried in vacuum using a specialised freeze dryer.

4.2.2.5. Critical point drying

This is the standard and recommended technique. A critical point dryer is used and the material is dried using pressurised liquid CO_2 (to replace the ethanol) at 37 ^{0}C. A critical point dryer (Biorad, Watford, England) was used at a temperature of 34-35 ^{0}C and the pressurized liquid CO_2 was at 80 lbs. The specimens were rinsed 4 times with 15 min in between rinses. It is during the rinsing that the liquid CO_2 replaces the ethanol.

4.2.2.6. Mounting specimens and making them electrically conductive

The final step is to mount dried specimens on metal stubs and using a sputter coater (Biorad SEM coating system, UK) to coat them with a metal such as gold.

The specimens were coated twice. The specimens were left in the sputter coater for 165 s.

4.2.3. SEM

The specimens are then analysed individually following placement onto a stage within the scanning electron microscope thereafter micrographs showing relevant data are taken.

4.3. RESULTS

Bacterial and yeasts cells can be seen in Fig. 1. A higher concentration of the microorganisms within the depression or crevice as compared to the flatter surface of the pot was clearly observed. This may suggest that the rate of biofilm formation is higher within depressions or rough surfaces as compared to flatter or smoother surfaces because such areas are not washed/ cleaned as easily as the flatter surfaces of the pot, hence more substrate material supporting microbial growth being available in such areas. This particular piece of pot was rinsed/ washed once after fermentation with normal tap water.

Fig. 2 shows a higher magnification of the same sample depicted in Fig. 1. What we note in this close up is the proximity of the bacteria and the yeasts, seemingly suggesting a symbiotic relationship which has been reported by several authors (Jespersen *et al*., 1994; Hounhouigan *et al*., 1993; Oyewole and Odunfa, 1990). The size of the yeast cells was approximately 4µm in diameter. Yeast size can vary greatly depending on the species, typically measuring 3 – 4 µm in diameter, although some yeasts can reach sizes bigger than 40 µm (Walker *et al*., 2002). The substance coating the microorganisms was thought to be remnants of the fermentation.

In Fig. 3, bacterial rods were in close association with the yeast cells, a trend that was observed with all the images obtained. The thick strands to which the microorganisms are attached were remnants of the fermented beverage. For this piece of pot, the preparation for SEM imaging was done without prior washing / rinsing with water. This may explain the observed thin and thick unidentified strands plus the dried out cocoon like features which should be remnants of the fermentation.

Madigan *et al*. (2003) defines biofilm as a microcolony of microbial cells attached to a surface. In Fig. 4, yeasts and bacterial cells appear to make up the

microcolonies that are attached to the pot surface. Looking at the individual colonies, it is interesting to note that some of the yeasts encircled the bacterial cells, whereas the bacterial cells were attached to the yeast cell. These are themes that are repeated in the various images obtained. We can also see areas on the pot surface where it was bare with no evidence of microbial activity. This might be due to what was stated earlier in relation to microbial activity within crevices as opposed to the flatter areas of the pot surface.

In Fig. 5, a higher magnification (x20000) of the pot surface as depicted in Fig. 4 is shown. It can clearly be seen that a distinct 'attachment' between the bacteria and yeasts existed. This lends further support to the likelihood of a symbiotic relationship existing between lactic acid bacteria and yeasts during fermentation, in this particular case, cereal fermentation. Bacteria are typically 0.5 – 5 μm in length and in this image the cells seen are approximately 2 μm in length.

Fig. 6 depicted a different area on the pot surface that was not washed prior to SEM analysis. Again, similar high concentrations of bacterial and yeast cells were observed in the same area. The yeasts present in Fig. 6 appeared different in morphology compared to the other images showing a smooth cell surface plus a more rounded appearance. This suggested the presence of different yeast species within the microbial ecology of this fermentation.

The image observed in Fig. 7 was from the same pot as the previous samples, the difference being that the pot had not been subjected to *umqombothi* fermentation immediately prior to SEM analysis. This was done to illustrate what would be observed if SEM analysis is carried out on the surface of a pot that has not 'freshly' been used for *umqombothi* fermentation. In other words, this was the typical 'dry' surface appearance of a pot that has been used for several fermentation processes on previous occasions. It clearly showed that after several fermentations, a biofilm will develop on the pot surface. These microorganisms present on the pot surface will develop as a bioflim and as a

result contribute to the initial microbiota of the consecutive fermentation processes, acting as starter cultures and give consistency to the final product.

Fig. 8 is a higher magnification of the pot surface compared to Fig. 7, taken from the top left hand point. Clearly, yeast cells along the left hand side of the image towards the top and bottom were visible. Towards the centre of the image (Fig. 8) bacterial cells that appeared like rods were visible. The biofilm layer in this image looked like a tangled, mashed mass as is true for the previous image (Fig. 7). However, despite being an old fermenting pot, being used more than two months ago for *umqombothi* fermentation, it is very clear from the images (Figs. 7 and 8) that a biofilm had developed on the surface.

4.4. DISCUSSION

4.4.1. Biofilm

In this study three of the pot specimens to be SEM analysed were immersed in a pot during active *umqombothi* fermentation, for its entire duration. The purpose of this procedure was to obtain fresh specimens (pot pieces), which on examination of their biofilm would give clear images of the microorganisms present (Figs. 1-6) as opposed to the fourth specimen which was not immersed in a fermentation prior to SEM analysis (Figs. 7-8). Images from both handling methods, after subjected to fermentation and without, were taken and compared. The differences observed emphasised the importance of using fresh specimens in this kind of study, as the biofilm developed on the fresh specimens clearly showed the microorganisms present.

