ISOLATION AND IDENTIFICATION OF THE MICROBIAL CONSORTIUM PRESENT IN FERMENTED MILKS FROM SUB-SAHARAN AFRICA

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DECLARATION

In this thesis, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

A wide variety of traditionally and commercially fermented milks are commonly consumed in various countries of Sub-Saharan Africa. Commercially fermented milk is produced on an industrial scale according to well-managed, standardised production processes and starters are used to initiate fermentation. Traditionally fermented milk is prepared domestically and fermentation occurs spontaneously at ambient temperatures. Lactic acid bacteria (LAB) are responsible for milk fermentation during which they convert the milk carbohydrates to lactic acid, carbon dioxide, alcohol and other organic metabolites. Acetic acid bacteria (AAB), yeasts and mycelial fungi have also been isolated from fermented milks.

In this study the microbial consortium present in three traditionally fermented milks, namely omashikwa from Namibia, masse from Mozambique and chekapmkaika from Uganda and two commercially fermented milks, namely chambiko from Malawi and omaere from Namibia, were isolated and enumerated on six different selective media that included MSR + C (specific for lactobacilli), KCA + TTC (specific for lactococci), KCA + V (specific for leuconostocs), MRS + E (specific for AAB), MEA (specific for mycelial fungi) and YPD (specific for yeasts).

No significant differences were found between the enumeration values obtained for the three chambiko samples, as well as for enumeration values obtained for the two omaere samples on each of the selective media, indicating low sample variance. Significant differences between enumeration values obtained for the three omashikwa samples were found on all six selective media. Significant differences between enumeration values of the three masse samples and both the chekapmkaika samples were also observed on the selective media. In addition to this, significant differences were observed between average enumeration values obtained for each media between the masse and chekapmkaika, the chambiko and omaere, as well as when the traditional and commercial milks were compared. According to the average enumeration values obtained on each media selective for LAB, the highest bacterial counts were detected on KCA + TTC medium for omaere (2.3×10^6 cfu.ml^{-1}), KCA + V for chambiko (1.8×10^5 cfu.ml^{-1}), KCA + TTC for omashikwa and MRS + C for masse and chekapmkaika (6.2×10^6 and 2.0×10^3 cfu.ml^{-1}, respectively).

After isolation and enumeration of the microbes present in each milk, bacterial isolates on the media selective for LAB and AAB were obtained according to the Harrison Disk method. These isolates were identified by amplifying a 1.5 kilobase (kb)

part of the 16S ribosomal RNA (rRNA) gene using the polymerase chain reaction (PCR), followed by DNA sequencing. The isolates were identified by comparing the sequences obtained to sequences listed in the NCBI database using the BLAST algorithm and searching for the closest relative.

The main LAB group present in the omaere was lactococci (94%), in chambiko and chekapmkaika it was lactobacilli (30% and 45%, respectively), in omashikwa it was enterococci (43%) and in masse it was leuconostocs (68%). The same microbial species were present on a number of the selective media used in this study. *Lactococcus* spp., *Enterococcus* spp. and *Lactobacillus* spp. were isolated from MRS + C, KCA + TTC, KCA + V and MRS + E and *Leuconostoc* spp. were isolated from MRS + C, MRS + E and KCA + V. Hygienic standards during traditional milk fermentation is often poor and, therefore, microbial contaminants were isolated from the traditional milk and these included *Acinetobacter johnsonii* and *Klebsiella pneumoniae* from KCA + V, *Mesorhizobium loti, Acinetobacter radioresistens, Escherichia coli, Staphylococcus* spp., *Kluyvera georgiana*, *Enterobacter* spp. and *Klebsiella oxytoca* from KCA + TTC*, Staphylococcus* spp. from MRS + C and *Bacillus* spp. from MRS + E. Since the media used for the isolation of the LAB and AAB in this study were not selective further identification of the enumerated microbes is of importance for the identification of the microbial groups present in each fermented milk.

The data obtained in this study clearly shows that fermented milks from Sub-Saharan Africa vary significantly from each other in terms of microbial numbers, microbial diversity and the dominant microbial groups present. The microbial diversity of the traditionally fermented milks was more diverse than the microbial diversity of the commercially fermented milks. LAB strains isolated from these traditionally fermented milks can be used to develop novel starters and as a result new commercially fermented dairy products with unique aromas, tastes and characteristics can be produced.

UITTREKSEL

‘n Wye verskeidenheid tradisioneel en kommersieel gefermenteerde melk produkte word algeneem verbruik in verskeie lande van Sub-Sahara Afrika. Kommersieel gefermenteerde melk word geproduseer op groot skaal, deur deeglik bestuurde gestandardiseerde produksieprosesse en ‘n beginkultuur word gebruik om fermentasie te inisieer. Tradisioneel gefermenteerde melk word tuis gemaak en fermentasie gebeur spontaan by kamertemperatuur. Melksuurbakterieë (MSB) is verantwoordelik vir melkfermentasie waartydens die bakterieë koolhidrate omskakel na melksuur, koolstofdioksied, alkohol en ander organiese sure. Asetaatsuurbakterieë (ASB), giste en miseliale fungi is ook al van gefermenteerde melk geïsoleer.

In hierdie studie is die mikrobiese konsortium teenwoordig in drie soorte tradisioneel gefermenteerde melk, naamlik omashikwa van Namibië, masse van Mosambiek en chekapmkaika van Uganda en twee soorte kommersieel gefermenteerde melk, naamlik chambiko van Malawi en omaere van Namibië, geïsoleer en getel op ses verskillende selektiewe groeimedia insluitend MRS + C (spesifiek vir lactobacilli), KCA + TTC (spesifiek vir lactococci), KCA + V (spesifiek vir leuconostocs), MRS + E (spesifiek vir ASB), MEA (spesifiek vir miseliale fungi) en YPD (spesifiek vir giste).

Geen betekenisvolle verskille is gevind tussen die mikrobiese tellings verkry vir die drie chambiko monsters nie, sowel as tussen die mikrobiese tellings verkry vir die twee omaere monsters, op elk van die selektiewe groeimedia, wat dui op lae monster variansie. Betekenisvolle verskille is gevind tussen die mikrobiese tellings verkry vir die drie omashikwa monsters op al ses selektiewe groeimedia. Betekenisvolle verskille is ook waargeneem tussen die mikrobiese tellings van die drie masse monsters en beide die chekapmkaika monsters op die selektiewe groeimedia. Daarbenewens is betekenisvolle verskille waargeneem tussen gemiddelde mikrobiese tellings verkry vir elke groeimedium tussen die masse en chekapmkaika, die chambiko en omaere asook toe die tradisionele en kommersiële melk produkte met mekaar vergelyk is. Volgens die gemiddelde mikrobiese tellings verkry op elk van die groeimedia selektief vir MSB, is die hoogste mikrobiese telling waargeneem op KCA + TTC medium vir omaere (2.3×10^6 kve.ml^{-1}), KCA + V vir chambiko (1.8×10^5 kve.ml^{-1}), KCA + TTC vir omashikwa en MRS + C vir masse en chekapmkaika (6.2×10^6 en 2.0×10^3 kve.ml^{-1}, respektiewelik).

Na die isolasie en tel van die mikrobes teenwoordig in elke melk is bakteriese isolate op die media selektief vir MSB en ASB verkry volgends die Harrison Disk metode. Hierdie isolate is geïdentifiseer deur amplifikasie van ‘n 1.5 kilobasis (kb)

gedeelte van die 16S ribosomale RNS (rRNS) geen deur gebruik te maak van die polimerase kettingreaksie gevolg deur DNS klonering. Die isolate is geïdentifiseer deur die gekloneerde insetsels se volgordes te vergelyk met volgordes beskikbaar op die NCBI webwerf deur van die BLAST algoritme gebruik te maak en die naas verwante insetsel op te spoor.

Die hoof MSB groep teenwoordig in die omaere was lactococci (94%), in chambiko en chekapmkaika was dit lactobacilli (30% en 45%, respektiewelik), in die omashikwa was dit enterococci (43%) en in die masse was dit leuconostocs (68%). Dieselfde mikrobiese spesies was teenwoordig op verskeie van die selektiewe groeimedia gebruik in hierdie studie. *Lactococcus* spp., *Enterococcus* spp. en *Lactobacillus* spp. is geïsoleer van MRS + C, KCA + TTC, KCA + V en MRS + E en *Leuconostoc* spp. is geïsoleer van MRS + C, MRS + E en KCA + V. Higiëniese standaarde tydens tradisionele melkfermentasie is dikwels swak en dus is mikrobiese kontaminante geïsoleer van die tradisionele melk produkte insluitend *Acinetobacter johnsonii* en *Klebsiella pneumoniae* van KCA + V, *Mesorhizobium loti, Acinetobacter radioresistens, Escherichia coli, Staphylococcus* spp., *Kluyvera georgiana*, *Enterobacter* spp. en *Klebsiella oxytoca* van KCA + TTC*, Staphylococcus* spp. van MRS + C en *Bacillus* spp. van MRS + E. Aangesien die media wat gebruik is vir die isolasie van die MSB en ASB in hierdie studie nie selektief was nie, is verdere identifikasie van die getelde mikrobes belangrik vir die identifikasie van die mikrobiese groepe teenwoordig in elke melk.

Die data verkry in hierdie studie dui aan dat gefermenteerde melk produkte van Sub-Sahara Afrika betekenisvol van mekaar verskil in terme van mikrobiese getalle, mikrobiese diversiteit en die dominante mikrobiese groepe teenwoordig. Die mikrobiese diversiteit van die tradisioneel gefermenteerde melk produkte was meer divers as die mikrobiese diversiteit van die kommersieel gefermenteerde melk produkte. MSB spesies geïsoleer van hierdie tradisioneel gefermenteerde melk produkte kan gebruik word om nuwe beginkulture te ontwikkel en gevolglik kan nuwe kommersieel gefermenteerde suiwelprodukte met unieke aromas, smake en eienskappe geproduseer word.

To my family and friends, for their love and inspiration

From left to right at the back: Laisve Lideikyte, Corli Witthuhn, MichelleCameron and Custodia Macuamule.
From left to right in front: Amy Strydom, Lionie Schutte and Donna Cawthorn.

“Education is the most powerful weapon which you can use to change the world.”

-Nelson Mandela-

“The function of education is to teach one to think intensively and to think critically. Intelligence plus character - that is the goal of true education.”

-Martin Luther King, Jr.-

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LIST OF ABBREVIATIONS

AAB – acetic acid bacteria
ANOVA – analysis of variance
BFAP – Bureau for Food and Agricultural Policy
DSMZ – Deutche Sammlung von Mikroorganismen und Zellkulturen
FAO/WHO – Food and Agriculture Organization/World Health Organization
GMP – Good Manufacturing Practice
KCA + TTC – potassium carboxymethyl cellulose agar and triphenyltetrazolium chloride
KCA + V – potassium carboxymethyl cellulose agar and vancomycin
LAB – lactic acid bacteria
LDH – lactate dehydrogenase
LPSN – List of Prokaryotic names with Standing in Nomenclature
MEA – malt extract agar
MIC – minimum inhibitory concentration
MRS + C – deMan Rogosa and Sharp-medium and cycloheximide
MRS + E – deMan Rogosa and Sharp-medium and ethanol
NC – no counts
NSLAB – non starter lactic acid bacteria
PCR – polymerase chain reaction
rRNA – ribosomal RNA
YPD – yeast peptone dextrose agar

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

A large variety of fermented food products are produced and consumed around the world. Fermentation serves to preserve raw foods and increases the diversity of available food products (Motarjemi, 2002; Ross *et al*., 2002). Cereals, oil seeds, milk, fish, meat and vegetables are raw foods that are fermented world-wide (Iwuoha & Eke, 1996; Lee, 1997). As part of the human diet, fermented foods can play an important role in maintaining a healthy intestinal tract and increase the acceptability of dairy products to lactose intolerant individuals (Bernardeau *et al*., 2008; Brown-Esters *et al*., 2012). In Africa, food fermentation is especially helpful to prevent malnutrition among infants and also to detoxify raw foods such as cassava which contain harmful chemicals (Edijala *et al*., 1999; Holzapfel, 2002).

Today most fermented food products in developed countries are produced commercially in large quantities though standardised and well controlled production processes. This usually occurs through fermentation which is initiated by adding defined starter cultures and results in high quality end-products which are consistently safe for consumption (Caplice & Fitzgerald, 1999). However, in Africa fermented foods are still frequently prepared in small quantities using traditional methods by rural communities through spontaneous fermentation or by adding a small amount of previously fermented product as a starter (Oyewole, 1997). Spontaneous fermentation can occur due to microbes inherent in the raw milk or by microbes from the environment or preparation equipment (Oyewole, 1997; Kebede *et al*., 2007). The characteristics of these products are influenced by the quality and the type of raw milk used, the production methods followed and the regional climatic conditions (Mensah, 1997; Wouters *et al*., 2002). During the preparation of traditionally fermented milks, good hygienic practises are often neglected and, therefore, these products are often of poor quality and spoilage microbes can be present (Bille *et al*., 2007; Aloys & Angeline, 2009). The microbial consortium present in traditionally fermented milk products is generally diverse which results in varied product quality with unique organoleptic properties (Holzapfel, 1997; Ross *et al*., 2002; Leroy & De Vuyst, 2004). Some of these traditionally fermented milks include sethemi (South Africa), omashikwa (Namibia), rob (Sudan) and ergo (Ethopia) (Abdelgadir, *et al*., 2001; Gonfa *et al*., 2001; Bille *et al*., 2007; Kebede *et al*., 2007). Amasi from South Africa and madila from Botswana are

both traditionally and commercially produced (Ohiokpehai, 2003; McMaster *et al*., 2005).

A wide variety of microbes can be responsible for the fermentation of milk including lactic acid bacteria (LAB), acetic acid bacteria (AAB), yeasts and mycelial fungi. The LAB genera generally present in fermented milk products are *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus* and *Pediococcus* (Temmerman *et al*., 2004; Zamfir *et al*., 2006). LAB initiate the process of fermentation whereby carbohydrates in the milk are oxidised into predominantly lactic acid, but alcohol, carbon dioxide and several other compounds can also be produced depending on the LAB strains present (Caplice & Fitzgerald, 1999; Ross *et al*., 2002). AAB have been isolated from kefir grains used to prepare traditionally fermented milk known as kefir (Witthuhn *et al*., 2005). AAB are also present in some commercial starters such as *Acetobacter orientalis* which is used in combination with *Lactococcus lactis* subsp*. cremoris* to produce fermented milk in Japan (Nakasaki *et al*., 2008). Yeasts present in fermented milk products generally enter the raw milk or cheese from the environment and their presence results in end-products with different physico-chemical characteristics in comparison to products where only LAB is present.

Information on starter cultures used in Sub-Saharan Africa is limited and very few of the microbial consortiums present in these traditionally fermented milks have been investigated. LAB strains isolated from the traditionally fermented milk can be used to construct new commercial starters and new fermented products with original characteristics can then be produced. In this study the microbial consortiums of five different fermented milk products from different countries in Sub-Saharan Africa were enumerated and further identifications made were focused on the LAB and AAB present in the fermented milks. These fermented milks include two commercially fermented milks, chambiko (Malawi) and omaere (Namibia) and three traditionally fermented milks, omashikwa (Namibia), masse (Mozambique) and chekapmkaika (Uganda).

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CHAPTER 2

LITERATURE REVIEW

A. Background

Milk from various ruminant species play an essential role in human nutrition and health, either through direct consumption of milk or the consumption of various types of dairy products (Ceballos *et al*., 2009). Milk is a source of important nutrients, including macronutrients such as sugars, lipids and proteins, as well as micronutrients, including various vitamins and minerals (Michaelidou, 2008; Ceballos *et al*., 2009; Fox, 2009). Other minor constituents also present in milk include enzymes, hormones and compounds such as alcohols, sulphides, diols and acrolein that are formed during the disintegration of macronutrients during milk processing (Fox, 2009; Huppertz *et al*., 2009).

Of the approximate 600 million tonnes per annum of milk produced in the world today, 85% is bovine milk, 11% is buffalo milk, 2% is caprine milk and 2% is ovine milk. Minimal amounts of milk are also produced from reindeer, camels, horses, yaks and donkeys (Fox, 2009). Dairy products, produced from these different milks, include fermented milk products and cheeses that are produced on a commercial scale or traditionally within communities (Lee, 1997; Kebede *et al.,* 2007). The popularity of different dairy products and their consumption varies between countries and people's personal taste. According to Milk South Africa, the South African commercial dairy market consisted of 40% concentrated and 60% liquid products in 2010. Hard and semi-hard cheeses were the predominant concentrated products consumed, followed by other cheese varieties, milk powder, butter, whey powder, condensed milk and buttermilk powder. The predominant liquid product consumed was pasteurised milk (52%), followed by UHT, sterilised milk, yoghurt, maas, buttermilk and flavoured milk. In South Africa traditionally fermented dairy products are especially popular among rural communities and these fermented milks are commonly consumed as is or with cereal products (Narvhus & Gadaga, 2003; Kebede *et al.*, 2007; Todorov *et al*., 2007).

Countries with the largest amount of dairy farms include India (78 million), Pakistan (7.4 million), Russia (3.2 million), Uganda, Kenya, China, Uzbekistan and the Ukraine (between 1.7 and 2.2 million). In more than 40% of countries globally, herds consists of an average of less than ten cows. South Africa is the only African country of eleven countries globally which have dairy herds of more than 100 cows (Coetzee,

2012a). A 0.5% or more annual decrease in the number of dairy farms, as has been observed around the world, has led to a decrease in milk production. The decrease in the number of farms was countered by an increase in the total milk produced per farm. However, this is only true for a few countries including South Africa, where a 7.5% or higher increase has been observed (Coetzee, 2012a). During the first ten months of 2011 the production of milk in South Africa was only 0.3% higher than in the same period of the previous year, while milk consumption increased by 5%. This trend is observed globally emphasising the pressure on milk production to support the high demand for dairy products (Anonymous, 2012a).

According to the Bureau for Food and Agricultural Policy (BFAP) (2008) the increase in producer costs in South Africa led to an increase in milk production and a new record was set of 2.65 million tons of fluid milk produced. In 2009 the annual milk production decreased to approximately 2.52 million tons, but an increase in production is predicted annually up to 2019 reaching approximately 2.75 million tons of milk. Over the next ten years it is expected that the dairy industry is going to be one of the fastest developing agricultural industries, where milk production will increase with an average of more than 2% per year to satisfy the increasing demand for fresh milk in third world countries. This correlates well with predictions of increasing dairy consumption in South Africa where by 2019 the average growth rate per year will be approximately 2.4% for cheese, 4.9% for skimmed milk powder and 5.9% for whole milk powder.