Madigan *et al*. (2003) define biofilm as a microcolony of microbial cells attached to a surface and encased in adhesive polysaccharides excreted by the cells. They further stated that it is the cell to cell communication that is critical in the development and maintenance of the biofilm. Attachment of a cell to a surface is signal for the expression of biofilm specific genes. These genes encode proteins that synthesize cell to cell signalling molecules and that begin polysaccharide formation. For instance, in *Pseudomonas aeruginosa,* a notorious biofilm former, the major signalling molecules are compounds called *homoserine lactones.* As these molecules accumulate, they function as chemostatic agents to recruit nearby *P. aeruginosa* cells (a mechanism called quorum sensing), and the biofilm develops (Madigan *et al*., 2003).

Dense biofilms can make structural analysis difficult as the microbiota beneath the uppermost layer can't be observed or quantified and those in the upper layers can easily be lost if harsh preparative methods are utilised (Surman *et al*., 1996).

Other problems with the visualisation of biofilm may be associated with the surface upon which the biofilm has formed and include the presence of a substrata composed of opaque materials and irregular surfaces, such as the pot surface that was analysed in this study, requiring optics with a large depth of field (Bakke and Olsson, 1986).

In this study, sample preparation in the form of fixation and dehydration was carried out prior to SEM analysis but as biological material, the biofilm tended to be sensitive to the harsh treatments required for visualisation with traditional electron microscopes (Little *et al.*, 1991; Goddard *et al*., 1993). The dehydration processes tended to have a shrinking effect on the biofilm and therefore care must be taken in the interpretation of these photomicrographs as in vivo biofilm structures.

Comparative visualisation by light microscopy and / or confirmatory biochemical tests may be required to aid in the discrimination between artefact and genuine structures (Lambe *et al*., 1988; Costertoon *et al*., 1986), however, the SEM does offer excellent resolution with the capacity to image complex shapes (Costerton, 1979).

4.4.2. Pot surface

In this study, pieces from a pot that had previously been used for several fermentations were subjected to an active *umqombothi* fermentation process. The cereal fermentation process was carried out in another pot and it is into this second pot that the pot pieces were immersed for the duration of the process. The same procedure was carried out by Habimana (2005), while working with *amasi,* a fermented milk product from South Africa.

Similarly, Madigan *et al*. (2003), reports that when a microscope slide is immersed in a microbial habitat, left for a period of time, and then retrieved and

examined by microscopy, the importance of the surface to microbial growth is apparent. A surface may itself also be a nutrient, such as a particle of organic matter where attached microorganisms catabolise nutrients directly from the surface of the particle (Madigan *et al*., 2003). Microcolonies naturally develop on such surfaces, leading to the formation of a biofilm, which is what we expected to find in the case of the pot examined and corroborated by the results obtained by the SEM analysis on the pot surface.

Madigan *et al*. (2003) further stated that periodic microscopic examination of immersed microscope slides can be used to measure growth rates of attached organisms in nature. This leads to the suggestion that future studies could study the growth rates of the microorganisms attached to surfaces of pots used for indigenous cereal fermentations with the aim of improving the process time and quality.

4.4.3. Yeast-bacteria association;

There was one recurrent theme in most of the images obtained namely the close proximity observed between the yeasts and the bacterial cells in the pot biofilm. This led to speculation of a symbiotic relationship existing between the yeasts and bacterial species found within the indigenous cereal fermentations, in this particular case, *umqombothi.*

In the study carried out on *umqombothi,* lactic acid bacteria were the group of bacteria that were found to be dominant in the fermentation (Chapter 2). Based on theses data and relevant studies on related African indigenous fermented foods (Hounhouigan *et al*.1993; Oyewole and Odunfa, 1990), it can be assumed that the bacteria seen in the images should be representatives of lactic acid bacteria.

In the images obtained, different yeast species were observed, though this was not confirmed, hence being subjective in nature. However, Kebede *et al*. (2007) found that within *sethemi*, naturally fermented South African milk, when prepared in a clay pot, had a wide diversity of yeasts, including species known to utilize milk constituents such as lactose and lactic acid.

Several authors have reported the coexistence and positive interactions involving yeasts and lactic acid bacteria in different African fermented foods (Jespersen *et al*., 1994; Hounhouigan *et al*.,1993; Oyewole and Odunfa, 1990). The stimulating effect of yeasts on lactic acid bacteria has been attributed to the provision of some compounds such as soluble nitrogenous compounds, B-vitamins, CO_2, pyruvate, propionate, acetate and succinate (Nout, 1991; Leroi and Pidoux, 1993). Vollmar and Meuser (1992) have also shown that yeasts multiplication is associated with an increase in acid formation of particular lactic acid bacteria in fermented products.

Similar symbiotic relationships based on acid- or alcohol fermentation occur when lactic acid bacteria are responsible for the lowering of the pH due to the secretion of organic acids allowing the yeast population to become competitive in the immediate environment, followed by yeast fermentation, like in various milk based fermentations such as *Leben*, *Dahi*, and *Koumiss* (Kosikowski, 1977; Vedamuthu, 1982; Steinkrauss, 1982; Bankole and Okagbue, 1992).

Repeated usage of a pot will lead to a characteristic microbial population that will probably not be as diverse as that found in a new pot and this in part being a result of the biofilm that develops on the interior surface of such a pot, which biofilm contains the dominant microbiota of a fermentation and as such succession will be quicker, similar to what happens during backslopping.

4.5. CONCLUSION

Biofilms are not homogenous in composition but are complex matrices composed of microcolonies interspersed with channels allowing the movement of fluids and nutrients. In this study we observed yeasts and bacterial cells in areas that had particles of what was thought to be substances from the fermentation such as small grains. This would serve as a source of nutrients supporting microbial growth. Different yeast species may be present within the biofilm studied hence contributing to the heterogeneous nature of the biofilm analysed.