B. Fermentation

Fermentation as a food processing technology dates as far back as 6000 BC, where it spread from its origin in the Middle East during the start of domestication of animals to the rest of the world (Caplice & Fitzgerald, 1999; Ross *et al*., 2002). Fermented food products originated through natural fermentation by the microbes present in the raw foods and these products became popular amongst the indigenous communities (Ross *et al*., 2002). Traditional fermentation methods were passed on from one generation to the next by using a relatively small amount of the previously fermented product as a starter culture for the following fermentation (Sanni, 1993). The latter process, also known as backslopping of the fermented product, resulted in the reduction of fermentation failure and conservation of the unique organoleptic properties (Caplice & Fitzgerald, 1999; Leroy & De Vuyst, 2004). Today most fermented products are produced on a commercial scale through highly developed equipment and industrial

processes where fermentation is initiated by defined starter cultures (Caplice & Fitzgerald, 1999).

In 1890 the first 'pure' starter (*Lactococcus lactis*) was used for the production of fermented milk and cheese in Germany and Denmark (Holzapfel, 1997). A starter culture is a product with high viable microbial counts and when added to certain foods, it accelerates fermentation leading to a final product with a desirable alteration in the aroma, texture and flavour profile (Holzapfel, 1997; 2002). Initially, starters were selected primarily according to the acidification rate and phage resistance. A better understanding of the metabolism and genomics of fermentation microbes led to improved strain selection to ensure product uniqueness (Leroy & De Vuyst, 2004). Although commercial starter cultures can ensure end-product safety and quality, traditional starters result in a fermented product with diverse sensory attributes due to the wide variety of microbes present (Holzapfel, 1997; Ross *et al*., 2002; Leroy & De Vuyst, 2004).

In most third world countries such as Africa, foods are still frequently fermented on house hold scale through spontaneous fermentations at ambient temperatures (Iwuoha & Eke, 1996; Oyewole, 1997). The quality of these traditionally prepared fermented foods is often poor. This is a result of neglected hygienic practises during preparation which leads to the presence of spoilage microbes, dirt and insects in the final product resulting in shortening of the shelf-life (Bille *et al*., 2007; Aloys & Angeline, 2009). Several aspects must be taken into account when a starter is selected for improving the product quality of traditional fermentations made on small scale in developing countries. Firstly, the production process must be managed to meet the desirable growth conditions for the starter bacteria to ensure fermentation and prevent contamination. This can be achieved by implementing the fundamental principles of Good Manufacturing Practice (GMP). Secondly, the sensory characteristics of the fermented food must meet the dietary habits and preferences of the target consumer communities. Thirdly, the starter strains must have the ability to utilise the carbohydrates in a specific raw food to produce the desired final product. And finally, the starter strain(s) must be able to reduce toxicological risks of foods if the raw product contains mycotoxins and toxic chemical compound (Edijala *et al*., 1999; Holzapfel, 2002; Aloys & Angeline, 2009). When selecting a starter the efficiency is dependent on the quality of the raw food, the culture age, storage and management procedures, such as temperature control at incubation and the presence of inhibitors. The presence of bacteriophages

can also affect starter culture efficiency, which can result in unsuccessful fermentation and final product losses (Giraffa *et al*., 2010).

In Africa, the most popular raw foods to be fermented are crops, cereals, oil seeds, roots and milk (Table 1) (Oyewole, 1997). Fish, meat and vegetables are also fermented in Africa, but not as frequently as in Europe and Asia (Lee, 1997; Oyewole, 1997). Recipes followed for the preparation of various traditionally fermented products vary and are influenced by the food type, cultural traditions and geographical regions (Mensah, 1997).

During fermentation, carbohydrates are oxidised (aerobically or anaerobically) by microbes, predominantly lactic acid bacteria (LAB). The end-products produced mainly include lactic acid, but also carbon dioxide and alcohol (Caplice & Fitzgerald, 1999; Ross *et al*., 2002). These microbes may also produce other organic acids such as acetic, propionic, formic and butyric acids, as well as enzymes, bacteriocins, aroma compounds and exopolysaccharides (Caplice & Fitzgerald, 1999; Leroy & De Vuyst, 2004). As a result, raw materials are converted to a safe product with a reduced pH and unique sensory characteristics (Sanni, 1993; Leroy & De Vuyst, 2004). Since only partial oxidation occurs, the fermented product still contains some carbohydrates and is, therefore, of nutritional value in the human diet (Caplice & Fitzgerald, 1999).

The four major fermentation processes include acetic acid, alkali, alcohol and lactic acid fermentation (Mensah, 1997). Vinegar, coffee, wine and cacoa are examples of fermented products where acetic acid fermentation takes place due to the presence of acetic acid bacteria (AAB) (De Vuyst *et al*., 2008; Sengun & Karabiyikli, 2011). Alkali fermentation occurs during the preparation of stink fish, as well as seed based fermented products such as dawadawa/iru, ugba and ogiri. The microbial species *Bacillus subtilis* is responsible for alkali fermentations (Sanni, 1993; Iwuoha & Eke, 1996; Mensah, 1997; Steinkraus, 1997). The fermentation process in these products is controlled by the ammonia produced during protein hydrolysis and the alkaline pH (Steinkraus, 1997). In West Africa, plant seeds that are often fermented in this manner include African locust bean, castor oil bean, sesame and melon seeds (Sanni, 1993). Fermented seed products are prepared firstly by removing the seed coats and boiling the remaining cotyledons. Salt is then added to the cooked seeds and placed on plant leaves in perforated calabashes or baskets. The container is then covered and left to ferment for two to three days. After the fermented product is dried it is ready to be used as a condiment with other dishes such as stews and soups (Mensah, 1997). In products such as beer, wine and bread, alcoholic fermentation takes place due to the

Table 1 Fermented foods prepared and consumed in Africa (Oyewole, 1993; Sanni, 1993; Iwuoha & Eke, 1996; Lee, 1997; Mensah, 1997; Gadaga *et al*., 1999; Blandino *et al*., 2003).

Raw food categories	Name of fermented products and raw food	Nature of fermented product	Country of preparation/ consumption
Cereal based non-alcoholic	Ogi (maize, millet or sorghum), Akamu (maize)	Porridge	Nigeria
	Koko or Akasa (maize)	Porridge	Ghana
	Kenkey, Banku (maize)	Dumplings	Ghana
	Uji (maize, millet or sorghum)	Porridge	Kenya
	Mawe (Maize)	Porridge	Benin
	Kisra (sorghum)	Bread	Sudan
	Mahewu/Magou (maize, wheat)	Non-alcoholic beverage	South Africa
Cereal based alcoholic	Bussa (maize, sorghum or millet)	Alcoholic beverage	Kenya
	Sekete (maize)	Beer	Nigeria
	Leting/Joala (maize or sorghum), Utshival amqomboti (sorghum)	Beer	South Africa
	Bouza (millet, wheat)	Alcoholic beverage	Egypt
	Otika (sorghum)	Alcoholic beverage	Nigeria
	Burukutu (sorghum)	Beer	West Africa
Starchy food non-alcoholic	Gari (cassava)	Flour	West Africa
	Agblima (cassava)	Dumpling	West Africa
	Lafun (cassava)	Flour	West Africa
	Fufu (cassava)	Paste	West Africa
Vegetable proteins	Ugba (oil been seed or sesame seed)	Flavourant	Nigeria
	Dawadawa/Iru (African locust bean)	Condiment	West and Central Africa
	Kawal (Cassia obtusifolla leaves)	Meat substitute	Sudan
	Ogiri (melon seed)	Condiment	Nigeria
Fruit juice alcoholic	Makumbi/Marula wine or beer (marula fruit)	Alcoholic beverage	Zimbabwe
	Mudetemwa (sand apple)	Alcoholic beverage	Zimbabwe
Meat and seafood	Afonnama (beef tripe)	Condiment	Nigeria
	Azu-okpo (fish)	Condiment	Nigeria
	Nsiko (crab)	Condiment	Nigeria

presence of yeasts which produces alcohol and carbon dioxide (Sicard & Legras, 2011). In Africa, a wide variety of traditionally prepared alcoholic beverages such as bussa, sekete, ogogoro, bouza, otika and bukurutu are produced from one type or a mixture of cereals including sorghum, wheat, maize and millet (Oyewole, 1993; Sanni, 1993; Lee, 1997; Blandino *et al*., 2003). Basic steps for the preparation of these beverages include germination of the cereal grains in water, followed by drying and milling into flour. The flower is then mixed with water, boiled and left to ferment (Sanni, 1993; Iwuoha & Eke, 1996; Blandino *et al*., 2003). In Zimbabwe traditionally prepared wine- or beer-like alcoholic beverages are also produced from fruit and are generically known as Makumbi (Gadaga *et al*., 1999). Fruits used for the preparation of these products include fruits from the marula tree (*Sclerocarya birrea* subsp*. caffra*), the buffalo thorn (*Ziziphus mauritiana*), the sand apple *(Parinari* curatellifolia) and the wild loquat (*Uapaca kirkiana)* (Gadaga *et al*., 1999; Mithöfer & Waibel, 2003; Nyanga *et al*., 2007). These beverages are mostly fermented by a combination of yeasts and LAB, with a resulting alcoholic and lactic acid fermentation (Sanni, 1993).

Most traditionally prepared fermented foods in Africa are a result of lactic acid fermentations by LAB, although other microbes can also be present (Oyewole, 1997). Examples of lactic acid fermented cereal based foods commonly prepared in Africa are ogi, kisra and mahewu (Lee, 1997; Blandino *et al*., 2003). Ogi is an important fermented cereal from West Africa used as a traditional weaning food, as a nutritious meal for sick people and as breakfast porridge (Oyewole, 1997). It is mostly prepared from maize, although millet and sorghum can also be used (Blandino *et al*., 2003). The grains are steeped for one to three days in a container, wet-milled and then wet-sieved. The ogi slurry can be fermented further before it is cooked to make porridge (Iwuoha & Eke, 1996; Blandino *et al*., 2003). Kisra is a type of fermented bread commonly prepared in Sudan by fermenting sorghum flour mixed with water into a thick dough. The dough is baked and consumed with stewed meat or vegetables (Blandino *et al*., 2003). Mahewu, a non-alcoholic maize based beverage is prepared by mixing maize porridge with water and adding either wheat, sorghum flour or millet malt before it is left to ferment spontaneously for approximately one day (Gadaga *et al*., 1999; Blandino *et al*., 2003). This product is mostly consumed by adults, but it is also used as a weaning food for infants. Mahewu is also produced commercially in Zimbabwe (Blandino *et al*., 2003).

The lactic acid fermentation of cassava (*Manihot esculenta* Crantz) roots result in a wide variety of nutritious products such as gari and agblima (Sanni, 1993; Mante *et*

al., 2003; Blagbrough *et al*., 2010). Gari, a type of fermented cassava flour is traditionally prepared by peeling cassava roots and grating them into a pulp. The cassava pulp is then placed in Hessian bags and compressed with rocks or wood to remove excess moisture. The bags are hung outside from a tree or hut for approximately four days during which fermentation takes place. Afterwards the dried pulp is sieved and roasted (Iwuoha & Eke, 1996; Mensah, 1997; Kostinek *et al*., 2005). Agblima is fermented cassava dumplings made from cassava flour similarly prepared to gari. The difference is the addition of a traditional starter culture, prepared by leaving cassava shavings in water for five days to increase the fermentation rate of the cassava pulp. The cassava flour is formed into dough and cooked in water to a stiff dumpling which is consumed with stews (Mensah, 1997; Mante *et al*., 2003). Fermentation of the raw cassava roots is important to ensure that cassava products are fit for human consumption. Cyanide, a toxic chemical compound potentially fatal to humans is found in raw cassava roots and, therefore, cassava must be processed before consumption to reduce the cyanide levels. This is achieved during fermentation, as well as during boiling or frying (Edijala *et al*., 1999; Aloys & Angeline, 2009). Fermentation of cassava is also important to increase the short shelf-life of the roots which is less than five days (Oyewole, 1997).

In Asia and Europe lactic fermented vegetables are commonly consumed and are a good source of minerals, vitamins, antioxidants and dietary fibre (Lee, 1997; Jevšnik *et al*., 2009). These products are commercially available, but also traditionally prepared. Since a wide variety of microbes are present on raw vegetables and pasteurization adversely affects product quality, salt is added to enhance the growth of LAB (Jevšnik *et al*., 2009). Well known fermented vegetable products in Asia and Europe include sauerkraut and kimchi, which are used as salads or side dishes (Lee, 1997; Kim & Chun, 2005; Xiong *et al*., 2012). Both these products are made from shredded cabbage and salt, but during kimchi preparation other ingredients such as radish, green onion, red pepper, garlic and ginger are added (ten Brink *et al*., 1990; Lee & Lee, 1993; Lee, 1997; Park *et al*., 2011). The salt concentration of sauerkraut is between 0.7 and 3.0%, while that of kimchi is between 3.0 and 5.0% (Lee, 1997). The lactic acid fermentation in both products is initiated by *Lactobacillus* spp., but in the preparation of kimchi the fermentation process is usually shorter (Lee, 1997).

In Asia the lactic acid fermentation of meat products is often enhanced by adding salt and an additional carbohydrate source such as sugar, flour, rice or millet to ensure a low pH (Lee, 1997; Rivera-Espinoza & Gallardo-Navarro, 2010). Examples of such

products include sai-krok-prieo prepared in Thailand and nem-chua prepared in Vietnam, which are both similar to salami commonly found in Europe (Lee, 1997). Fermented sausages are generally made from ground raw meat, often pork, fat, curing agents (nitrate/nitrite), sugar, spices and salt which are mixed and stuffed into a casing. The sausage is then fermented by LAB, mainly *Lactobacillus* and *Pediococcus* spp. Yeasts and mycelial fungi can also be present, especially in traditionally prepared sausages. After fermentation, which can last for several days to several months, the sausage is dried before it is consumed (Lücke, 1994; Lee, 1997; Caplice & Fitzgerald, 1999; Rivera-Espinoza & Gallardo-Navarro, 2010; Papavergou, 2011). In Europe fermented sausages are made by adding starter cultures which shorten the fermentation process and ensure product safety and quality (Lücke, 1994). If nitrate is used as a curing agent staphylococci and micrococci are also added to the LAB starter to guarantee nitrate reductase activity (Lücke, 1994; Hugas & Monfort, 1997; Hammes, 2012).

In the north-eastern coastal regions of Korea a variety of traditionally fermented fish products are still consumed today, generically referred to as sikhae. Sikhae, different from stink fish, is a lactic acid fermented food where *Lactobacillus* and *Leuconostoc* spp. are mainly responsible for fermentation (Lee, 1997; Rhee *et al*., 2011). The dominance of LAB can be explained by the inclusion of garlic (3-4%) during sikhae preparation, which has an inhibitory effect on other bacteria including species of *Bacillus, Pseudomonas* and *Micrococcus* (Lee, 1997). Garlic may also provide LAB with fermentable carbohydrates along with the millet usually added to sikhae (Paludan-Müller *et al*., 1999). The salt concentration of sikhae (8%) also creates favourable growth conditions for LAB (Lee, 1997).

The lactic acid fermentation of milk is popular in many African countries (Mensah, 1997; Abdelgadir *et al*., 1998). Most milk fermentations in Africa are traditionally prepared by undefined starters at home from raw milk by backslopping, spontaneous fermentation from microbes in the environment, LAB inherent in the raw milk or by preparation in used fermentation containers (Oyewole, 1997; Kebede *et al*., 2007). Sheep, goat or mainly cow milk are used for the production of fermented milk in Africa (Narvhus & Gadaga, 2003). Fermented milks have a characteristic semi-solid and curdled texture, because the casein proteins in the milk are dispersed in the liquid product where an increase in viscosity occurs due to physical and chemical changes that takes place during fermentation (Wood, 1994; Gonfa *et al*., 2001).

In Eastern and Southern Africa, excluding Zimbabwe and Kenya, about 80-90% of milk produced on rural farms is consumed by the tribal people themselves (Kebede *et al*., 2007). Traditionally fermented dairy products consumed in rural African communities include products similar to cottage-cheese, butter and a wide variety of fermented milks (Iwuoha & Eke, 1996; Abdelgadir *et al*., 1998; Gadaga *et al*., 1999; Aloys & Angeline, 2009). Examples of traditionally prepared products that all resemble cottage cheese are hodzeko from Zimbabwe, warankasi from Nigeria and jibna-beida from Sudan (Abdelgadir *et al*., 1998; Gadaga *et al*., 1999; Aloys & Angeline, 2009). Traditionally prepared butter products include maishanu from Nigeria, amavuta from Burundi and samin from Sudan (Abdelgadir *et al*., 1998; Gadaga *et al*., 1999; Aloys & Angeline, 2009). Information on starter cultures used to produce these products in Sub-Saharan Africa is limited (Holzapfel, 2002).

In Asia, koumiss is an example of a traditionally fermented milk, while in Europe fermented milks such as kefir, yoghurt and viili are popular, as well as a wide variety of cheeses (Toba, 1990; Wood, 1994; Garrote *et al*., 1997; Kücükcetin *et al*., 2003; Liu, 2003; Irigoyen *et al*., 2005; Bouamra-Mechemache *et al*., 2008; Xie *et al*., 2011). Over the last century, fermentation of dairy products has been well researched in Europe where safe and nutritious products are commercially produced on a large scale through defined processes (Lee, 1997). Small scale fermented food producers in developing countries have so far relied primarily on improvements regarding product safety and quality through years of experience, by changing production processes as problems are identified, rather than through scientific research (Valyasevi & Rolle, 2002).

Antimicrobial activity of fermented foods

Developed countries have the resources to preserve food through freezing and canning. However, the main preservation techniques in developing countries are dehydration, salting and fermentation. This is because of the accessibility and low cost of these preservation methods (Steinkraus, 1994; Oyewole, 1997; Holzapfel, 2002; Motarjemi, 2002). As a result of inadequate sewage disposal facilities and contaminated water sources in developing countries, pathogens are frequently present in raw milk and on other raw foods. The high ambient temperatures and lack of refrigeration facilities in these countries leads to multiplication of the pathogenic microbes and to higher risk of infection (Mensah, 1997; Motarjemi, 2002; Gran *et al*., 2003). Pathogenic microbes that have been found in raw milk and naturally fermented raw milk include *Escherichia coli, Vibrio cholerae, Shigella* spp., *Staphylococcus aureus, Yersinia* spp., *Listeria*

monocytogenes, Mycobacterium tuberculosis, Mycobacterium bovis, Salmonella spp*., Brucella abortus, Campylobacter jejuni* and *Bacillus cereus* (Gran *et al*., 2003; Herreros *et al*., 2005; Mufandaedza *et al*., 2006).

During fermentation the growth of pathogens, as well as other spoilage organisms, are frequently inhibited through antimicrobial components produced by LAB (Varadaraj *et al*., 1993; Adams & Nicolaides, 1997; Herreros *et al*., 2005; Park *et al*., 2005). By incorporating LAB which produces antimicrobial components in commercial starter cultures the use of chemical preservatives such as sodium benzoate and sodium metabisulphite can be reduced (Joseph & Akinyosoye, 1997; de Mendonça *et al*., 2001; Herreros *et al*., 2005). These antimicrobial components produced include organic acids, hydrogen peroxide, carbon dioxide, acetaldehyde, diacetyl, ethanol and bacteriocins (Adams & Nicolaides, 1997; Caplice & Fitzgerald, 1999; Ross *et al*., 2002; Herreros *et al.,* 2005; González *et al*., 2007).