The detection of biofilm formation by the use of a method such as scanning electron microscopy is laborious, often requiring numerous steps for sample preparation as well as observation of many fields to avoid observer error. It may be subjective too, as images that showed the desired trend may be picked in preference to ones that show dissimilar trends.

It follows that a combination of different techniques available may be required to overcome the problem of recognising artefacts and to give the most accurate picture of the true biofilm structure and organisation. This study confirmed that biofilm formation does take place on the interior surface of the pots used in indigenous cereal fermentations and it showed the microorganisms present in this biofilm to be predominantly yeasts and bacteria.

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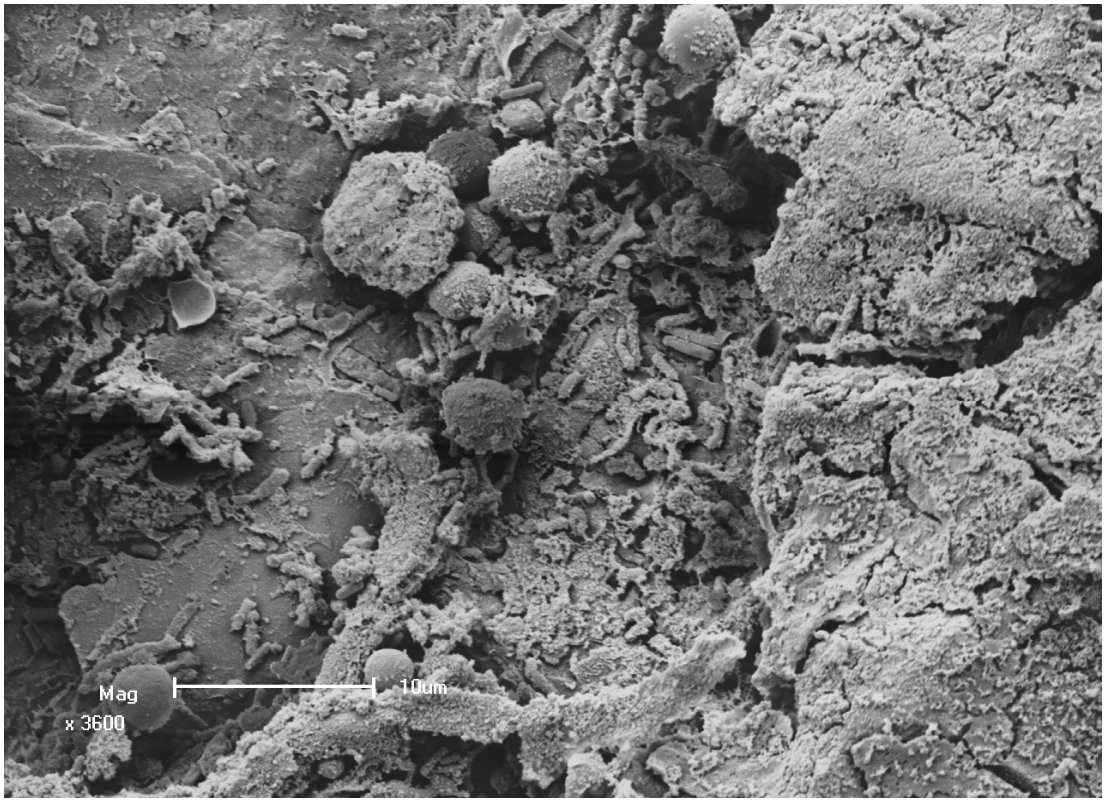

Fig. 1: Pot surface - washed once with normal tap water after subjected to *umqombothi* fermentation.

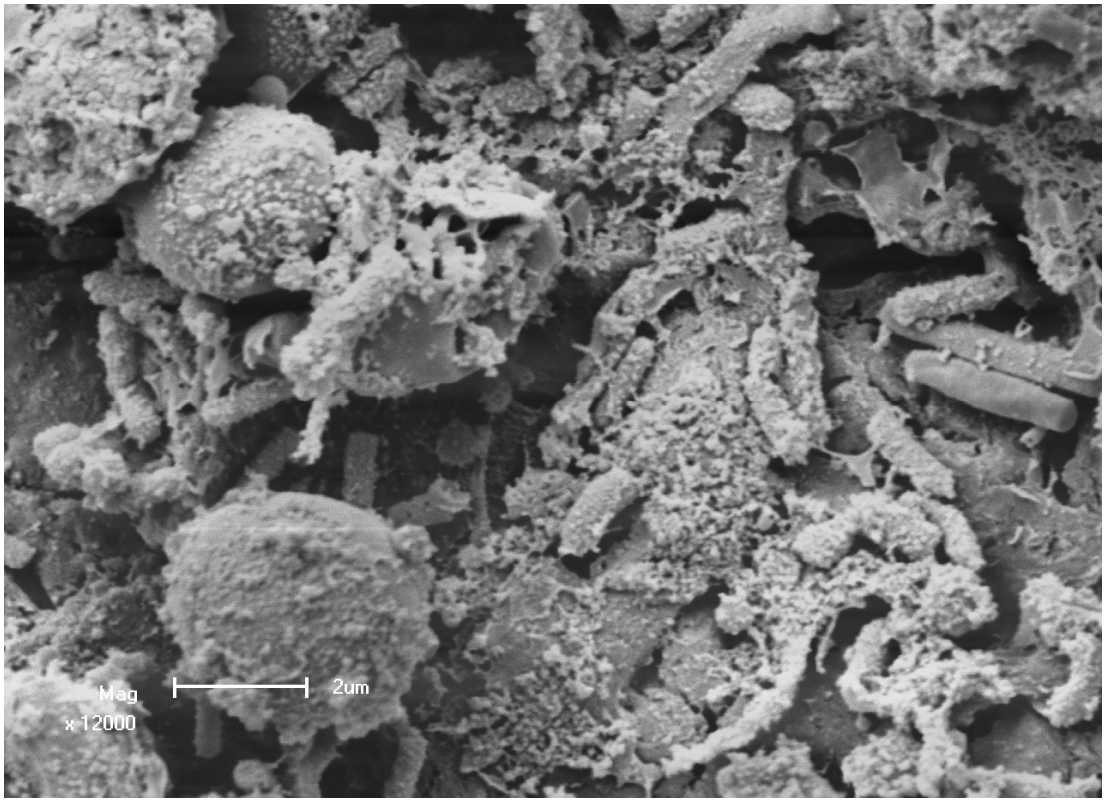

Fig. 2: Pot surface - washed once with normal tap water after subjected to *umqombothi* fermentation.