Organic acids

During food fermentation LAB produce organic acids as a result of carbohydrates that are metabolised. The predominant acid produced by LAB is lactic acid. Other acids such as acetic acid and propionic acid can also be formed by bacteria present during fermentation (Caplice & Fitzgerald, 1999; Dalié *et al*., 2010). The presence of organic acids in a food medium results in a reduction of the pH (Adams & Nicolaides, 1997; Mante *et al*., 2003; Mufandaedza *et al*., 2006; Charlier *et al*., 2009). The reduced pH results in unfavourable growth conditions for a wide variety of pathogens and spoilage microbes whereas LAB are more tolerant to lower pH environments (Ross *et al*., 2002). For example, the approximate pH tolerance for *Escherichia coli* is between 4.4 and 9.0, where some *Lactobacilli* can tolerate pH environments of between 3.0 and 7.2 (Mensah, 1997).

The inhibitory effect of organic acids depends on the amount of acid in its undissociated form present in the food medium (Charlier *et al*., 2009). The undissociated form of an acid can easily diffuse over the cytoplasmic membrane of the spoilage microbes. The higher pH of the intracellular environment results in the dissociation of the acid and a proton is released. This increase in protons leads to a decrease of the pH of the cytoplasm and causes inactivation of pH sensitive enzymes and structural changes in cellular membranes which have inhibiting or lethal effects (Schnürer & Magnusson, 2005; González *et al*., 2007; Dalié *et al*., 2010). Organic acids differ in their ability to inhibit microbes depending on their individual pKa values (Adams,

1990). Acids with a high pKa dissolve only partially in an aqueous food medium, which results in a higher amount of undissociated acid present (Schnürer & Magnusson, 2005). Acetic acid (pKa 4.8) and propionic acid (pKa 4.9) are, therefore, stronger antimicrobials than lactic acid (pKa 3.9) (Charlier *et al*., 2009).

Hydrogen peroxide

LAB possess flavoprotein which oxidises to produce hydrogen peroxide (H_2O_2) in the presence of oxygen (Adams, 1990). The absence of the catalase enzyme in LAB which disintegrates H_2O_2 results in the accumulation of H_2O_2 in the fermented food medium (Caplice & Fitzgerald, 1999). The oxidation of protein structures and membrane lipids of spoilage microbes present such as *Staphylococcus aureus* and *Pseudomona* spp. mediates the inhibitory effect of H_2O_2 (Adams, 1990; Adams & Nicolaides, 1997, Caplice & Fitzgerald, 1999). Fortunately the fermenting LAB are more resistant to the inhibiting effects of H_2O_2 in comparison to other Gram-negative bacteria (Caplice & Fitzgerald, 1999). The amount of H_2O_2 produced depends on the availability of oxygen in the food medium at the beginning of fermentation, keeping in mind that lactic acid fermentation essentially occurs under anaerobic conditions (Adams, 1990; Adams & Nicolaides, 1997). Sufficient amounts of H_2O_2 must be produced to have inhibitory effects to meet the minimum inhibitory concentration (MIC) which differs between microbial species and strains (Dalié *et al*., 2010). For example, the MIC for *Staphylococcus aureus* was found to be 5 - 6 $mg.ml^{-1}$, far less than the MIC for *Lactococcus lactis* (125 $mg.ml^{-1}$) (Adams & Nicolaides, 1997). Hydrogen peroxide is also responsible for the activation of the antimicrobial lactoperoxidase system in milk, which involves the production of molecules inhibitory to Gram-negative bacteria such as hypothiocyanite during the catalysation of thiocyanate by a lactoperoxidase. This system explains the inhibitory effect of H_2O_2 when it is present in non-lethal amounts in fermented milk (Adams, 1990; Dalié *et al*., 2010).

Carbon dioxide

Heterofermentative LAB produces carbon dioxide (CO_2) as an end-product of hexose fermentation (Caplice & Fitzgerald, 1999). The antimicrobial effect of CO_2 is achieved in two ways. Firstly, an anaerobic environment is created which favours the growth of anaerobic LAB and some yeasts, but inhibits obligated aerobic microbes such as mycelial fungi and Gram-negative bacteria (Eklund, 1984; Lindgren & Dobrogosz, 1990; Adams & Nicolaides, 1997). Secondly, a rise in the CO_2 pressure may result in

inefficient cell membrane transport mechanisms, which mediate pH changes of intracellular and extracellular environments and inhibit enzymatic reactions (Adams & Nicolaides, 1997; Caplice & Fitzgerald, 1999).

Diacetyl

Diacetyl produced by heterofermentative LAB during fermentation can have antimicrobial effects (Ross *et al*., 2002). Diacetyl (2,3-butanedione), an end-product of citrate metabolism is important for flavour and aroma formation in dairy products, especially butter. This compound also inhibits various microbes such as *Escherichia coli, Salmonella* spp*., Staphylococcus aureus, Bacillus* spp*., Mycobacterium tuberculosis* and *Aeromonas hydrophila* (Adams & Nicolaides, 1997; Caplice & Fitzgerald, 1999). The antimicrobial mechanism of diacetyl is active at a low pH and believed to be the cause of the disruption of arginine utilisation (Caplice & Fitzgerald, 1999; Schnürer & Magnusson, 2005). Although diacetyl is a well known antimicrobial, the concentration produced is often too low to have a measurable lethal effect (Adams & Nicolaides, 1997; Caplice & Fitzgerald, 1999; Dalié *et al*., 2010). An increase of this component during fermentation to ensure antimicrobial activity can compromise the organoleptic properties of the fermented food (Schnürer & Magnusson, 2005). For example, for inhibition of Gram-negative bacteria 200 $mg.kg^{-1}$ diacetyl is needed where acceptable levels of diacetyl in dairy products are between 2 - 7 $mg.kg^{-1}$ (Adams & Nicolaides, 1997, Schnürer & Magnusson, 2005).

Bacteriocins

Bacteriocins are proteins or peptides that are ribosomally produced by bacterial species and strains (Garneau *et al*., 2002). Numerous LAB synthesize bacteriocins which have varying spectrums of inhibition on closely related Gram-positive bacteria and certain yeast strains (Topisirovic *et al*., 2006; Charlier *et al*., 2009). Inhibition of pathogenic foodborne bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* by bacteriocins lead to the realisation of their potential role as natural food preservatives (Adams & Nicolaides, 1997; Caplice & Fitzgerald, 1999; Van der Merwe *et al*., 2004; Charlier *et al*., 2009).

Classification of bacteriocins produced by LAB can be done by dividing them into four groups according to their structure, chemical properties and function (Garneau *et al*., 2002; Topisirovic *et al*., 2006). The four groups include *Class I*, *Class II*, *Class III* and *Class IV* (Garneau *et al*., 2002; Topisirovic *et al*., 2006). *Class I* and *Class II*

bacteriocins are mostly associated with LAB commonly found in food (Caplice & Fitzgerald, 1999).

Nisin is the best characterized bacteriocin and is used as a food preservative in dairy products, brewing, packaged and canned meats and in sausages world-wide (Stiles & Holzapfel, 1997; Casalta & Montel, 2008; Sobrino-López & Martín-Belloso, 2008). Nisin is produced by *Lactococcus lactis* subsp*. lactis* and classified as a lantibiotic (*Class I*). This antimicrobial peptide consisting of 34 amino acids has a broad spectrum of activity against Gram-positive bacteria, as well as *Clostridium botulinum* and its spores (Stiles & Holzapfel, 1997; Ross *et al*., 2002; Sobrino-López & Martín-Belloso, 2008). It is stable in foods with a low pH and levels used in food products are between 2.5 and 100 ppm (Caplice & Fitzgerald, 1999). Nisin inhibits bacteria by creating pores in the outer cellular membranes, which causes depolarization of the membranes and results in leaking of intracellular materials (Cleveland *et al*., 2001; Ross *et al*., 2002). Other bacteriocins may inhibit microbes by disrupting cell membrane synthesis (Cleveland *et al*., 2001).

Health benefits

Almost one third of the human diet consists of fermented foods, which emphasise the importance of these products to human health. Fermented milk and fermented cereal products are of the most important, because they are produced and consumed in the largest amounts (Campbell-Platt, 1994). In Africa fermented food may help to decrease foodborne diseases by improving product safety. Fermented food may also contribute to reducing hunger by adding nutritional value to food and increasing the bioavailability of nutrients (Motarjemi, 2002; Nah & Chau, 2010).

The consumption of milk, a highly nutritious beverage, is made more acceptable to lactose-intolerant individuals through milk fermentation due to the conversion of lactose to lactic acid. For example, in yoghurt 25 - 50% of the lactose is converted to lactic acid and the end concentration of lactose is reduced to approximately 4% (Steijns, 2008; Brown-Esters *et al.,* 2012). Previous studies have concluded that adults from African and Asian decent are characteristically lactose-intolerant. This may be a result of dairy herding not being practiced by their ancestors due to ecological and environmental factors which then resulted in the lactose-intolerant phenotype being transferred from one generation to the next (Bloom & Sherman, 2005).

Fermented foods play an important role in the nutritional status of populations in Africa. In many African countries infants often suffer from malnutrition due to food

shortages, poor bioavailability of nutrients and low nutritional value of the available foods (Oyewole, 1997). Malnutrition usually leads to illnesses in young children such as kwashiorkor and marasmus. Protein deficiencies in the diet cause kwashiorkor which leads to a weak immune system. Protein and energy deficiencies cause marasmus resulting in poor growth and extensive muscle and fat loss (Steinkraus, 1997). Fermentation is a cost effective way to enrich food with essential amino acids and vitamins which can help prevent malnutrition (Holzapfel, 2002; Motarjemi, 2002).

Fermented food often includes LAB strains with probiotic properties. The FAO/WHO defines probiotics as “live micro-organisms which, when administered in adequate amounts, confer health benefits on the host”. The health benefits of probiotics for humans include protection against inflammatory bowel diseases and gastrointestinal infections. Probiotics can be used instead of antibiotics in the treatment of enteric infections and simultaneously reduces diarrhea caused by antibiotics. Probiotic cultures regulate human intestinal bacteria and inhibit harmful bacteria that can be present in the intestines. They also support the body’s immune system, modulation of allergic diseases and treatment of infections formed during pregnancy (Bernardeau *et al*., 2008; Giraffa *et al*., 2010). Examples of LAB in fermented milks that have probiotic properties are *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum* (Adams, 1990). Consumption of dairy products is the best way to provide the human body with probiotic bacterial strains. However, currently there are limited amounts of probiotic strains available which can be used for commercial applications (Bernardeau *et al*., 2008; Giraffa *et al*., 2010).

C. Fermented milks of Sub-Saharan Africa

A diverse variety of traditionally fermented milk products are available in Sub-Saharan Africa, each with unique organoleptic properties (Steinkraus, 1994; Kebede *et al*., 2007). These products are made by local farmers in the communities (Gadaga *et al*. 1999; 2000; Mathara *et al*., 2004; Bille *et al*., 2007). Very little information is available on the properties of some of these products. No information has been reported as yet on the microbial consortiums of the Sub-Sahara African fermented milks kwerionik, katanik, chekapmkaika, mass, omaere, chambiko, madila, urubu, makamo and macunda.

Chekapmkaika and kwerionik

In Uganda, milk fermentation is mostly carried out by the pastoral tribes including the Bahima in Western Uganda, fermenting mostly Zebu cow milk, and the agro-pastoral tribes including the Itesot and the Sebei in Eastern Uganda, fermenting longhorn Ankole cow milk. Goat milk is not often used for the production of fermented milk products since the dairy breeds have only been recently introduced to the country. The gourds used for fermentation are mostly smoked by using plant materials such as grass or hard wood. In Eastern Uganda smoking chips or charcoal are sometimes used along with plant materials. Kwerionik is a fermented milk product traditionally prepared in Eastern Uganda from raw cow milk. The raw milk is placed in smoked gourds and left to ferment at ambient temperatures for three to seven days. Kwerionik is a curdled milk product consumed within seven days. If kwerionik is kept for eight to 28 days it is known as katanik and chekapmkaika is kwerionik which has been kept for 29 days up to a year. Whey is constantly removed from the chekapmkaika and fresh or boiled milk is added every second day or once a week depending on the ambient temperatures. The storage quality is enhanced by removal of the top layer which is likely to contain mycelial fungi before the addition of milk. This product is consumed during the dry seasons with blood or porridge (personal communication, Moses Matovu, Food Bioscience Research Centre, Uganda).

Masse and homemade yoghurt

Masse is a well known fermented milk beverage from Mozambique, made by leaving raw cow milk in a pot overnight at ambient temperatures or for a few days to ferment spontaneously. Masse is an unsweetened curdled milk product with a strong acidic taste and a firm semi-fluid consistency which is consumed within seven days. Homemade yoghurt is prepared by adding one cup of yoghurt from the previous day to approximately 20 L of boiled milk in a pot. The milk is then left to ferment for a day at ambient temperatures. Homemade yoghurt is consumed as a beverage and occasionally sugar is added. This is different from masse in that the fermented milk has a less acidic taste and a more creamy texture (personal communication, Custodia Macuamule, University of Stellenbosch, South Africa).

Omashikwa

Omashikwa is a traditionally prepared buttermilk product made by local farmers, especially the women of the Owambo and Herero tribes in the rural areas of Namibia

(Bille *et al*., 2002; 2007). Omashikwa is sold directly from the farmers to the public or on the local markets to generate a source of income for their families. Due to the inconsistency in the quality of the fermented milk, omashikwa is often bought after sensory evaluation by the consumer. It is consumed as a beverage or as a condiment along with sorghum, maize, millet flour gruel or porridge (Bille *et al*., 2007).

Omashikwa is made by placing 20 L of raw milk and 12 - 15 pieces of root of the *Omunkunzi* tree (*Boscia albitrunca*) in rinsed plastic containers, calabashes or gourds and adding 400 ml of previously made omashikwa as a starter. The mixture is then left to ferment at ambient temperatures (27º - 36 ºC) for two to three days. When the milk is fermented the roots are removed before churning of the product for two to three hours. During this process the butter that forms on top of the fermented product is removed and the remaining buttermilk is referred to as omashikwa. No whey removal is needed during the production of omashikwa since it is already a viscous product with a thick consistency. This can be due to the presence of exopolysaccharide producing microbes or gum-like compounds secreted from the *Omunkunzi* roots. Omashikwa has a slimy texture with an acidic (approximate pH of 3.3), bitter taste and a rooty flavour (Bille *et al*., 2002; 2007).

Other fermented milks

Amasi is a well known fermented milk consumed in South Africa, Zimbabwe and Lesotho and it is often consumed with maize porridge as a main meal or with ground sorghum between meals (McMaster *et al*., 2005; Gadaga *et al*., 1999; 2000; Todorov *et al*., 2007). In all three countries it is traditionally prepared, but in South Africa it is also produced on a commercial scale. Commercially produced amasi is a sweetened curdled milk product with a shelf life of 21 days at 4 ºC and traditionally produced amasi is an unsweetened thick curdled milk with a shelf-life of three days at room temperature and a pH between 3.6 and 4.2 (McMaster *et al*., 2005; Todorov *et al*., 2007). Traditional amasi is prepared in the rural communities by keeping raw cow milk in a pot or gourd to ferment spontaneously at ambient temperatures for two to three days. Sometimes a small amount of previously made amasi is added to the raw milk to accelerate the fermentation process. The whey is drained through a hole in the pot or gourd after fermentation has occurred (Gadaga *et al*., 1999; 2000; 2001a; Todorov *et al*., 2007).

Another fermented milk both traditionally prepared and commercially available is madila in Botswana (Ohiokpehai, 2003). Traditional madila is prepared by straining raw cow or occasionally goat milk into an enamel bucket and then leaving the milk to

ferment for 24 h. Thereafter, the fermented milk is added to previously fermented milk which has been placed in a woven polypropylene sack, where after the sack is hung from a beam for a few days to ensure drainage of extra whey. After the concentrated madila in the woven sack is mixed with fresh milk in a four to one ratio, the madila is ready to be consumed or sold on the market (Ohiokpehai & Jagow, 1998; Ohiokpehai, 2003; Parry-Hanson *et al*., 2009). Madila is a liquid or semi-solid curdled product often flavoured with fruit juice or artificial colorants (Ohiokpehai, 2003).

Other fermented milks commonly prepared in Sub-Saharan Africa include urubu from Burundi, nono/nunu from Nigeria or Northern Ghana, fermented milk from the Fulani community in Burkina Faso, kule noato from the East African Rift Valley, sussa/suusac from Somalia or Kenya and ergo from Ethiopia. Cow milk is mostly used for the preparation of these milks, except for sussa which is made from camel milk and occasionally fermented milk from the Fulani community is made from goat milk. These fermented milks are made according to similar methods to masse although kule noato, ergo and sometimes sussa are prepared in smoked containers (Isono *et al*., 1994; Gonfa *et al*., 2001; Mathara *et al*., 2004; Savadogo et al., 2004; Lore *et al*., 2005; Patrignani *et al*., 2006; Farah *et al*., 2007; Aloys & Angeline, 2009; Akabanda *et al*., 2010; Okonkwo, 2011). The smoked fermentation vessels contribute to the distinct flavour of the fermented milk and may also have an effect on the fermenting microbes present (Lore *et al*., 2005). It has also been found that by using smoked vessels it slows down souring and reduces coliform numbers (Gonfa *et al*., 2001).

In Sub-Saharan Africa fermented milk is often used as a basis to prepare other traditional fermented dairy products. Makamo (known as kivuguto in Rwanda) is a traditionally fermented milk from Uganda used to make macunda and mashita. Makamo is prepared by adding makamo from the previous day to raw or boiled cow milk and then leaving the milk to ferment for two to three days at ambient temperatures in a smoked gourd. Lemon juice is sometimes added to accelerate souring. The consistency of makamo is thicker than milk and it is consumed as a beverage or a dessert. Makamo can be kept for four days to a week. To prepare macunda and mashita, makamo is churned for up to an hour to facilitate separation of fat globules from the fermented milk. The churned makamo is then filtered through a sieve to separate the Mashita from the Macunda. Macunda is a viscous white buttermilk with a short shelf-life of two days and consumed by women and children. Mashita directly translated means oil and is the fat globules that form on the top of the macunda. It is yellow in colour and has a unique smell. Mashita is often boiled into ghee (personal

communication, Moses Matovu, Food Bioscience Research Centre, Uganda). Other examples of traditionally fermented milks churned to obtain traditional buttermilk and butter are pendidam in Cameroon, rob in Sudan and ergo in Ethiopia (Abdelgadir *et al*., 1998; 2001; Pamela *et al*., 1999; Gonfa *et al*., 2001).