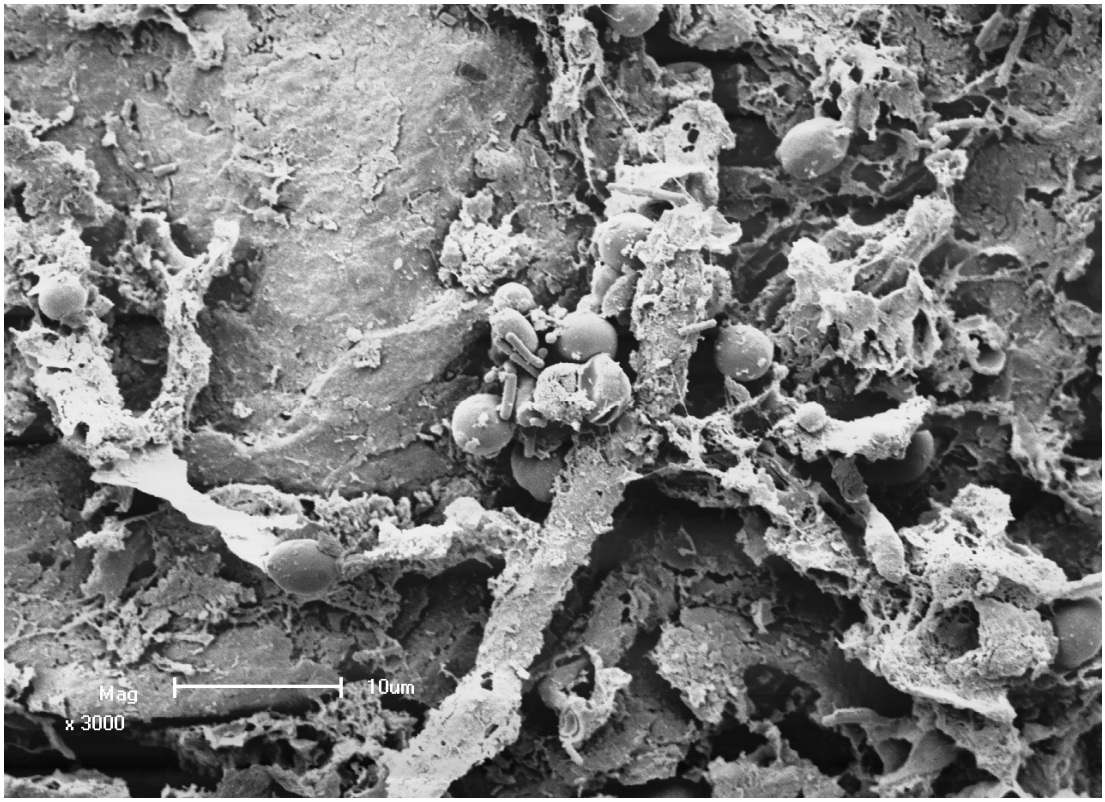

Fig. 3: Pot surface – not washed after subjected to *umqombothi* fermentation.

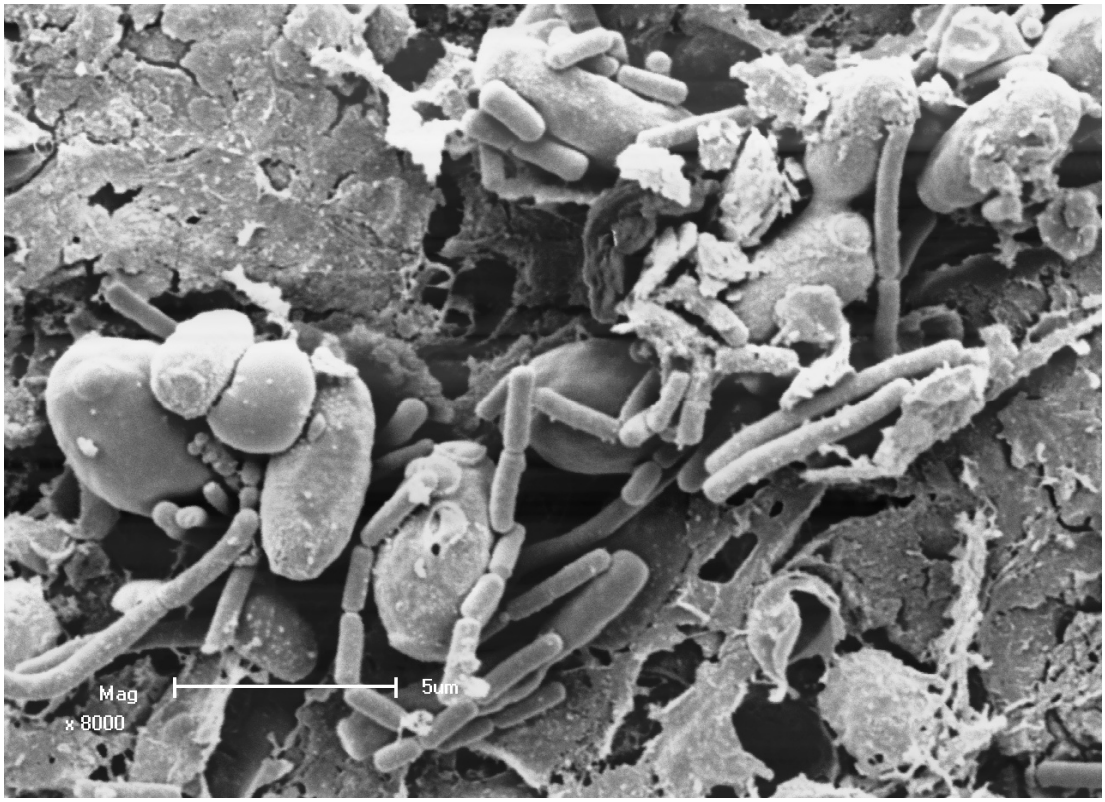

Fig. 4: Pot surface: not washed after subjected to *umqombothi* fermentation.

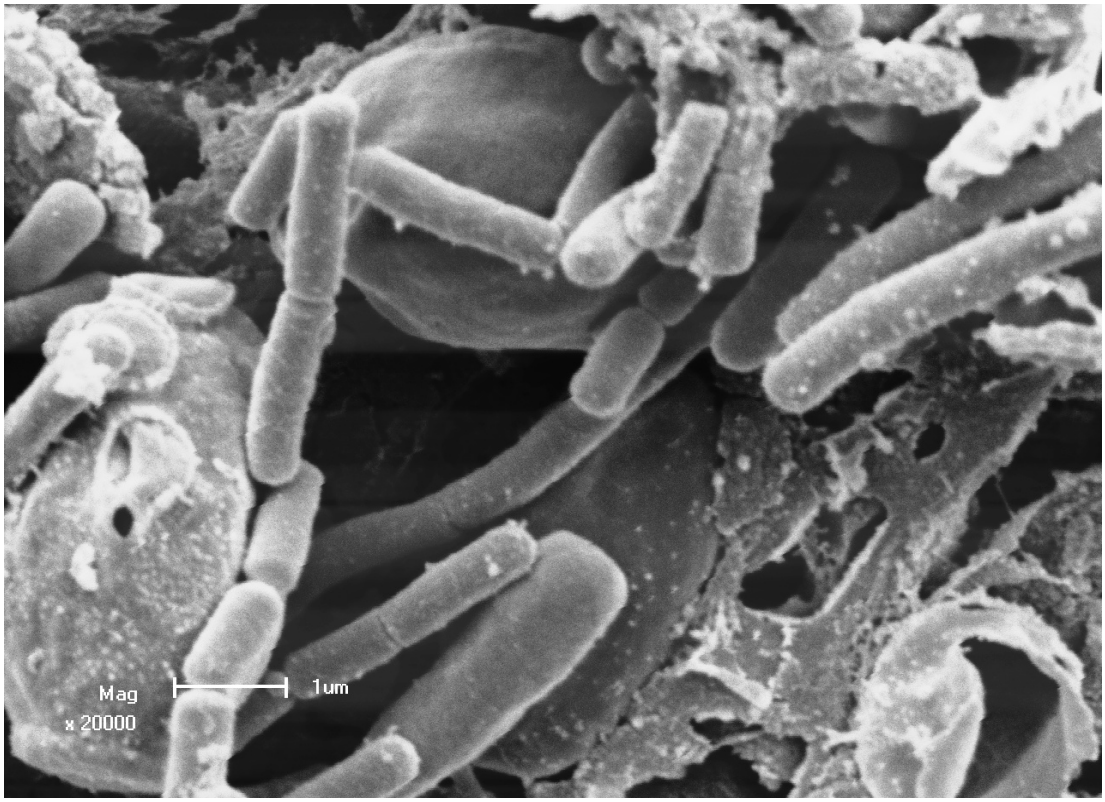

Fig. 5: Pot surface – not washed after subjected to *umqombothi* fermentation.