D. Microbes responsible for milk fermentation

Lactic acid bacteria

Bacteria that taxonomically belong to the heterogeneous group of LAB all have the following characteristics: Gram-positive; catalase-negative; acid tolerant; devoid of cytochromes; aerotolerant; non-sporulating; and they are strictly fermentative rods or cocci which produce lactic acid as the major product from the energy-yielding fermentation of sugars (Stiles & Holzapfel, 1997; Temmerman *et al*., 2004; Wessel *et al*., 2004). The LAB genera generally associated with the fermentation of a variety of foods are *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus, Pediococcus, Oenococcus, Teragenococcus, Cronobacterium* and *Weissella* (Table 2) (Adams & Martau, 1995; Stiles & Holzapfel, 1997; Temmerman *et al*., 2004; Wessel *et al*., 2004). These bacteria are not only isolated from fermented foods but they are also found in a wide variety of environmental habitats including raw food products (Adams & Marteau, 1995; Wouters, *et al*., 2002; Furet *et al*., 2004). LAB are also natural inhabitants of the oral cavity and the gastrointestinal track of mammals, living in a complex symbiosis with the host (Adams & Marteau, 1995; Furet *et al*., 2004).

Among the LAB group there are two distinct carbohydrate fermentation pathways (Fig. 1). Based on these two fermentation pathways, LAB can be sub-categorised into two distinct groups, homofermentative or heterofermentative (Zúñiga *et al.*, 1993; Caplice & Fitzgerald, 1999). The Embden-Meyerhof-Parnas pathway or Glycolysis where lactic acid is produced as the major or only end-product of homolactic metabolism and the 6-phosphogluconate/phosphocetolase pathway where in addition to lactic acid, other end-products including CO_2 and ethanol are produced during heterolactic metabolism (Caplice & Fitzgerald, 1999). Homofermentative LAB include *Streptococcus, Lactococcus, Enterococcus, Pediococcus* and heterofermentative LAB include *Weissella and Leuconostoc* (Zúñiga *et al.*, 1993; Ross *et al*., 2002).

Some LAB have the ability to catabolise lactic acid to acetic acid and CO_2. Lactic acid catabolism can occur under aerobic conditions where lactic acid is oxidised by NAD^+-independent lactate dehydrogenase (LDH) by *Lb. plantarum, Lb. carvatus, Lb.*

Table 2 Prevalent lactic acid bacteria genera responsible for the fermentation of various types of food (Temmerman *et al*., 2004; Zamfir *et al*., 2006).

Food type	**Fermenting LAB genera**
Milk	*Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus, Pediococcus*
Meat	*Lactobacillus, Leuconostoc, Pediococcus*
Vegetables	*Lactobacillus, Pediococcus*
Cereal	*Lactobacillus*
Wine	*Oenococcus*

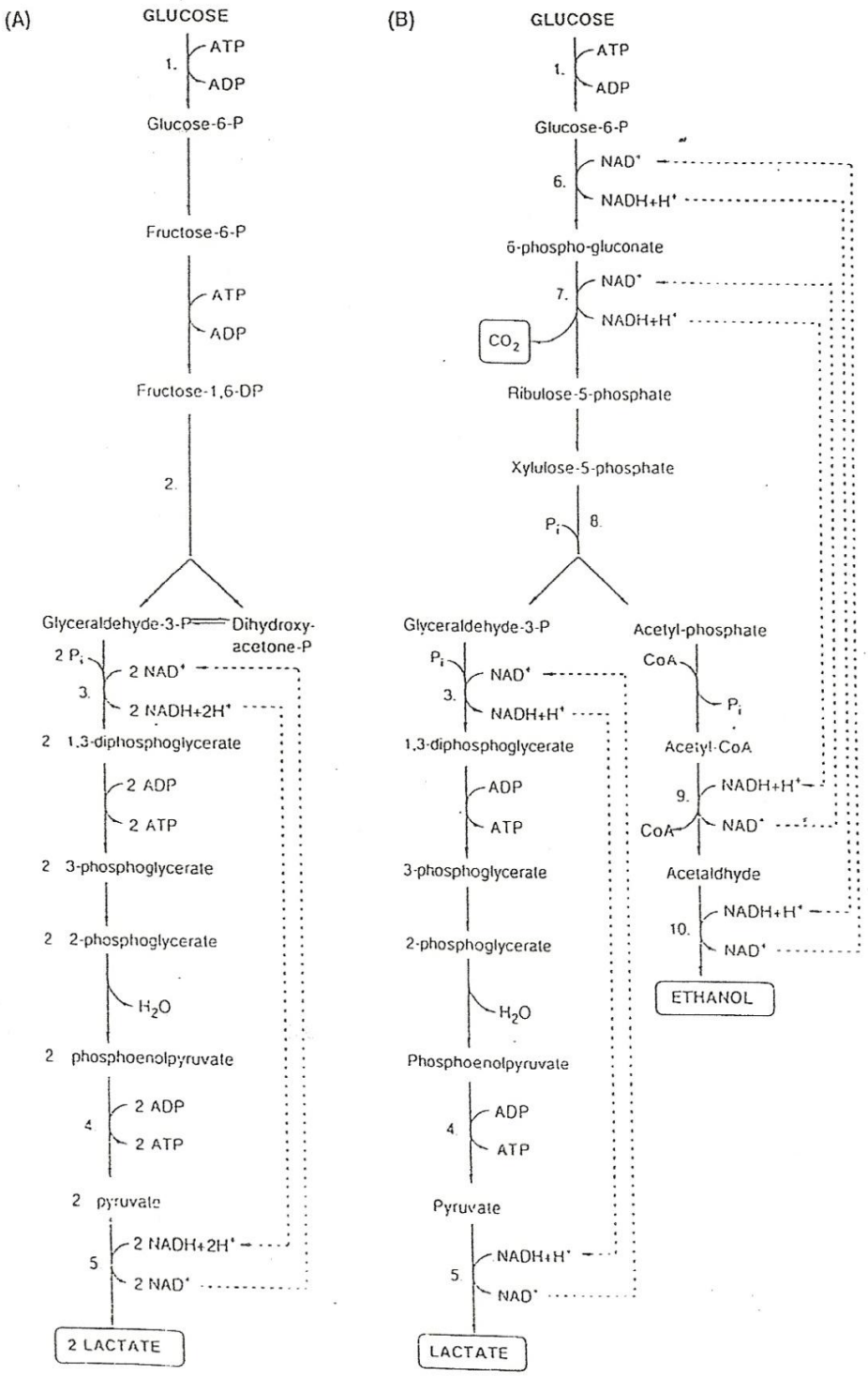

Figure 1 Glucose fermentation in homofermentative LAB through Glycolysis (A) and glucose fermentation in heterofermentative LAB through the Embden-Meyerhof-Parnas pathway (B) (Axelsson, 1993).

casei and *Lb. sake* (Liu, 2003). Certain LAB such as *Lb. buchneri, Lb. brevis* and *Lb. plantarum* also catabolises lactic acid by NAD^+-independent LDH, but under anaerobic conditions (Liu, 2003).

LAB present in dairy fermentations can generally be classified in terms of optimum growth temperatures, namely mesophilic and thermophilic LAB. Mesophilic LAB grows optimally between 20º - 30 ºC and thermophilic LAB between 30º - 45 ºC. Therefore, mesophilic LAB is often isolated from traditional fermented dairy products from the colder Northern and Western European countries and thermophilic LAB from traditional fermented dairy products from hotter sub-tropical regions (Wouters *et al*., 2002).

Lactobacillus *and* Streptococcus

Of all the LAB the genus *Lactobacillus* contains the most species (Giraffa *et al*., 2010). Currently the genus consists of 174 species and 27 subspecies (Deutche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), 2012; List of Prokaryotic names with Standing in Nomenclature (LPSN), 2012). This heterogeneous group is Gram-positive, rod-shaped, strictly fermentative, aciduric or acidophilic, non-endospore forming bacteria which grow well in anaerobic environments, although they are aerotolerant (Stiles & Holzapfel, 1997; Bernardeau *et al*., 2008; Giraffa *et al*., 2010). Lactobacilli have been isolated from a variety of habitats including the intestinal tract of mammals, plant material, raw milk and sewerage (Stiles & Holzapfel, 1997; Giraffa *et al*., 2010).

The genus *Lactobacillus* can be subdivided into three groups based on sugar fermentation, namely facultative heterofermentative *(Group I)*, obligated heterofermentative *(Group II)* and obligated homofermentative *(Group III)* (Table 3) (Stiles & Holzapfel, 1997; Bernardeau *et al*., 2008). Lactobacilli from *Group I* ferment hexoses to lactic acid and pentoses to lactic acid and acetic acid, and gas is not produced from glucose, but from gluconate. *Group II* bacteria produce carbon dioxide, lactic acid, acetic acid and/or ethanol from hexoses, and produce gas from glucose. Lactobacilli from *Group III* do not ferment gluconate or pentoses, but ferment glucose to lactic acid. *Lactobacillus* spp. from all three of these groups can take part in food fermentation. However, lactobacilli from the obligated heterofermentative group are often responsible for food spoilage (Stiles & Holzapfel, 1997).

LAB that belong to the genus *Streptococcus* is Gram-positive, catalase-negative, anaerobic, aerotolerant, coccus-shaped cells grouped in linear chains (Stiles & Holzapfel, 1997; Delorme, 2008). In this genus only one species, *Streptococcus*

Table 3 Lactobacilli associated with dairy products divided into three major groups based on their sugar fermentation (Delfederico *et al*., 2006; Zamfir *et al*., 2006; Bernardeau *et al*., 2008; El-Baradei *et al*., 2008; Schleifer, 2009; Gawad *et al*., 2010).

Facultative heterofermentative *(Group I)*	**Obligated heterofermentative *(Group II)***	**Obligated homofermentative *(Group III)***
Lb. delbrueckii subsp. *delbrueckii*	*Lb. animalis*	*Lb. brevis*
Lb. delbrueckii subsp. *bulgaricus*	*Lb. casei*	*Lb. buchneri*
Lb. delbrueckii subsp. *lactis*	*Lb. curvatus*	*Lb. fermentum*
Lb. delbrueckii subsp. *indicus*	*Lb. cypricasei*	*Lb. hilgardii*
Lb. acidophilus	*Lb. paracasei* subsp. *paracasei*	*Lb. kefiri*
Lb. crispatus	*Lb. plantarum*	*Lb. parakefiri*
Lb. gasseri	*Lb. rhamnosus*	*Lb. reuteri*
Lb. helveticus		
Lb. johnsonii		
Lb. kefiranofaciens		
Lb. salivarius		

thermophilus (synonym*: Sc. salivarius* subsp*. thermophilus*) is “generally recognised as safe” (GRAS) and found in dairy environments (Delorme, 2008; De Vuyst & Tsakalidou, 2008; DSMZ, 2012). *Streptococcus thermophilus* is the only *Streptococcus* spp. that is used as a starter along with one or more LAB strains from the genus *Lactobacillus.* These mixed strain starter cultures are used in various dairy fermentations, including the production of yoghurt, fermented milks and Italian and Swiss-type cheeses (Stiles & Holzapfel, 1997; Delorme, 2008).

Lactobacillus delbrueckii subsp*. bilgaricus* and *Sc. thermophilus* have been isolated form commercially produced fermented milk such as laban from Lebanon and Columbian yoghurt (Chammas *et al*., 2006; Vélez *et al*., 2007; Giraffa *et al*., 2010). Some strains of *Lb.delbrueckii* subsp*. bulgaricus* and *Sc. thermophilus* can produce exopolysaccharides which enhance the product texture by stabilising the yoghurt gel and lowering syneresis (Wouters *et al*., 2002; Sodini *et al*., 2004). The chemical structures of exopolysaccharides from yoghurt starter bacteria vary and the major monomers identified are glucose, rhamnose and galactose (Wouters *et al*., 2002).

Flavour compounds present in milk fermented with *Lb. delbrueckii* subsp*. bulgaricus* strains includes 2-butanol, dimethyl disulfide and acetic acid. In milk fermented with *Sc. thermophilus* 2,3-pentanedione, acetoin and diacetyl are present. The flavour profile of milk fermented with both *Lb. delbrueckii* subsp*. bulgaricus* and *Sc. thermophilus* includes hexanoic acid, acetone, butanoic acid and acetaldehyde. The latter is the most important flavour compound in yoghurt, mainly produced by *Lb. delbrueckii* subsp*. bulgaricus* as an end-product of threonine metabolism during milk fermentation (Chammas *et al*., 2006).

During growth in milk *Sc. thermophilus* produces folic acid. The amount of folic acid produced is strain-dependent and the concentration of this acid in fermented milk products can decrease due to utilisation of folic acid by certain strains of *Lb. delbrueckii* subsp*. bulgaricus* (Wouters *et al*., 2002). Folic acid has health benefits such as the prevention of birth defects and potential decrease of cardiovascular diseases (Cornel *et al*., 2005). Therefore, it is important to construct a yoghurt starter culture of the optimal combination of strains to ensure a desirable end-product with a higher folic acid concentration (Wouters *et al*., 2002).

Thermophilic LAB strains have good acidifying activity as 10 $ml.L^{-1}$ of inoculum reduce the initial pH (6.6) of milk in 6 h at 42 ºC to a pH of 5.3 (Chammas *et al*., 2006). According to Chammas *et al*. (2006) the maximum acidification rate of *Sc. thermophilus* (0.0085 $pH.min^{-1}$) is much lower than that of the *Lb. delbrueckii* subsp*. bulgaricus*

(0.0111 $pH.min^{-1}$). Using *Sc. thermophiles,* an acidified product can be produced if *Sc. thermophilus* is used in combination with *Lb. delbrueckii* subsp*. bulgaricus* (Stiles & Holzapfel, 1997).

Various *Lactobacillus* spp. have been isolated from traditionally fermented milk products (Bernardeau *et al*., 2008). *Lactobacillus delbrueckii* subsp*. bilgaricus* commonly present in fermented milks have been isolated from rayeb (10.5% total microbial isolates) and zabady (from 2 of 11 samples analysed), which are traditionally fermented milks from Egypt, as well as from traditionally prepared laban from Lebanon (Chammas *et al*., 2006; El-Baradei *et al*., 2008; Gawad *et al*., 2010). From zabady low amounts of other lactobacilli have also been isolated, including *Lb. johnsonii.* Some *Lb. johnsonii* strains have probiotic properties and have the potential to enhance the therapeutic value of a product (El-Baradei *et al*., 2008). *Lactobacillus acidophilus* and *Lb. helveticus* were also found in rayeb where the largest percentage (30%) of the microbial consortium consisted of lactobacilli (Gawald *et al*., 2010). In traditionally fermented milk products *Lactobacillus* spp. are frequently isolated in conjunction with members of the genus *Streptococcus* (Bernardeau *et al*., 2008). In both zabady and rayeb *Sc. thermophilus* was also present. Other *Streptococcus* species including *Sc. durans* and *Sc. acidomonas* were also isolated from rayeb, but these species are seen as contaminants in fermented milk and many cause foodborne diseases (Delorme, 2008; El-Baradei *et al*., 2008; Gawad *et al*., 2010).

Kefir is fermented milk traditionally prepared in the eastern parts of Europe by using kefir grains as a starter culture. A diversity of lactobacilli have been isolated from kefir grains such as *Lb. acidophilus*, *Lb. brevis*, *Lb. casei*, *Lb. fermentum*, *Lb. helveticus*, *Lb. kefiri*, *Lb. parakefiri*, and *Lb. kefiranofaciens* along with *Sc. thermophilus* (Witthuhn *et al*., 2005; Mainville *et al*., 2006). *Lactobacillus kefiranofaciens* generates the kefiran polymer which forms the unique matrix of the kefir grain (Mainville *et al*., 2006).

Lactobacilli including *Lb. plantarum* and *Lb. delbrueckii* subsp*. lactis* have been isolated from traditionally prepared amasi from South Africa and Zimbabwe. A wide variety of other lactobacilli were also isolated from Zimbabwean amasi including *Lb. helveticus, Lb. casei* subsp*. casei* and *Lb. casei* subsp*. pseudoplantarum* (Gadaga *et al*., 1999; 2000; McMastera *et al*., 2005; Todorov *et al*., 2007). Nono/nunu traditionally prepared in Nigeria and Ghana is another example of traditionally fermented milk from which a wide variety of lactobacilli including *Lb. brevis*, *Lb. delbrueckii*, *Lb. plantarum*, *Lb. casei* and *Lb. fermentum* have been isolated (Okonkwo, 2011).

Lactococcus

In 1985 the genus *Lactococcus* was suggested after reclassification of specific species from the genera *Lactobacillus* and *Streptococcus* through chemotaxonomic analysis and 16S rRNA sequencing (Casalta & Montel, 2008). Lactococci are coccus-shaped Gram-positive, non-motile LAB that are homofermentative and produce strictly L(+) lactic acid from glucose (Stiles & Holzapfel, 1997; Casalta & Montel, 2008). Currently the genus *Lactococcus* consists of seven species, including *Lc. lactis* that is divided into four subspecies, namely *Lc. lactic* subsp*. lactis*, *Lc. lactis* subsp*. cremoris*, *Lc. lactis* subsp*. hordniae* and *Lc. lactis* subsp*. tructae* (DSMZ, 2012; LPSN, 2011). Lactococci are generally isolated from plant surfaces and animal skin. Mesophilic lactococci are often isolated from raw milk due to contamination from the environment and equipment used during milking (Casalta & Montel, 2008; Walther *et al*., 2008). *Lactococcus raffinolactis* and *Lc. garvieae* have been isolated from the dairy environment, but *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* are the two lactococci species most frequently isolated from raw milk along with other dairy products such as Camembert, Pecorino, Serra and Vanco cheeses (Stiles & Holzapfel, 1997; Casalta & Montel, 2008). *Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* are commonly used as a starter culture for the commercial production of fermented milks such as viili produced in Finland and amasi produced in South Africa (Toba *et al*., 1990; McMastera *et al*., 2005). These strains are also used as single or multiple strain starters often mixed with other LAB for commercial dairy fermentations such as butter, sour cream and cheeses (Casalta & Montel, 2008). A mixed strain starter culture is used to produce Gouda cheese composed of *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris, Lc. lactis* subsp*. lactis* biovar *diacetylactis* and *Leuconostoc* spp*.* (Wouters *et al*., 2002).

The selection of the *Lc. lactis* strains in starter cultures are based mainly on their ability to acidify milk by producing L(+) lactic acid, but also their contribution to desirable product properties (Wouters *et al*., 2002). *Lactococcus lactis* subsp. *cremoris* and *Lc. lactis* subsp*. lactis* synthesise exopolysaccharides which improve the texture, viscosity and consistency of fermented milks. The exopolysaccharide polygalactan, is a homopolysaccharide synthesised by *Lc. lactis* subsp*. lactis* (Ruas-Madiedo *et al*., 2002a; 2002b; Casalta & Montel, 2008). Volatile aroma compounds produced by these LAB during growth such as alcohols, ketones and aldehydes, contribute to the flavour of the fermented product (Casalta & Montel, 2008; Ziadi *et al*., 2010). For example the tart green-apple flavour associated with acetaldehyde and the buttery flavour associated

with diacetyl are popular flavours contributors in fermented dairy products (Holler & Steele, 1995; Kleerebezem *et al*., 2000).