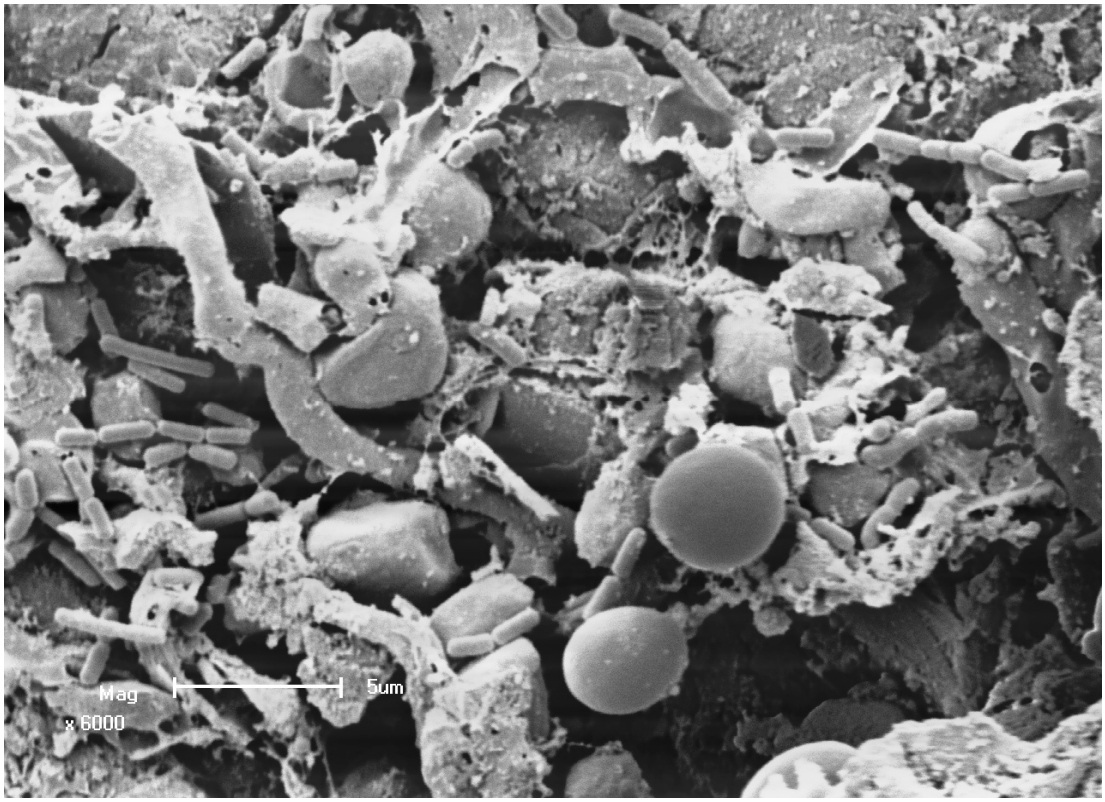

Fig. 6: Pot Surface – not washed after subjected to *umqombothi* fermentation.

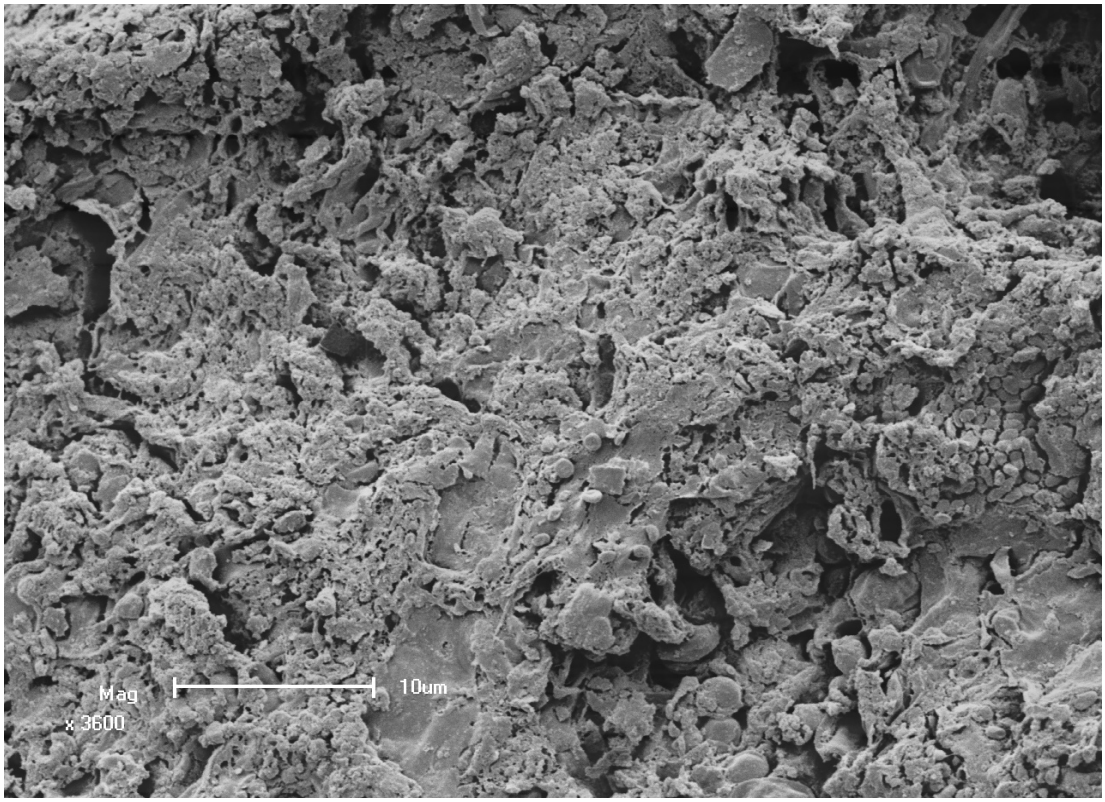

Fig. 7: Pot surface – previously used pot, not subjected to additional *umqombothi* fermentation.

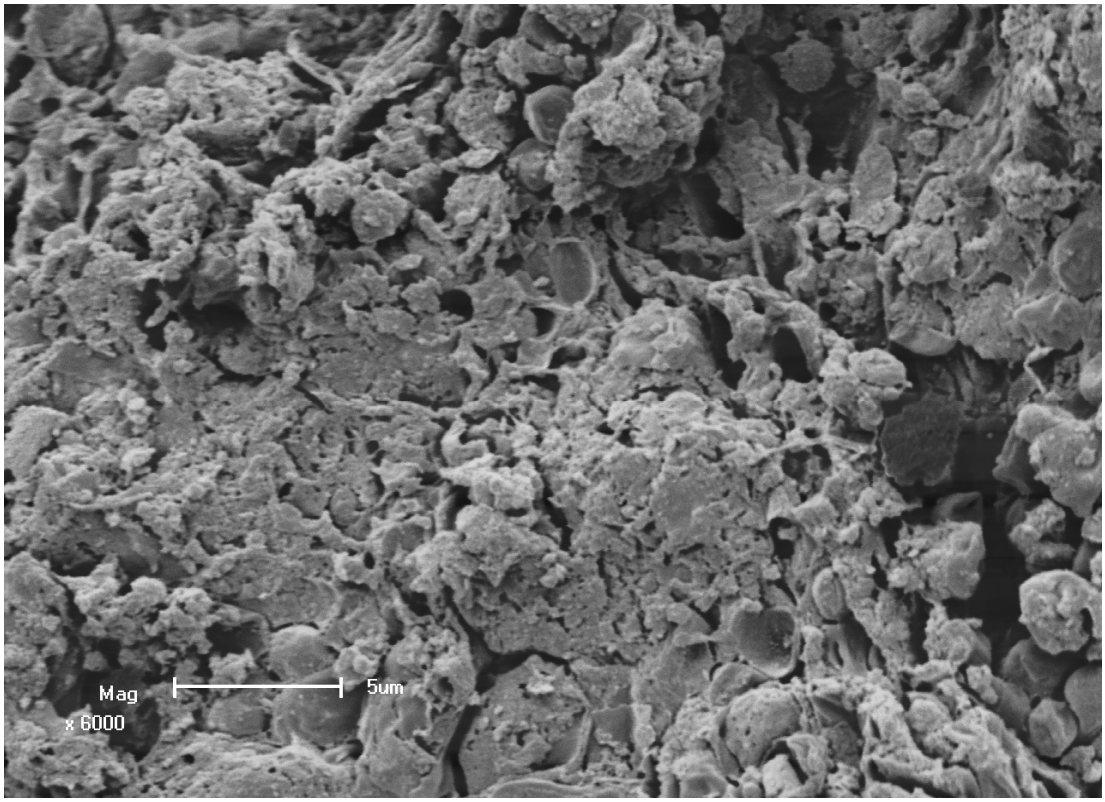

Fig. 8: Pot surface – previously used pot, not subjected to additional *umqombothi* fermentation.

Chapter 5

General Discussion and Conclusion

GENERAL DISCUSSION

Throughout the World there are many different types of fermented foods in which a range of different substrates are metabolized by a variety of microorganisms to yield products with unique and appealing characteristics. In many of these foods, the microbiological and biological bases of the fermentation processes are poorly understood.

This study highlights the fact that production of indigenous cereal fermented beverages in Southern Africa is still largely a traditional art associated with poor hygiene, inconsistent quality presentation and short shelf life. The preparation of these indigenous beverages generally depends on a spontaneous or chance inoculation by naturally occurring lactic acid bacteria. The improved control of such fermentations and product characteristics is therefore strongly recommended and this includes, but is not limited to, the use of purified starter cultures with appropriate technological properties.

It is likely that the basic microbiological analyses in conjunction with the appropriate technological developments will, in the first instance at least, be sufficient to achieve these objectives, and the goal here would be to further improve reliability and product quality through optimization of starter culture performance and to eliminate those factors that impede the fermentation process.

Indigenous cereal fermentations in Southern Africa are slightly different in terms of process variations such as preparation time, cereal types used and the combination of these cereals but it is true to say that they are basically similar. This is supported by the fact that one finds similar microbial ecology in different traditional beverages, for instance *umqombothi* from South Africa and *munkoyo* from Botswana.

The development of the correct flavour characteristics is a critical factor in the production of a range of fermented foods. However, the specific mechanisms by which flavour is generated are not fully understood, although the principal components contributing to flavour, such as protease, peptidase and lipase activities are known (Caplice and Fitzgerald, 1999). In this study, it was realised that a number of the indigenously fermented cereal beverages had similar tastes and this was thought to arise due to similar fermentable substrates and fermentation process procedures.

The general aim of this study was to determine the microbial diversity associated with indigenous cereal fermented products in Southern Africa. Associations of lactic acid bacteria (LAB) and yeast were found to be responsible for fermentation of *umqombothi*. A similar trend was observed in *mahewu.* Species of the genera *Candida, Saccharomyces* and *Debaryomyces* and *Dekkera* were the predominant yeasts in the microbial ecology of these two cereal fermented beverages.

Yeasts are unicellular fungi, and most of them are classified within the Ascomycetes. Yeast cells are usually spherical, oval, or cylindrical, and cell division generally takes place by budding. In the budding process, a new cell forms as an outgrowth of the old cell; the bud gradually enlarges and then separates (Madigan *et al*., 2003). The SEM images obtained in this study showed yeast cells with different morphology. The budding process as well as yeast cell scars present after budding, were evident in some of the SEM images seen.

On a commercial scale the most important yeasts are the baker's and brewer's yeast, which are members of the genus *Saccharomyces.* Yeasts have been greatly improved through the years by careful selection and genetic manipulation by industrial microbiologists. Indeed, *S. cerevisiae* has been studied as a model

eukaryote for many years and was the first eukaryote to have its genome completely sequenced (Madigan *et al.*, 2003).

The lactic acid bacteria (LAB) are a group composed of 13 genera of Gram-positive bacteria at this time; *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Lactosphaera*, *Leuconostoc*, *Oenococcus, Pediococcus*, *Paralactobacillus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, *Weisella* (Jay *et al*., 2005). Although the lactic acid group is loosely defined with no precise boundaries, all members share the property of producing lactic acid from hexoses and as fermenting organisms, they lack functional heme-linked electron transport systems or cytochromes. They obtain their energy by substrate level phosphorylation while oxidizing carbohydrates and they do not have a functional Krebs cycle (Jay *et al*., 2005).

Due to the alcoholic nature of several indigenous cereal fermented beverages, it is suggested that predominant LAB involved are heterofermentative in nature, that is, they produce equal molar amounts of lactate, carbondioxide, and ethanol from hexoses. This is as opposed to LAB in the homofermentative group where the lactics produce lactic acid as the major or sole product of glucose fermentation.

The occurrence of yeasts together with LAB in indigenously fermented cereal products has led to suggestions of possible interactions between these groups of microorganisms. Understanding the roles of these microorganisms in these products will therefore help in the quest to develop cultured products that have similar characteristics to these indigenously fermented cereal products. Several authors have reported the co-existence and positive interactions involving yeasts and lactic acid bacteria in different African fermented foods (Jespersen *et al*., 1994; Hounhouigan *et al*., 1993; Oyewole and Odunfa, 1990).