Lactococci have been isolated from a wide variety of dairy products. Thirty five artisanal dairy products from Europe were analysed in a study where 38% of the bacterial strains isolated belonged to the genus *Lactococcus* and accounted for the largest LAB group present (Casalta & Montel, 2008). A study on traditionally available dairy products from Romania showed that in more than 90% of the raw milk samples, 69% of the fermented milk samples, 80% of the sour cream samples and 52% of the cheese samples *Lc. lactis* subsp. *lactis* where found (Zamfir *et al*., 2006). *Lactococcus lactis* was the most prevalent microbe present in Tibetan kefir, comprising of 58-70% of the total microbial consortium analysed. Although lactococci have been detected in kefir grains they are present in low numbers, the high numbers detected in the kefir beverage is due to the poor adherence of lactococci to the kefir grains and consequently they fall into the kepi beverage (Jianzhong *et al*., 2009). Subspecies such as *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* have been isolated from kefir (Witthuhn *et al*., 2005; Mainville *et al*., 2006; Jianzhong *et al*., 2009). Lactococci have been isolated from traditionally prepared fermented milks from Sub-Saharan Africa including rob, ergo and the Fulani communities' traditional fermented milk (Abdelgadir *et al*., 2001; Gonfa *et al*., 2001; Savadogo *et al*., 2004).

Leuconostoc

Leuconostoc are Gram-positive, non-endospore forming, non-motile, facultatively anaerobic, catalase-negative cocci or oval shaped bacteria often present in short chains or in pairs (Hemme & Foucaud-Scheunemann, 2004; Ogier *et al*., 2008). All the species in this genus are resistant to the antibiotic vancomycin, a useful characteristic for isolation of these bacteria (Hemme & Foucaud-Scheunemann, 2004; Ogier *et al*., 2008). Currently the genus *Leuconostoc* consists of 22 species and 3 sub-species (DSMZ, 2012; LPSN, 2011). Most of the *Leuconostoc* strains favour growth between 4º - 10 ºC and also grow at 30 ºC, but no growth occurs at 45 ºC (Hemme & Foucaud-Scheunemann, 2004). Although the members of the genus *Leuconostoc* are classified as opportunistic pathogens, they are GRAS for use in food fermentations. *Leuconostoc* spp. are used in industrial dairy starters, but more often these bacteria disseminate to dairy environments and are often present in traditionally prepared fermented milks and as non starter lactic acid bacteria (NSLAB) in raw milk cheeses (Stiles & Holzapfel, 1997; Hemme & Foucaud-Scheunemann, 2004; Ogier *et al*., 2008).

These bacteria are heterofermentative and predominantly metabolise the D(-) lactate isomer from glucose along with carbon dioxide and ethanol and/or acetate through the phosphoketolase pathway (Stiles & Holzapfel, 1997; Hemme & Foucaud-Scheunemann, 2004). Production of the D(-) lactic acid distinguishes *Leuconostoc* from similar heterofermentative lactobacilli which produces both D(-) and L(+) lactic acid and lactococci which produces L(+) lactic acid (Stiles & Holzapfel, 1997). In milk the co-metabolism of lactose and citrate by *Leuconostoc* leads to the production of diacety as well as acetone (Hemme & Foucaud-Scheunemann, 2004; Ogier *et al*., 2008). Diacetyl can be further transformed by these bacteria to 2,3-butanol and acetoin. This undesirable transformation can be prevented in fermented milks by cooling the milk after aroma formation (Hemme & Foucaud-Scheunemann, 2004).

Species from this genus have been isolated from various traditionally fermented milks and kefir. *Leuconostoc citreum* has been isolated from Egyptian zabady and *Ln. mesenteriodes* subsp. *cremoris* has been isolated from European kefir (Mainville *et al*., 2006; El-Baradei *et al*., 2008). *Leuconostoc* was the predominant LAB group present in traditionally prepared fermented milk samples collected from various households in South Africa and Namibia. Species isolated included *Ln. lactis, Ln. citreum* and *Ln. mesenteriodes* subsp. *dextranicum* (Beukes *et al*., 2001). The latter two species were also found in traditionally prepared South African amasi (Todorov *et al*., 2007). *Leuconostoc* have been isolated from a wide variety of traditionally prepared fermented milks from Sub-Saharan Africa, including sethemi, kule noato, sussa/suusac and ergo (Gonfa *et al*., 2001; Mathara *et al*., 2004; Farah *et al*., 2007; Kebede *et al*., 2007).

Enterococcus

Enterococci are coccus-shaped, Gram-positive, facultative anaerobic, oxidase-negative, non-endospore forming, catalase-negative bacteria that occurs in chains, pairs or singly (Giraffe, 2003; Foulquié Moreno *et al*., 2006). They are homofermenters and produce L(+) lactic acid from glucose and are also able to metabolise amino acids and citrate (Stiles & Holzapfel, 1997). The genus *Enterococcus* consists of 41 species of which *Ec. faecium, Ec. faecalis*, *Ec. durans*, *Ec. hirae* and *Ec. casseliflavus* are isolated from raw milk and dairy products (Franz *et al*., 1999; Cortés *et al*., 2006; Ogier & Serror, 2008; DSMZ, 2012; LPSN, 2011). *Enterococcus faecalis* and *Ec. faecium* are commonly isolated from faeces as they are inhibitors of the gastrointestinal tract in humans and animals. Enterococci enter the food and dairy environment from other primary habitats such as faeces, soil, plants and water (Batish *et al*., 1984; Franz *et al*., 1999; Giraffa,

2003; Foulquié Moreno *et al*., 2006; Ogier & Serror, 2008). The reason for their adaptability to various environments is that these bacteria can grow in high salinity, extreme pH (4.0 - 9.6), temperatures between 10º - 45 ºC and survive 30 min of heating at 60 ºC. Consequently they are present in dairy products made from raw, but also heat-treated milk (Giraffa, 2003; Foulquié Moreno *et al*., 2006; Ogier & Serror, 2008).

Bacteria of the genus *Enterococcus* are, unlike most LAB, not GRAS. This is due to their association with faecal contamination of primarily water and their role as opportunistic pathogens causing clinical human infections such as meningitis, bacteremia and endocarditis. However, enterococci have a history of being safe to use in food fermentations (Gardiner *et al*., 1999; Cortés *et al*., 2006; Foulquié Moreno *et al*., 2006; Ogier & Serror, 2008). As a result of the controversy on their safety they are not reliable hygiene indicators in food products (Foulquié Moreno *et al*., 2006). Each strain must be identified and characterised to assure the safety of the application of the strain in dairy products (Klein, 2003). *Enterococcus faecium* K77D is an example of an enterococci strain that is approved to be used as commercial starter culture in Denmark (Gardiner *et al*., 1999).

Enterococci usually occur as NSLAB in various types of Southern European artisanal cheeses made from raw and pasteurised milk such as Water-buffalo Mozzarella, Venaco, Cheddar, Hispanico Feta and Cebreiro, as well as in African fermented food (Franz *et al*., 1999; Giraffa, 2003; Ogier & Serror, 2008). The positive contributions enterococci provide in dairy fermentations include the production of antimicrobial bacteriocins, known as enterocins and their role in cheese ripening, which include the improvement of the aroma, flavour and texture of cheese (Franz *et al*., 1999; Leroy *et al*., 2003; Foulquié Moreno *et al*., 2006).

Enterococcus is often isolated from traditionally fermented milks made from raw milk. *Enterococcus faecium* and *Ec. faecalis* have been isolated from Egyptian zabady made from raw cow milk and all the *Enterococcus* isolates from rayeb made from raw buffalo milk were identified as *Ec. faecium* (El-Baradei *et al*., 2008; Gawad *et al*., 2010). *Enterococcus durans* was isolated along with other *Enterococcus* spp. from traditionally prepared Romanian milks (Zamfir *et al*., 2006). Enterococci have been isolated from traditionally prepared Sub-Sahara African fermented milks including ergo, kule noato and fermented milk from the Fulani communities (Gonfa *et al*., 2001; Mathara *et al*., 2004; Savadogo *et al*., 2004).

Pediococcus

The genus *Pediococcus* currently consists of 15 species which are spherical Gram-positive LAB arranged in tetras or pairs (Stiles & Holzapfel, 1997; Pfannebecker & Fröhlich, 2008; DSMZ, 2012; LPSN, 2011). These bacteria are facultatively aerobic, catalase-negative, non-motile and non-endospore forming (Pfannebecker & Fröhlich, 2008; Schleifer, 2009). Carbohydrate utilization patterns in this genus differ between species, but most pediococci produce D(-) and L(+) lactic acid from glucose (Stiles & Holzapfel, 1997). Some species in this genus can withstand extreme environmental conditions, such as high temperatures, pH and NaCl concentrations. These bacteria are often isolated from plants, a variety of fermented foods such as sauerkraut, fermented sausages and as spoilage microbes from beer (Stiles & Holzapfel, 1997; Gurira & Buys, 2005).

Even though pediococci grow inadequately in milk due to their irregular utilization of lactose, *Pc. pentosaceus* and *Pc. acidilactici* have been isolated from dairy products (Stiles & Holzapfel, 1997; Gurira & Buys, 2005). Pediococci have been isolated from fermented milks such as traditionally prepared Ethiopian ergo (Gonfa *et al*., 2001).

Acetic acid bacteria

This heterogeneous group has undergone various taxonomy changes in the last three decades. Currently the AAB is classified into twelve main genera which belong to the family Acetobacteraceae, containing the following genera *Acetobacter, Gluconacetobacter, Gluconobater, Asaia, Acidomonas, Granulibacter, Ameyamaea, Neoasaia, Kozakia, Saccharibacter, Swaminathania* and *Tanticharoenia* (Cleenwerck & De Vos, 2008; Yamada & Yukphan, 2008; Sengun & Karabiyikli, 2011). AAB are obligated aerobic, non-endospore forming, catalase-positive and oxidase-negative, Gram-negative or Gram-variable, spherical to rod-shaped organisms present as single cells, pairs or chains (De Vuyst, *et al*., 2008; Sengun & Karabiyikli, 2011). The cell size is between 0.8 - 4.5 µm long and 0.4 - 1 µm wide and grow optimally at a pH between 5 and 6.5, but can also survive at a pH between 3 and 4 (De Vuyst, *et al*., 2008; Sengun & Karabiyikli, 2011). AAB can be non-motile or motile with polar or peritrichous flagella (Cleenwerck & De Vos, 2008). They are widespread in nature and isolated from flowers, fruits, herbs and cereals. Industrially AAB are predominantly used for vinegar production, but can also cause spoilage if present in wine, ciders and beer (De Vuyst, *et al*., 2008; Sengun & Karabiyikli, 2011). They are difficult to isolate due to habitat specificity and individual growth media requirements (Sengun & Karabiyikli, 2011).

Acetobacter have been isolated from dairy products, as *A. syzygii*, *A. aceti* and *A. rasens* have been isolated from kefir grains (Witthuhn *et al*., 2005; da Cruz Pedrozo Miguel *et al*., 2010). From mashita, traditionally prepared butter fat prepared in Uganda, *A. aceti, A. lovaniensis, A. orientalis* and *A. pasteurianus* have been isolated (Ongol & Asano, 2009). AAB are used as commercial starter bacteria, for example *A. orientalis* in combination with *Lc. lactis* subsp*. cremoris* is used as starter culture to produce fermented milk in Japan (Nakasaki *et al*., 2008).

Yeasts and mycelial fungi

Yeasts are aerobic, eukaryotic, unicellular organisms that can grow in various niches such as soil, seawater, fruits, plants, algae and are also found in the intestinal tract and on the skin of animals (Garotte *at al*., 1997; Jacques & Casaregola, 2008). These heterotrophic organisms utilise organic carbon to produce alcohol and carbon dioxide and they are often present in processed food with a high amount of sugar content (Jakobsen & Narvhus, 1996; Gadaga *et al*., 2001b). Their ability to grow at a low pH may be the reason why yeasts can occur in food already fermented by bacteria (Jacques & Casaregola, 2008).

Yeasts are often present in dairy products, because they enter raw milk or cheese from the environment and processing equipment which leads to an end-product which differ in physico-chemical properties from those made with pure LAB starters (Viljoen, 2001; Wouters *et al*., 2002; Narvhus & Gadaga, 2003). Yeast species that grow well in milk, can utilise lactose or galactose and assimilate lactic, citric and succinic acid, grow well at low temperatures, can survive in high salt environments and metabolise proteins and fats (Viljoen, 2001; Álvarez-Martín *et al*., 2008; Jacques & Casaregola, 2008).

European fermented milk, kefir and koumiss, are examples of milk fermented with starters consisting of both LAB and yeasts (Lopandic *et al*., 2006). In the centre of kefir grains, yeasts are the dominant microbes, their presence results in the symbiotic relationships with LAB present and contributes to the characteristic aroma and texture of kefir due to carbon dioxide production (Wouters *et al*., 2002; Garotte *at al*., 1997). These yeasts include *Kluyveromyces marxianus, Candida kefir, C. lambica, Saccharomyces cerevisiae, S. exiguous, S. delbrueckii* and *Torula kefir* (Wouters *et al*., 2002; Witthuhn *et al*., 2005; Mainville *et al*., 2006). In koumiss thermophilic LAB and species of *Saccharomyces* are responsible for fermentation. *Saccharomyces unisporus*, a lactose-non-fermenting yeast is also present and utilise galactose produced by most LAB to produce alcohol (Wouters *et al*., 2002). The metabolism of

yeasts in fermented milk probably differs from their metabolism on the outside of cheese. It is possible that yeasts can alter their metabolism to adapt to milk as a food substrate, where higher concentrations of carbohydrates are available, but a more anaerobic environment is maintained by the LAB co-cultures (Narvhus & Gadaga, 2003).

In fermented dairy products, yeasts can cause spoilage due to excessive growth and cause off-flavours, excessive gas production and discoloration (Jakobsen & Narvhus, 1996; Lopandic *et al*., 2006; Jacques & Casaregola, 2008). The addition of fruit, sugar, honey or nuts to fermented milk such as yoghurt encourages spoilage because these products can be contaminated or provide more organic carbon for yeast growth (Jakobsen & Narvhus, 1996; Narvhus & Gadaga, 2003).

Yeasts have been isolated from various traditionally fermented milks from Sub-Saharan Africa. From 30 samples of traditionally prepared Zimbabwean amasi, 20 different yeast species were isolated, predominantly including *Saccharomyces dairenensis, S. cerevisiae, Candida lusitaniae* and *C. colliculosa* (Gadaga *et al*., 2000). The type of fermentation container used for the preparation of South African sethemi influenced the yeast consortium present. *Debaryomyces hansenii* and *S. cerevisiae* were predominantly present in sethemi from the clay containers and *Cryptococcus curvatus* in sethemi from the nickel and plastic containers (Kebede *et al*., 2007).

Geotrichum candidum is a filamentous fungus with a yeast-like to a mycelial-like form (Pottier *et al*., 2008). This fungus can be present on the surface of raw milk which has been fermented for a few days, as a branched hyphae layer. *Geotrichum candidum* has been isolated from kefir grains, Finnish fermented milk viili and soft, semi-hard and semi-fresh cheese (Garotte *at al*., 1997; Witthuhn *et al*., 2005; Pottier *et al*., 2008). Slow growth of *G. candidum* on top of the creamy viili due to a limited oxygen supply in the container helps to maintain a pH under 5 resulting in a mild tasting smooth product (Wouters *et al*., 2002). This microbe is present on the outside of the kefir grains, but does not influence the grain performance or sensory attributes of the kefir beverage (Schoeman, 2001). In cheese *G*. *candidum* is present as an adjunct starter culture which grows well during the early stages of cheese ripening in symbiosis with other starter bacteria and contribute to cheese texture, aroma, flavour and appearance (Pottier *et al*., 2008).

E. Conclusions

A wide variety of traditionally fermented milks are prepared on small scale in various countries of Sub-Saharan Africa. The microbial consortium of these products is mostly diverse and LAB, often together with yeasts, is present. The isolation and identification of microbes responsible for fermentation of some of the traditionally fermented milks from Sub-Saharan Africa still needs to be performed. On an industrial scale, these microbes (mainly LAB) can be used to develop new starter cultures to produce fermented milk products with similar aroma, flavour and texture characteristics. To ensure successful incorporation of the microbial strains isolated from the traditional fermented milks into commercial starter cultures, the original starter composition must be acknowledged and the role of each strain must be investigated and understood.

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CHAPTER 3

ENUMERATION OF THE MICROBIAL CONSORTIUM PRESENT IN FERMENTED MILKS FROM SUB-SAHARAN AFRICA

Abstract

Different traditionally and commercially fermented milks are commonly consumed in various countries of Sub-Saharan Africa. Commercially fermented milk is produced on an industrial scale according to well controlled standardised production processes. Traditionally fermented milks are domestically prepared and are a result of spontaneous fermentation at ambient temperatures. The microbial consortium of a large variety of the traditionally fermented milk from Sub-Saharan Africa has not been investigated or little information is available. In this study the microbial counts present in three traditionally fermented milks, omashikwa, masse and chekapmkaika and two commercially fermented milks, chambiko and omaere were determined by using six different selective growth media. Based on the average enumeration values obtained on the media used for the isolation of lactic acid bacteria (LAB), the highest counts were observed on KCA + V medium for chambiko (1.8×10^5 cfu.ml^{-1}), KCA + TTC medium for omaere (2.3×10^6 cfu.ml^{-1}), KCA + TTC medium for omashikwa and MRS + C medium for masse and chekapmkaika (6.2×10^6 and 2.0×10^3 cfu.ml^{-1}, respectively).

No significant differences were found between the enumeration values obtained for the three chambiko samples, as well as for the two omaere samples on each of the six media. Variances within the sample sets of these two products were, therefore, low. All the enumeration values obtained for the three omashikwa samples differed significantly from one another on all selective, indicating large variances within the sample set. Sample variances were also observed between the three masse, as well as the two chekapmkaika samples. Significant differences were found in microbial counts detected on each of the six media when comparing the average enumeration values of the omaere and chambiko, and masse and chekapmkaika, as well as between the commercial and traditional milks.