Several microbial interactions involving yeasts have been suggested in other types of fermented products such as blue cheese, white mould cheese, kefir and koumiss (Subramanian and Shankar, 1985; Fleet, 1990; Jakobsen and Narvhus, 1996). Interaction between *Lactobacillus hilgardii* and *S*. *florentinus* isolated from sugary kefir grains has also been reported, where the yeast stimulated the LAB through production of carbon dioxide, pyruvate, propionate and succinate (Leroi and Pidoux, 1993). In addition, some LAB release galactose into the medium as a by-product of lactose metabolism (Marshall, 1987; Davidson and Hillier, 1995; Tamime and Marshall, 1997), which may be utilised by galactose assimilating, but lactose negative, yeasts.

Although the beneficial aspects of the lactic acid bacteria to human and animal health are unquestioned, some of these bacteria are associated with human illness. This subject was reviewed by Aguiree and Collins (1993), who noted that around 68 reports of lactobacilli in human clinical illness were made over about a 50 year period. Several species of leuconostocs were implicated in about 27 reports in 7 years, the pediococci in 18 reports over 3 years, and the enterococci in numerous reports (Jay *et al*., 2005).

CONCLUSION

The very impressive scientific and technological developments that have been made with lactic acid bacteria over the past number of years are likely to relieve many of the bottlenecks encountered with their full and efficient application in food fermentations in the near future. However, the scientific community and the industrial user need to be aware of consumer concerns regarding recombinant DNA technology especially when in involves food products. Thus, there is a need to ensure that there will be clear consumer benefits arising from the manipulation of these bacteria and also that the traditional positive attitude associated with fermented foods is not compromised by the exciting biotechnological development.

Like with any other fermentation process the understanding of the microbial ecology of cereal fermentations needs the knowledge of the fermentation substrates, that is, the grains or seeds of the various cereal plants, as well as the products obtained thereof. When considering the multitude of foods from cereals one has to recognize that their greater part has been subjected to fermentation processes taking place at least one step of their generation. This is because fermentation is basically a process that proceeds under the influence of activities exerted by enzymes and or microorganisms. An understanding of both activities, which are important in cereal fermentation, will make a vital contribution towards the improvement of indigenous cereal fermentations in Africa.

As has been done for a variety of African fermented foods, studies could be carried out on the optimisation of process variables such as the cooking temperature and time, for *umqombothi* and *mahewu*. 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.

Further investigations need to be carried out before it can be categorically stated that yeasts and the lactic acid bacteria do exist in a symbiotic relationship in the biofilm from fermentation pots but the preliminary evidence collected during this study suggests that this is a distinct possibility.

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. This in turn necessitates a more thorough understanding of the microorganisms involved, in terms of the type and their specific activities, to ensure more reliable and predictable fermentation processes.

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Chapter 6

Summary

Several aspects related to cereal fermentations were reviewed in the literature section of this study. These aspects included among others, the history of indigenous cereal fermentation, factors that affect cereal fermentation, potential microbiological hazards of cereal fermentation, recent advances in related industry, and the future of fermented foods. A wide variety of recipes exists across the African continent and the beers are known by many local names. In particular this study focussed on *mahewu* and *umqombothi*, both made from a combination of maize and sorghum, fermented beverages consumed in South Africa. Improved process technologies such as the use of starter cultures can lead to improved product characteristics and consequently better health attributes for the consumer of these indigenously fermented products.

During the fermentation processes for *umqombothi* and *mahewu,* yeasts, lactic acid bacteria, moulds and enterobacteriaceae were present at the start of the process but as fermentation progressed yeasts and lactic acid bacteria were the dominant microorganisms. This was over a 48 h, and 3 day, fermentation period for the *umqombothi* and *mahewu* respectively. Home-, township-, and laboratory-made samples of *umqobothi* were compared while for mahewu, the comparison was between the home- and laboratory-made samples. Results from different production sites were not significantly different although there were instances that called for improved hygiene as contaminants were found in some samples.

Yeasts isolated from *umqombothi* were identified and these included; *Candida ethanolica, C. haemuloni, C. sorbophila, Dekkera anomala, Dekkera bruxellensis, Saccharomycopsis capsularis* and *Saccharomyces cerevisiae*. The yeasts isolated from *mahewu* were identified and the predominant strains were *Candida haemuloni, Candida sorbophila, Debaryomyces hansenii, Saccharomyces capsularis and Saccharomyces cerevisiae*. Confirmation of the yeast identity was done using sequence analysis of the D1/D2 domain using primer pairs NL-1(5'-GCATATCAATAAGCGGAGGAAAAG) and NL-4(5'-GGTCCGTGTTTCAAGACGG).

Scanning electron microscopy was performed on the interior surface of a pot used for indigenous cereal fermentation. The images obtained confirmed the presence of biofilm on the surface and the microorganisms present in this biofilm were predominantly yeasts and bacteria. The observed proximity of yeasts to the bacterial cells lent further support to the suggestion of a symbiotic relationship existing between these microorganisms in indigenous cereal fermentations. Biofilms are not homogenous in composition but are complex matrices composed of microcolonies interspersed with channels allowing the movement of fluids and nutrients. It therefore follows that a combination of different techniques that are available may be required to give the most accurate picture of the true biofilm structure and organisation.

Indigenous cereal fermented products are an integral part of the diet of many African households. Several advantages do arise from the fermentation of cereals and improvement of this process will not only lead to better health attributes but also the production of fermented beverages with guaranteed and consistent quality. This augurs well for the producers of fermented products as more consumers become aware of the health properties of fermented products and this trend is only likely to improve in the future.

Keywords: *umqombothi*, *mahewu*, yeasts, lactic acid bacteria, biofilm, symbiotic relationship