Introduction

Various tribal communities from different countries of Sub-Saharan Africa consume fermented milk traditionally prepared by leaving raw milk to ferment spontaneously

(Oyewole, 1997). Examples of such traditionally fermented milks include omashikwa from Namibia, masse from Mozambique and chekapmkaika from Uganda. The preparation of these fermented milks occurs at ambient temperatures in gourds, pots and smoked gourds, respectively (Oyewole, 1997; Bille *et al*., 2002; 2007). In Africa, cow milk is mostly used for the preparation of traditionally fermented milk (Narvhus & Gadaga, 2003). Microbes responsible for the spontaneous milk fermentation are present in the environment, used fermentation containers, on preparation utensils or inherent in the raw milk (Oyewole, 1997; Todorov *et al*., 2007). Kebede *et al*. (2007) found that the type of fermentation vessel used during the production of sethemi increased the diversity of the yeast population present. The microbial consortium of traditionally fermented milks is generally diverse and influenced by factors such as the regional climatic conditions and the duration of the fermentation process (Savadogo *et al*., 2004; Akabanda *et al*., 2010). The microbial consortium influences the unique organoleptic properties of different traditionally fermented milk products (Steinkraus, 1994; Mathara *et al*., 2004).

Commercially fermented milks are also produced in Sub-Saharan Africa such as chambiko in Malawi and omaere in Namibia. Commercially fermented milk is produced by inoculating pasteurised cow milk with specific starter cultures under controlled environmental conditions (Caplice & Fitzgerald, 1999). The microbial diversity in commercially fermented milk is often low because commercial starter cultures mostly consist of only one or two bacterial strains (Stiles & Holzapfel, 1997; Chammas *et al*., 2006).

Studies to enumerate and isolate the microbial populations present in traditionally fermented milks from Sub-Saharan Africa by using selective growth media have been done on Zimbabwean amasi (Gadaga *et al*., 2000), traditionally fermented milks from South Africa (Beukes *et al*., 2001), Ghanaian nunu (Akabanda *et al*., 2010), fermented milk from the Fulani community in Burkina Faso (Savadogo *et al*., 2004), Kenyan suusac (Lore *et al*., 2005) and South African sethemi (Kebede *et al*., 2007). Lactic acid bacteria (LAB) that have been isolated from Sub-Sahara African fermented milks include species from the genera *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus* and *Pediococcus* (Abdelgadir *et al*., 2001; Gonfa *et al*., 2001; Mathara *et al*., 2004; Kebede *et al*., 2007; Okonkwo, 2011). Although it has not been reported that acetic acid bacteria (AAB) have been isolated from fermented milks prepared in Sub-Saharan Africa, AAB including *Acetobacter aceti*, *A. syzygii* and *A. rasens* have been found in kefir grains which are used to ferment milk to produce kefir

(Witthuhn *et al*., 2005; da Cruz Pedrozo Miguel *et al*., 2010). Therefore, it may be possible that AAB are also present in fermented milk from Sub-Saharan Africa.

The increase in popularity of dairy products among consumers (Bureau for Food and Agricultural Policy, 2012) emphasises the need to extend the diversity of dairy products by developing new starter cultures. LAB and AAB strains present in these traditionally fermented milks can be isolated and used to develop new commercial starter cultures. As a result, new fermented dairy products with unique aromas, tastes and characteristics can be produced. The aim of this study was to enumerate the microbial consortium present in the three traditionally fermented milks, namely omashikwa from Namibia, masse from Mozambique and chekapmkaika from Uganda and two commercially fermented milks, namely chambiko from Malawi and omaere from Namibia.

Materials and methods

Sample collection

Five types of fermented milks were chosen from different Sub-Sahara African countries and these included two commercially fermented milks, chambiko from Malawi and omaere from Namibia, as well as three traditionally prepared fermented milks, namely omashikwa from Namibia, masse from Mozambique and chekapmkaika from Uganda. The commercial and traditional fermented milk products examined in this study were all prepared using cow milk. The minimum sample size required of each of the fermented milks was 100 ml.

Three 250 ml sachets of commercial chambiko and two 500 ml cartons of commercial omaere were purchased at a local retailer in Malawi and Namibia, respectively. Three 200 ml samples of traditionally prepared omashikwa and two 100 ml samples of traditionally prepared chekapmkaika were collected from a tribal community where these fermented milks are prepared and consumed. Three 200 ml samples of the traditionally prepared masse were purchased from a local market in Mozambique.

Each traditional fermented milk sample represented a different batch and was collected in 50 ml sterile Falcon tubes. All samples were frozen after collection or purchase. All the samples were received within three days from sampling, with the exception of chekapmkaika which was received after five days. All the fermented milk samples were stored at 4 ºC for a maximum of three days before analysis. The pH

value of each sample was measured using a calibrated pH-meter (H1221 Calibration Check Microprocessor, Spraytech).

Isolation and enumeration of the microbial consortium

Each fermented milk product was properly mixed to ensure homogenisation of the microbes present in the fermented milk. For each of the fermented milks 1 ml of each sample was pipetted aseptically into 9 ml (1:10 dilution) of sterile saline solution (0.85% (w/v) NaCl) in a McCartney bottle. The mixture was mixed with a vortex (Gemmy Industrial Corporation, Taiwan) for 5 min. A dilution series of 10^{-1} to 10^{-6} was made in sterile saline solution and 1 ml of each of these serial dilutions was pipetted in duplicate into appropriately marked Petri dishes. This was done for six different selective media (Table 1) which was pour plated into Petri dishes filled with the serial dilutions and properly mixed for 30 s. Each fermented milk sample was analysed in duplicate. The viable microbial counts of the bacteria, yeasts and mycelial fungi suspended in each serial dilution were determined and expressed in colony forming units per millilitre ($cfu.ml^{-1}$) of fermented milk. The media that were used for the isolation and enumeration of lactobacilli (MRS + C), lactococci (KCA + TTC) and leuconostocs (KCA + V) were incubated anaerobically in 3 L glass bottles (Consol) in the presence of a gas-generating kit (Anaerocult A system, Merck) at 30 ºC for 5 to 10 days. The selective media that was used for the isolation of mycelial fungi (MEA) and yeasts (YPD) were incubated aerobically at 25 ºC for 3 to 5 days. The media used for the isolation of AAB (MRS + E) was incubated at 30 ºC for 3 to 5 days.

Statistical analysis

Statistical analysis was done using Stata/IC 10.0 from StataCrop LP. All enumeration values analysed were transformed to $\log_{10}$ values due to the abnormal nature of the data. A one way analysis of variance (ANOVA) was performed on enumeration values (duplicate means) obtained for each sample per medium for each fermented milk. If the P (P1) value obtained for each sample set was significant (P1 value < 0.05) it indicated that at least one of the enumeration values differed from another. On those sample sets where P1<0.05, a two way ANOVA was performed to determine which samples differed significantly (P2<0.05) from each other. A two way ANOVA was also performed on the averaged enumeration values obtained on each medium for the chambiko, omaere, masse and chekapmkaika. If the P (P3) value obtained was smaller than 0.05 the averaged enumeration values for each fermented milk per medium differed significantly.

Table 1 Selective media used for the isolation and enumeration of the microbes present in the five different fermented milks from Sub-Saharan Africa.

Selective media[a]	Composition	Selected microbes
MRS + C	MRS-medium (Merck) with 3% (v/v) ethanol (Merck). Prepared by adding 100 µg.ml^{-1} cycloheximide (Sigma) soluble in ethanol (Merck) (stock solution concentration of 50 mg.ml^{-1}) after sterilisation of the MRS-medium (pH 6.2) (Pintado *et al*., 1996).	Lactobacilli
KCA + TTC	KCA-medium (g.l^{-1}): tri-sodium citrate.2H_2O (Saarchem) 2.0; gelatine (Merck) 2.5; sodium chloride (Merck) 4.0; yeast extract (Merck) 5.0; lactose (Merck) 5.0; glucose (Merck) 5.0; calcium lactate.5H_2O (Saarchem) 8.0; calcium citrate (Merck) 10.0; agar (Merck) 15.0; tryptone (Merck) 20.0 and carboxymethyl cellulose (Merck) (1.5% v/w) 100ml. Add 1 g TTC (Merck) diluted in 1 ml dH_2O after sterilisation of the KCA-medium (pH 6.6) (Nickels & Leesment, 1964; Beloti *et al*., 1999).	Lactococci
KCA + V	KCA-medium with 30 µg.ml^{-1} vancomycin (Sigma) diluted in dd$H_2$0 (stock solution concentration of 50 mg.ml^{-1}) added to the KCA-medium after sterilisation (pH 6.6) (Benkerroum *et al*., 1993).	Leuconostocs
MRS + E	MRS-medium (Merck) with 2% (v/v) ethanol (Merck) (Bester, 2009).	AAB
MEA	MEA-medium (Merck) (pH 5.5).	Mycelial fungi
YPD	YPD-medium (g.l^{-1}): Yeast extract (Merck) 10.0; Peptone (Merck) 20.0; Glucose (Merck) 20.0 (pH 6.5) (DSMZ, 2012).	Yeasts

[a] The following abbreviations were used: MRS = deMan, Rogosa and Sharpe-medium, KCA = potassium carboxymethyl cellulose agar, TTC = triphenyltetrazolium chloride, MEA = malt extract agar, YPD = yeast peptone dextrose agar, C = cycloheximide, V = vancomycin, E = ethanol.

Results and discussion

Enumeration values

It has been shown that by using standard plating procedures to determine the diversity of microbes present in a food product, it is possible that only a portion of the true microbial population will be enumerated due to the partial recovery of microbes (Witthuhn *et al*., 2005). To ensure that a wide spectrum of the microbes present is indeed counted after plating, six different selective media were used, each supporting the growth of a specific group of microbes.

Enumeration values of commercial chambiko and omaere

The enumeration values (cfu.ml^{-1}) obtained from the three samples of chambiko on each of the six selective media are presented in Table 2. For sample 1 the highest microbial counts was obtained on KCA + V medium (3.2 x 10^5 cfu.ml^{-1}), used for the isolation of leuconostocs. The highest enumeration values for sample 2 (2.2 x 10^5 cfu.ml^{-1}) and 3 (2.3 x 10^5 cfu.ml^{-1}) of chambiko were obtained on YPD medium that is selective for yeasts. In all three samples of chambiko the lowest enumeration values were observed on MEA medium selective for mycelial fungi. With values of 1.4 x 10^4, 1.1 x 10^4 and 1.3 x 10^4 cfu.ml^{-1}, respectively.

The enumeration values (cfu.ml^{-1}) obtained from the two samples of omaere on each of the six selective media are summarised in Table 3. For both samples 1 and 2 of omaere the highest enumeration values were obtained on YPD medium (1.8 x 10^7 and 1.6 x 10^7 cfu.ml^{-1}) used for the isolation of yeasts. For the omaere 2.0 x 10^6 cfu.ml^{-1} for sample 1 and 2.0 x 10^6 cfu.ml^{-1} for sample 2 were the lowest enumeration values obtained on the medium used for the isolation of lactobacilli (MRS + C). No microbial growth was detected on the medium used for the isolation of *Leuconostoc* spp. (KCA + V).

None of the enumeration values obtained for samples 1, 2 and 3 of chambiko on each growth medium differed significantly from one another (P1 value > 0.05). This was also true for omaere where no significant differences were found between the enumeration values obtained for samples 1 and 2 per growth medium. Therefore, it indicates that there is low variance between the three chambiko samples, as well as between the two omaere samples collected from different batches. Furthermore, similarities between the chambiko and omaere samples were also observed after measuring their pH values. All three chambiko samples had the same pH value of 4.2

Table 2 Enumeration values (cfu.ml^{-1}) obtained for commercial chambiko.

Selective medium[a]	Sample 1[b]	Sample 2[b]	Sample 3[b]	P1 value[c]
MRS + C	2.0 x 10^5	8.9 x 10^4	1.3 x 10^5	0.4811
KCA + TTC	7.4 x 10^4	2.7 x 10^4	2.1 x 10^4	0.4105
KCA + V	3.2 x 10^5	1.1 x 10^5	1.2 x 10^5	0.209
MRS + E	2.6 x 10^4	2.2 x 10^4	2.1 x 10^4	0.0744
MEA	1.4 x 10^4	1.1 x 10^4	1.3 x 10^4	0.6097
YPD	2.5 x 10^5	2.2 x 10^5	2.3 x 10^5	0.8331

[a] The following abbreviations were used: MRS = deMan, Rogosa and Sharpe-medium, KCA = potassium carboxymethyl cellulose agar, TTC = triphenyltetrazolium chloride, MEA = malt extract agar, YPD = yeast peptone dextrose agar, C = cycloheximide, V = vancomycin, E = ethanol.

[b] Average enumeration value obtained from duplicate media plates.

[c] P1 value < 0.05 indicates that at least one of the mean values differs significantly from another (using one way ANOVA test).

Table 3 Enumeration values (cfu.ml^{-1}) obtained for commercial omaere.

Selective medium[a]	Sample 1[b]	Sample 2[b]	P1 value[c]
MRS + C	2.0 x 10^6	2.0 x 10^6	0.8778
KCA + TTC	2.4 x 10^6	2.1 x 10^6	0.1286
KCA + V	NC	NC	-
MRS + E	1.6 x 10^7	1.2 x 10^7	0.1986
MEA	3.0 x 10^6	2.9 x 10^6	0.3650
YPD	1.8 x 10^7	1.6 x 10^7	0.2192

[a] The following abbreviations were used: MRS = deMan, Rogosa and Sharpe-medium, KCA = potassium carboxymethyl cellulose agar, TTC = triphenyltetrazolium chloride, MEA = malt extract agar, YPD = yeast peptone dextrose agar, C = cycloheximide, V = vancomycin, E = ethanol, NC = no counts.

[b] Average enumeration value obtained from duplicate media plates.

[c] P1 value < 0.05 indicates that at least one of the mean values differs significantly from another (using one way ANOVA test).

and both the omaere samples had a pH value of 4.3. If the pH values and microbial counts per medium of the different samples of a specific fermented milk are the same it could indicate that each batch is exposed to starters with similar concentrations of the same microbes, similar fermentation periods and similar process conditions. Consistency of these parameters is emulated when producing a fermented product in a on a commercial scale (Dave & Shah, 1996; Mayssoun & Nadine, 2010). Therefore, both the chambiko and omaere are produced by a standardised production process which results in a consistent product.

The average enumeration values obtained for chambiko illustrated in Fig. 1 gives an indication on which media the highest and lowest microbial growth occurred. The average enumeration values in decreasing order are as follows: 2.3 x 10^5 (YPD); 1.8 x 10^5 (KCA + V); 1.4 x 10^5 (MRS + C); 4.1 x 10^4 (KCA + TTC); 2.3 x 10^4 (MRS + E); and 1.3 x 10^4 cfu.ml^{-1} (MEA). According to these values, the three main microbial groups present in chambiko in decreasing order are yeasts, leuconostocs and lactobacilli. In chambiko, the highest enumeration value for all the media used for the isolation of LAB were obtained on KCA + V medium used for the isolation of leuconostocs. Therefore, the predominant microbes present in the starter culture used for the chambiko could possibly be strains from the genus *Leuconostoc*.

The average enumeration values obtained for omaere on the six selective media are illustrated in Fig. 2. The highest level of microbial growth was detected on YPD (1.7 x 10^7 cfu.ml^{-1}) followed by MRS + E (1.4 x 10^7 cfu.ml^{-1}), MEA (3.0 x 10^6 cfu.ml^{-1}), KCA + TTC (2.3 x 10^6 cfu.ml^{-1}) and MRS + C medium (2.0 x 10^6 cfu.ml^{-1}). Therefore, it may be that yeasts, AAB and mycelial fungi are the three largest microbial groups present in omaere. The largest LAB group present may be lactococci due to a higher average enumeration value obtained on the KCA + TTC medium. This can indicate that the predominant microbes present in the starter used for the omaere are strains from the genus *Lactobacillus*.

By observing Fig 1 and 2 it is clear that there are differences in the overall microbial counts present in the chambiko and omaere. The results after performing a two way ANOVA between the average enumeration values obtained for the omaere and the chambiko on each of the selective media confirmed that the average enumeration values obtained on five of the selective media, including MRS + C, KCA + TTC, MRS + E, MEA and MRS + E for the omaere were significantly larger ($P3=0.0001<0.05$) than the average enumeration values on these media obtained for the chambiko. However, the average enumeration value obtained on the KCA + V medium for the omaere was

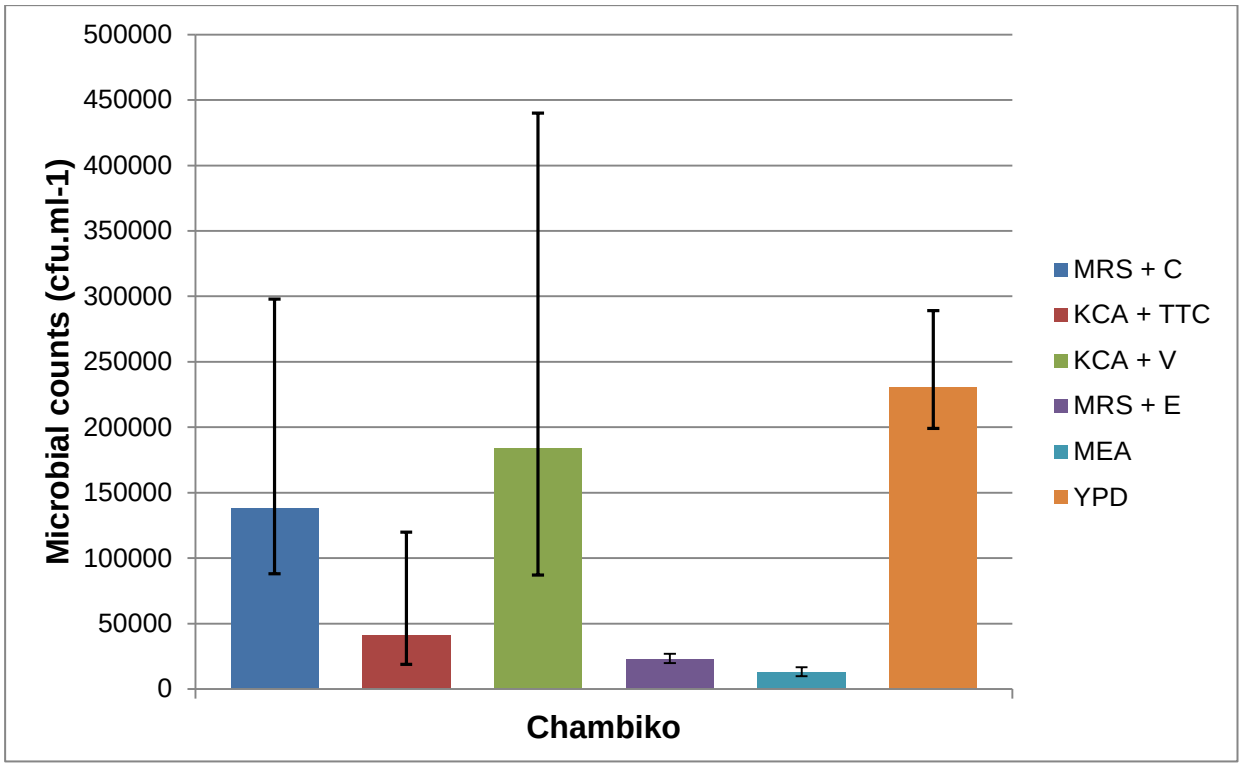

Figure 1 The microbial levels in three samples of commercial chambiko analysed in duplicate on six different selective media and deviation bars indicating the maximum and minimum enumeration value obtained per medium.

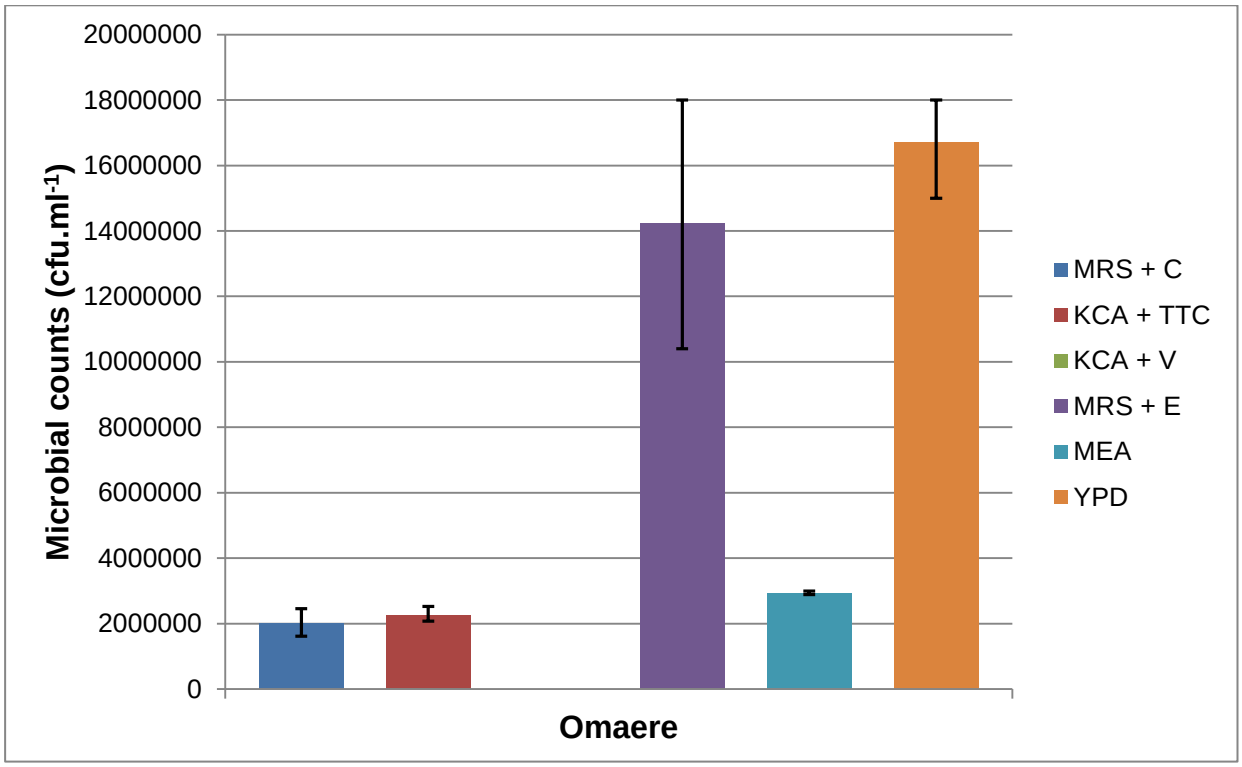

Figure 2 The microbial levels in two samples of commercial omaere analysed in duplicate on six different selective media and deviation bars indicating the maximum and minimum enumeration value obtained per medium.

significantly smaller (P3=0.0135<0.05) than the average enumeration value obtain on this medium for the chambiko. The higher microbial counts present in the omaere relative to the chambiko can be due to a higher concentration of microbes present in the starter used for omaere production. It may also be possible that the chambiko and omaere have been exposed to different storage periods and temperatures which can affect microbial growth (Mayssoun & Nadine, 2010).

Enumeration values of traditional omashikwa, masse and chekapmkaika

Information on the microbial consortium present in the fermented milk omashikwa is limited and no information is available on the microbes present in masse and chekapmkaika. Little is also known about starter traditions used to prepare these milks.

The enumeration values (cfu.ml^{-1}) obtained for the three samples of omashikwa are presented in Table 4. The highest enumeration values obtained from samples 2 (1.0 x 10^{6} cfu.ml^{-1}) and 3 (1.4 x 10^{6} cfu.ml^{-1}) were on the KCA + TTC medium used for the isolation of lactococci. From sample 1 the highest enumeration value was obtained on YPD (1.6 x 10^{7} cfu.ml^{-1}) selecting for yeasts, followed by enumeration values on MRS + C (1.0 x 10^{7} cfu.ml^{-1}) selecting for lactobacilli. The lowest enumeration values for all three omashikwa samples were obtained on MEA used for the isolation of mycelial fungi. These enumeration values are 1.3 x 10^{6} cfu.ml^{-1} for sample 1, 2.4 x 10^{4} cfu.ml^{-1} for sample 2 and 2.9 x 10^{4} cfu.ml^{-1} for sample 3.

Since P1 was found to be smaller than 0.05 for all six selective media, the one way ANOVA was followed by a two way ANOVA for all the omashikwa samples to determine to what extent the means of the enumeration values obtained per medium differed. The enumeration values obtained on all six selective media for samples 2 and 3 are significantly smaller (P2≤0.0084<0.05) compared to the enumeration values obtained for sample 1. No significant differences (P2=0.8734>0.05) were found between the microbial counts on MRS + C between samples 2 and 3. On all the other media significant differences (P2≤ 0.0242<05) of the microbial counts occurred between samples 2 and 3, but these differences were smaller than found between each of the samples and sample 1. Therefore, the microbial counts present in samples 2 and 3 are more comparable to each other than they are to sample 1. This variance between samples may be contributed to the inconsistency in preparation methods of traditionally fermented milks. This may indicate the possibility of exposure of different batches to varying environmental conditions such as temperature changes and hygiene standards, influencing the composition and growth of the microbial population present.

Table 4 Enumeration values (cfu.ml^{-1}) obtained for traditional omashikwa.

Selective medium[a]	Sample 1[b]	Sample 2[b]	Sample 3[b]	P1 value[c]
MRS + C	$1.0 \times 10^{7}{}_{i}$	$8.3 \times 10^{4}{}_{j}$	$1.8 \times 10^{5}{}_{j}$	0.0003
KCA + TTC	$4.2 \times 10^{6}{}_{i}$	$1.0 \times 10^{6}{}_{j}$	$1.4 \times 10^{6}{}_{k}$	0.0001
KCA + V	$2.8 \times 10^{6}{}_{i}$	$1.5 \times 10^{5}{}_{j}$	$4.9 \times 10^{5}{}_{k}$	0.0001
MRS + E	$9.8 \times 10^{6}{}_{i}$	$1.4 \times 10^{5}{}_{j}$	$2.4 \times 10^{5}{}_{k}$	0.004
MEA	$1.3 \times 10^{6}{}_{i}$	$2.4 \times 10^{4}{}_{j}$	$2.9 \times 10^{4}{}_{k}$	0.0021
YPD	$1.6 \times 10^{7}{}_{i}$	$2.9 \times 10^{5}{}_{j}$	$2.5 \times 10^{5}{}_{k}$	0.0001

[a] The following abbreviations were used: MRS = deMan, Rogosa and Sharpe-medium, KCA = potassium carboxymethyl cellulose agar, TTC = triphenyltetrazolium chloride, MEA = malt extract agar, YPD = yeast peptone dextrose agar, C = cycloheximide, V = vancomycin, E = ethanol.

[b] Average enumeration value obtained from duplicate media plates.

[c] P1 value < 0.05 indicates that at least one of the mean values differs significantly from another (using one way ANOVA test).

[ijk] Enumeration values (duplicate means) obtained for each sample on the same medium followed by a different letter are significantly different (P2<0.05).

The pH values measured for samples 2 (3.4) and 3 (3.4) of omashikwa are the same compared to the pH value measured for sample 1 (4.2). The higher pH value for sample 1 may be a reason for the higher microbial numbers in this sample. Microbes sensitive to acidic environments may not be present in the samples with low pH values. Traditional omashikwa previously analysed by Bille *et al*. (2007) had an average pH value of 3.3, closer to the pH values of samples 2 and 3. These two samples are probably better representatives of traditional omashikwa. Due to the large variance between samples 1 and the other two samples, the average enumeration values were not determined. Evaluating the enumeration values of each sample individually the main microbial group present in omashikwa may be lactococci due to high microbial counts on KCA + TTC for both samples 2 and 3.

The enumeration values (cfu.ml^{-1}) obtained for the three samples of traditional masse are given in Table 5. For samples 1 and 3 the highest enumeration values were obtained on the MRS + C (7.1 x 10^6 and 7.9 x 10^6 cfu.ml^{-1}, respectively) followed by enumeration values obtained on MRS + E, which was 6.3 x 10^6 cfu.ml^{-1} for sample 1 and 7.0 x 10^6 cfu.ml^{-1} for sample 3. The highest enumeration value for sample 2 was obtained on the MRS + E (5.3 x 10^6 cfu.ml^{-1}). The lowest enumeration values obtained for all three masse samples were obtained on KCA + TTC used for the isolation of lactococci. These enumeration values ranged from 2.9 x 10^5 to 1.9 x 10^6 cfu.ml^{-1}.

No significant differences ($P1>0.05$) were found between enumeration values of masse samples obtained from the MEA and YPD media. Since $P1<0.05$ for the media MRS + C, KCA + TTC, KCA + V and MRS + E the one way ANOVA performed on enumeration values for these media was followed by a two way ANOVA to determine the means in which these enumeration values obtained on each medium differed from one another. Enumeration values for sample 2 were significantly smaller ($P2\leq0.0314<0.05$) than those for sample 1 on the MRS + C, KCA + TTC, KCA + V and MRS + E media. Enumeration values for sample 3 was significantly larger ($P2\leq0.0408<0.05$) than those for sample 2 on MRS + C, KCA + V and MRS + E. However, the enumeration value for sample 3 on KCA + TTC was significantly smaller ($P2=0.0005<0.05$) than the enumeration value on sample 2. No significant differences ($P2=0.1020>0.05$) were found between the enumeration values of samples 3 and 1 obtained for MRS + C. Enumeration values on KCA + TTC and KCA + V from sample 3 were significantly smaller ($P2\leq0.0383<0.05$) than those for sample 1. The enumeration value on MRS + E of sample 3 was significantly larger ($P2=0.0260<0.05$) than the enumeration value for sample 1. The variances observed between the masse samples

Table 5 Enumeration values (cfu.ml^{-1}) obtained for traditional masse.

Selective medium[a]	Sample 1[b]	Sample 2[b]	Sample 3[b]	P1 value[c]
MRS + C	$7.1 \times 10^{6}{}_{i}$	$3.7 \times 10^{6}{}_{j}$	$7.9 \times 10^{6}{}_{i}$	0.017
KCA + TTC	$1.9 \times 10^{6}{}_{i}$	$1.4 \times 10^{6}{}_{j}$	$2.9 \times 10^{5}{}_{k}$	0.0002
KCA + V	$2.9 \times 10^{6}{}_{i}$	$2.3 \times 10^{6}{}_{j}$	$2.6 \times 10^{6}{}_{k}$	0.0213
MRS + E	$6.3 \times 10^{6}{}_{i}$	$5.3 \times 10^{6}{}_{j}$	$7.0 \times 10^{6}{}_{k}$	0.0104
MEA	2.9×10^{6}	2.8×10^{6}	3.0×10^{6}	0.2069
YPD	2.1×10^{6}	1.8×10^{6}	2.1×10^{6}	0.4456

[a] The following abbreviations were used: MRS = deMan, Rogosa and Sharpe-medium, KCA = potassium carboxymethyl cellulose agar, TTC = triphenyltetrazolium chloride, MEA = malt extract agar, YPD = yeast peptone dextrose agar, C = cycloheximide, V = vancomycin, E = ethanol.

[b] Average enumeration value obtained from duplicate media plates.

[c] P1 value < 0.05 indicates that at least one of the mean values differs significantly from another (using one way ANOVA test).

[ijk] Enumeration values (duplicate means) obtained for each sample on the same medium followed by a different letter are significantly different (P2<0.05).

may be ascribed to the variable temperature conditions and starters characteristic of traditional fermentations. If the sample variance between the masse samples are compared with the sample variance of the omashikwa samples it is clear that masse samples were more similar. The lower sample variance between the masse samples is reflected in the pH values of the samples, where the average pH of the three masse samples was 5.7 with pH values ranging from 5.6 to 5.8. This small difference in the pH values of the masse samples indicate the likelihood of more similar fermentation periods and conditions present during preparation of the different masse batches than the different omashikwa batches. This shows that traditional fermentations can be consistent.

According to the average enumeration values obtained for masse, as illustrated in Fig. 3, the highest number of microbes were isolated on MRS + C (6.2×10^6 cfu.ml^{-1}) and MRS + E (6.2×10^6 cfu.ml^{-1}). Therefore, it is possible that the two largest microbial groups present are lactobacilli and AAB. Lower average enumeration values were obtained on MEA (2.9×10^6 cfu.ml^{-1}), KCA + V (2.6×10^6 cfu.ml^{-1}) and YPD (2.0×10^6 cfu.ml^{-1}). The lowest average enumeration values were obtained on KCA + TTC (1.2×10^6 cfu.ml^{-1}). It is expected that low concentrations of lactococci are present in masse due to low microbial growth on the KCA + TTC used for isolation of these LAB.

The enumeration values (cfu.ml^{-1}) obtained from each of the two samples of traditional chekapmkaika are summarised in Table 6. The highest enumeration values for sample 1 and 2 were obtained on MRS + E (9.0×10^3 and 5.9×10^3 cfu.ml^{-1}, respectively). On YPD medium lower enumeration values were obtained for samples 1 (6.8×10^3 cfu.ml^{-1}) and 2 (5.1×10^3 cfu.ml^{-1}). No growth was detected on both KCA + TTC and KCA + V for both samples of chekapmkaika. Therefore, it is unlikely that lactococci and leuconostocs will be present in either of these samples. The enumeration values obtained for the MRS + C were 1.8×10^3 cfu.ml^{-1} for sample 1 and 2.2×10^3 cfu.ml^{-1} for sample 2.

No significant differences ($P1>0.05$) were found between enumeration values on the MEA and YPD media obtained from the chekapmkaika samples. Since $P1<0.05$ for the media MRS + C and MRS + E, the one way ANOVA performed on enumeration values was followed by a two way ANOVA. The enumeration value for sample 2 on MRS + C was significantly larger ($P2=0.0115<0.05$) than the enumeration value obtained for sample 1. On MRS + E the enumeration value obtained for sample 2 was significantly smaller ($P2=0.0086<0.05$) than the enumeration value obtained for sample 1. In the two chekapmkaika samples a significant variance was only detected on two of

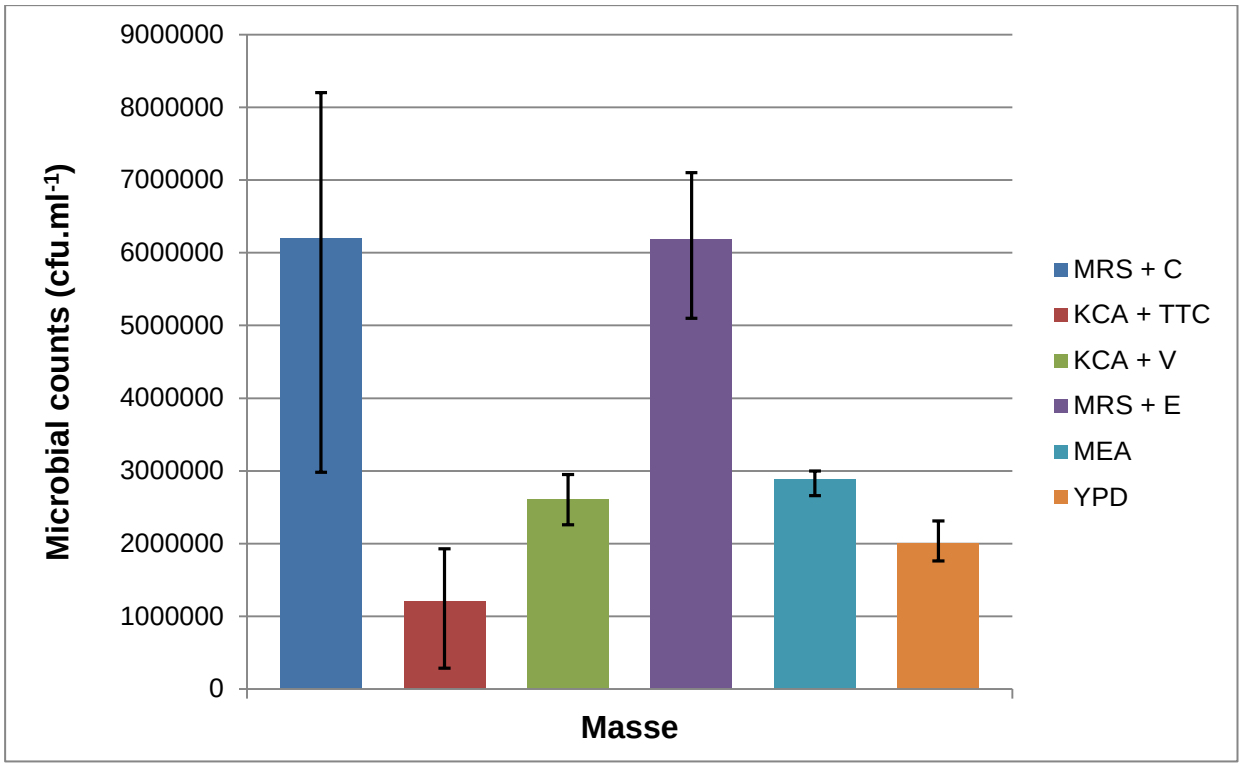

Figure 3 The microbial levels in three samples of traditional masse analysed in duplicate on six different selective media and deviation bars indicating the maximum and minimum enumeration value obtained per medium.

Table 6 Enumeration values (cfu.ml^{-1}) obtained for traditional chekapmkaika.

Selective medium[a]	Sample 1[b]	Sample 2[b]	P1 value[c]
MRS + C	$1.8 \times 10^{3}_{i}$	$2.2 \times 10^{3}_{j}$	0.0229
KCA + TTC	NC	NC	-
KCA + V	NC	NC	-
MRS + E	$9.0 \times 10^{3}_{i}$	$5.9 \times 10^{3}_{j}$	0.0172
MEA	7.1×10^{2}	5.7×10^{2}	0.1316
YPD	6.8×10^{3}	5.1×10^{3}	0.0698

[a] The following abbreviations were used: MRS = deMan, Rogosa and Sharpe-medium, KCA = potassium carboxymethyl cellulose agar, TTC = triphenyltetrazolium chloride, MEA = malt extract agar, YPD = yeast peptone dextrose agar, C = cycloheximide, V = vancomycin, E = ethanol, NC = no counts.

[b] Average enumeration value obtained from duplicate media plates.

[c] P1 value < 0.05 indicates that at least one of the mean values differs significantly from another (using one way ANOVA test).

[ij] Enumeration values (duplicate means) obtained for each sample on the same medium followed by a different letter are significantly different (P2<0.05).

the media (MRS + C and MRS + E). The pH values for the two samples were also similar (3.6 and 3.7, respectively). The similarities of the two samples can be due to a standardised traditional preparation process.

The average enumeration values obtained for chekapmkaika on the six selective media (Fig. 4) are MRS + E (7.5 x 10^3 cfu.ml^{-1}), YPD (5.9 x 10^3 cfu.ml^{-1}), MRS + C (2.0 x 10^3 cfu.ml^{-1}) and MEA (6.4 x 10^2 cfu.ml^{-1}). It is possible that the predominant microbial group present in chekapmkaika is AAB. Yeasts and mycelial fungi may also be present due to microbial counts obtained on the YPD and MEA media. MRS + C was the only medium used for the isolation of LAB and it is, therefore, likely that lactobacilli are the only LAB group present in chekapmkaika.

From Fig 3 and 4 it can be seen that there are differences in the overall microbial counts present in the masse and chekapmkaika. Results obtained after performing a two way ANOVA between the average enumeration values obtained from the masse and the chekapmkaika on each of the selective media confirmed that the average enumeration values obtained on all six selective media for the masse were significantly larger ($P3 \leq 0.0061 < 0.05$) than the average enumeration values on these media obtained for the chambiko. The lower average pH value of the chekapmkaika (3.7) compared to the average pH value of the masse samples (5.7) can be a reason for the lower microbial counts in the chekapmkaika because microbes sensitive to acidic environments cannot grow. The low pH of the chekapmkaika can be a result of the age of the product (minimum of 29 days) during which fermentation continues in comparison with the masse where fermentation only took place for a few days before consumption.

Significant differences also occurred between the average enumeration values obtained for the traditionally fermented products and the commercially fermented products. The average enumeration values obtained on all six media for the traditional chekapmkaika were significantly lower than the average enumeration values obtained for both the commercial omaere ($P3 = 0.0001 < 0.05$) and chambiko ($P3 \leq 0.0383 < 0.05$). The average enumeration values obtained on KCA + TTC, MRS + E and YPD for the traditional masse were significantly smaller ($P3 \leq 0.0126 < 0.05$) than the average enumeration values obtained for the omaere. No significant difference ($P3 = 0.1695 > 0.05$) was found between the average enumeration values obtained for the masse and omaere on MEA. The average enumeration values obtained on MRS + C and KCA + V for the masse were significantly larger ($P3 \leq 0.0383 < 0.05$) than the average enumeration values obtained for the omaere. This was also the case for the KCA + V, since there was no microbial growth on KCA + V for the omaere. The

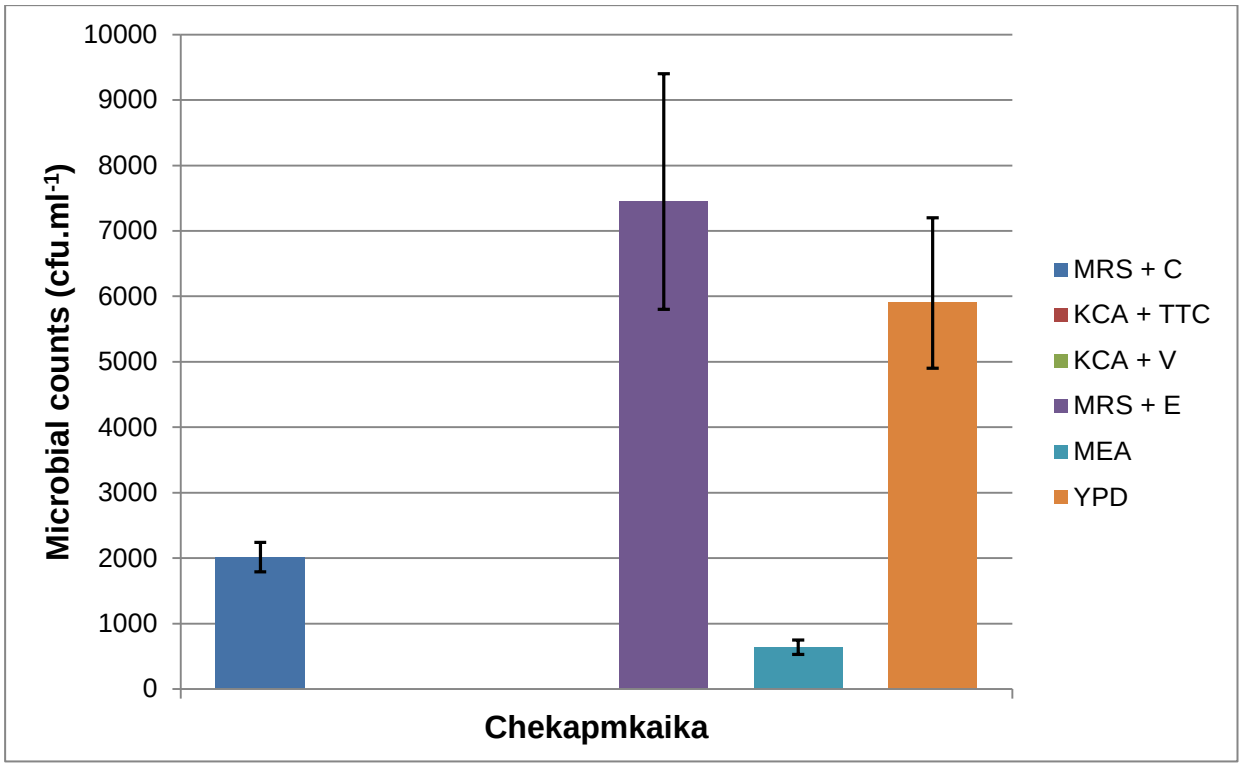

Figure 4 The microbial levels in two samples of traditional chekapmkaika analysed in duplicate on six different selective media and deviation bars indicating the maximum and minimum enumeration value obtained per medium.

average enumeration values obtained on all six media for the traditional masse were significantly larger ($P3 \leq 0.0015 < 0.05$) than the average enumeration values obtained for both the commercial omaere and the chambiko.

Conclusions

The results obtained in this study shows that samples of different fermented milks from Sub-Saharan Africa may differ significantly from each other in terms of microbial numbers or pH values. Significant variations were found between microbial counts obtained for the omashikwa samples on all the media, the masse samples on four of the media and the chekapmkaika samples on two of the media, possibly due to the generally inconsistent preparation of traditionally fermented milk. Large variation in pH values were found between some omashikwa samples. Insignificant variations in the pH values and microbial counts occurred between the different samples of both the commercial chambiko and omaere, possibly a result of standardised production of commercially fermented milk.

As expected, different fermented milks were found to have different microbial counts on each selective media, possibly indicating which microbial groups are present if these media are highly selective. The microbial counts obtained for the five milks on the different media generally differed significantly from one another. Selective growth media isolations are not always specific and more than one microbe may grow on each media. Therefore, further identification of microbes obtained on each media is important before the microbial consortium of each traditionally and commercially fermented milk can be characterised.

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CHAPTER 4

SELECTION AND IDENTIFICATION OF THE BACTERIAL CONSORTIUM PRESENT IN FERMENTED MILKS FROM SUB-SAHARAN AFRICA

Abstract

A wide variety of traditionally and commercially fermented milks are commonly consumed in various countries of Sub-Saharan Africa. However, the microbial consortium of a large variety of the traditionally fermented milks from Sub-Saharan Africa has not been identified. Microbes present in three traditionally fermented milks, namely omashikwa, masse and chekapmkaika and two commercially fermented milks, namely chambiko and omaere were isolated. The media used were specific for lactic acid bacteria (LAB), namely MRS + C (lactobacilli), KCA + TTC (lactococci) and KCA + V (leuconostocs), as well as MRS + E that was selective for acetic acid bacteria (AAB). In this study the number of bacterial colonies selected from specific media plates for further identification was determined by means of the Harrison Disk method. The isolates obtained from the different fermented milks on the six media were identified using the polymerase chain reaction (PCR) and DNA sequencing. A 1.5 kilobase (kb) part of the 16S ribosomal RNA (rRNA) gene was amplified, sequenced and identified using the BLAST search option to find the closest relative in the NCBI database. The predominant LAB group present in chambiko and chekapmkaika was found to be *Lactobacillus* spp., in omaere it was *Lactococcus* spp., in omashikwa it was *Enterococcus* spp. and in the masse it was found to be *Leuconostoc* spp. During the preparation of traditionally fermented milk hygienic practices are often neglected. In traditionally fermented milks evaluated in this study microbial contaminants were identified and included *Mesorhizobium loti, Escherichia coli, Kluyvera georgiana, Acinetobacter* spp*., Staphylococcus* spp., *Enterobacter* spp., *Klebsiella spp.* and *Bacillus* spp. LAB and AAB strains present in traditionally fermented milks can be used to develop novel commercial starter cultures which can be used to produce new fermented products with unique aromas, tastes and characteristics.

Introduction

A variety of microbes have been isolated from fermented milks, including lactic acid bacteria (LAB), acetic acid bacteria (AAB), yeasts and mycelial fungi (Gadaga *et al*.,

2000, Witthuhn *et al*., 2005, Zamfir *et al*., 2006). The LAB present in fermented milk is responsible for the fermentation by converting the carbohydrates to mainly lactic acid, but carbon dioxide, alcohol and other organic acids can also be produced (Caplice & Fitzgerald, 1999; Ross *et al*., 2002). LAB also produce a wide variety of antimicrobial compounds such as organic acids, hydrogen peroxide, carbon dioxide, acetaldehyde, diacetyl, ethanol and bacteriocins. During fermentation the growth of pathogens and other microbial contaminants are frequently inhibited by these antimicrobial components (Adams & Nicolaides, 1997; Caplice & Fitzgerald, 1999; Ross *et al*., 2002; Herreros *et al.,* 2005; Park *et al*., 2005; González *et al*., 2007). LAB species that have been isolated from fermented milks from Sub-Saharan Africa belonged to the genera *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus* and *Pediococcus* (Abdelgadir *et al*., 2001; Gonfa *et al*., 2001; Mathara *et al*., 2004; Kebede *et al*., 2007; Okonkwo, 2011). AAB that have been isolated from traditionally prepared kefir include *Acetobacter aceti*, *A. syzygii* and *A. rasens* (Witthuhn *et al*., 2005; da Cruz Pedrozo Miguel *et al*., 2010). *Acetobacter orientalis* in combination with *Lactococcus lactis* subsp. *cremoris* is used as a starter for commercially fermented milk in Japan (Nakasaki *et al*., 2008). Microbial contaminants are frequently isolated from traditionally fermented milk, as well as from raw milk and compromise product quality and safety (Huis in't Veld, 1996). Some of these microbial contaminants are pathogenic and can cause food-borne diseases. Pathogenic microbes that have been isolated from raw and fermented milk include *Escherichia coli, Vibrio cholerae, Shigella* spp*., Staphylococcus aureus, Yersinia* spp*., Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium bovis, Salmonella* spp*., Brucella abortus, Campylobacter jejuni* and *Bacillus cereus* (Gran *et al*., 2003; Herreros *et al*., 2005; Mufandaedza *et al*., 2006).

LAB strains present in traditionally fermented milk products has the potential to be used as new starters with unique antimicrobial properties ensuring the safety of fermented foods. New starters can also be used to develop fermented food products with sensory characteristics similar to that of the traditional products. Identification of microbes present in the fermented Sub-Sahara African milks chambiko, omaere, omashikwa, masse and chekapmkaika has never been reported to our knowledge. Therefore, the aim of this study was to identify the microbial consortium present in each of the three traditionally fermented milks, namely omashikwa from Namibia, masse from Mozambique and chekapmkaika from Uganda and two commercially fermented milks, namely chambiko from Malawi and omaere from Namibia by using polymerase chain reaction (PCR) and DNA sequencing of part of the 16S ribosomal RNA (rRNA) gene.

Material and methods

Strain selection and cultivation

The number of bacterial colonies selected from specific media plates for further identification was determined by means of the Harrison Disk method (Harrigan & McCance, 1976). This method ensures that a significant representation of the predominant colonies present in each one of the fermented milks is selected for identification. The Harrison Disk method allows selection of multiple colonies from a single media plate and by using this selection method a representation of the predominant microbial group or groups is obtained.

Samples used for enumeration of the commercially fermented milks included three sachets of chambiko and two cartons of omaere purchased at a local retailer in Malawi and Namibia, respectively. Samples used for enumeration of the traditionally fermented milks included three samples of omashikwa from Namibia, two samples of chekapmkaika from Uganda which were collected from a tribal community where these fermented milks are prepared and consumed and three samples of masse which were purchased from a local market in Mozambique. The microbes present in each milk sample was isolated and enumerated. Colonies were selected from media plates used for the isolation of LAB and AAB, including MRS + C, KCA + TTC, KCA + V and MRS + E (Table 1).

After selection (Harrigan & McCance, 1976) of the bacterial colonies from the different media for each of the fermented milks, the selected colonies were streaked out on media plates used for cultivation in order to obtain pure cultures. MRS media plates were used for the cultivation of LAB colonies selected from the MRS + C, KCA + TTC and KCA + V media. AAB colonies selected from the MRS + E media were cultivated on MRS + E media plates. All the media plates used for cultivation were incubated for three days at 30 ºC. To ensure that the selected colonies were pure cultures they were Gram-stained and inspected microscopically.

DNA extraction

The DNA of the selected bacterial colonies was isolated according to the TZ-method as described by Wang and Levin (2006). Bacterial cells were collected from the media plates with an inoculation loop and placed in a boil proof eppendorf tube containing 250 µL ddH_2O and mixed thoroughly with a vortex (Gemmy Industrial Corp., Taiwan) before the addition of 250 µL double strength TZ (2 x TZ). Double strength TZ

Table 1 Selective media used for the isolation and selection of the microbes present in the five different fermented milks from Sub-Saharan Africa.

Selective media[a]	Composition	Selected microbes
MRS + C	MRS-medium (Merck) with 3% (v/v) ethanol (Merck). Prepared by adding 100 µg.ml^{-1} cycloheximide (Sigma) soluble in ethanol (Merck) (stock solution concentration of 50 mg.ml^{-1}) after sterilisation of the MRS-medium (pH 6.2) (Pintado *et al*., 1996).	Lactobacilli
KCA + TTC	KCA-medium (g.l^{-1}): tri-sodium citrate.2H_2O (Saarchem) 2.0; gelatine (Merck) 2.5; sodium chloride (Merck) 4.0; yeast extract (Merck) 5.0; lactose (Merck) 5.0; glucose (Merck) 5.0; calcium lactate.5H_2O (Saarchem) 8.0; calcium citrate (Merck) 10.0; agar (Merck) 15.0; tryptone (Merck) 20.0 and carboxymethyl cellulose (Merck) (1.5% v/w) 100ml. Add 1 g TTC (Merck) diluted in 1 ml dH_2O after sterilisation of the KCA-medium (pH 6.6) (Nickels & Leesment, 1964; Beloti *et al*., 1999).	Lactococci
KCA + V	KCA-medium with 30 µg.ml^{-1} vancomycin (Sigma) diluted in dd$H_2$0 (stock solution concentration of 50 mg.ml^{-1}) added to the KCA-medium after sterilisation (pH 6.6) (Benkerroum *et al*., 1993).	Leuconostocs
MRS + E	MRS-medium (Merck) with 2% (v/v) ethanol (Merck) (Bester, 2009).	AAB

[a] The following abbreviations were used: MRS = deMan, Rogosa and Sharpe-medium, KCA = potassium carboxymethyl cellulose agar, TTC = triphenyltetrazolium chloride, C = cycloheximide, V = vancomycin, E = ethanol.

is a mixture consisting of 5 mg.ml^{-1} sodium azide (Merck) and 4% (v/v) Triton X-100 (Merck) in 0.1 M Tris-HCL (Fluka) at pH 8. The eppendorf tube was then placed in a boiling water bath for 10 min to ensure cell lyses. In order to obtain the supernatant was used as DNA template the eppendorf tubes were centrifuged (Eppendorf Centrifuge 5415D, Germany) at 10000 *g* for 5 min.

PCR amplification

The primers F8 (5'- CAC GGA TCC AGA CTT TGA TYM TGG CTC AG -3') and R1512 (5'- GTG AAG CTT ACG GYT AGC TTG TTA CGA CTT -3') (Felske *et al*., 1997) were used to amplify a 1.5 kilobase (kb) fragment of part of the 16S rRNA gene of the selected bacterial colonies. PCR amplifications were preformed in a PCR mixture with a total volume of 25 µL consisting of 1.5 µL (50 mM) $MgCl_2$ (Bioline), 2.5 µL 10 X buffer (Bioline), 1 µL 99% (v/v) DMSO (Merck), 1 µL (400 nM) dNTPs (Promega), 1 µL (400 nM) of both primer F8 and R1512, 0.5 µL (5 U) *Taq* DNA polymerase (Bioline), 2 µL DNA template and 14.5 µL ddH_2O. Amplification parameters of the PCR reaction included an initial denaturation at 92 ºC for 3 min followed by, 35 cycles of denaturation at 92 ºC for 30 s, annealing at 54 ºC for 30 s, and elongation at 68 ºC for 60 s and the final elongation step at 72 ºC for 7 min (Felske *et al*., 1997).

DNA sequencing and identification

The successful PCR amplification products were sequenced using an ABI 3130xl Genetic Analyser (Applied Biosystems, Foster City, USA). Sequences obtained were compared to sequences listed in NCBI database using the BLAST algorithm and identified according to the closest relative (Altschul *et al*., 1997). The DNA sequences that were used for the identification of the microbes using the BLAST search option were a minimum of 700 base pairs in length.

Results and discussion

Microbial species present in chambiko, omaere, omashikwa, masse and chekapmkaika identified after comparing their sequence data to sequences listed in the NCBI database are given in Tables 2 to 6.

Identification of isolates from commercial chambiko and omaere

Identification results of the microbial strains isolated from chambiko is listed in Table 2. From commercially produced chambiko the LAB identified all belonged to the genus

Table 2 Identification of the microbial strains isolated from the chambiko.

Isolate number	Identification	Isolation medium	% Similarity[z]
1MRS + C	*Lactobacillus paracasei* subsp. *paracasei*	MRS + C	98%
4MRS + C	*Lactobacillus paracasei* subsp. *paracasei*	MRS + C	85%
12MRS + C	*Lactobacillus paracasei* subsp. *paracasei*	MRS + C	98%
3MRS + C	*Lactobacillus casei*	MRS + C	97%
7MRS + C	*Lactobacillus casei*	MRS + C	97%
8MRS + C	*Lactobacillus casei*	MRS + C	94%
9MRS + C	*Lactobacillus casei*	MRS + C	97%
10MRS + C	*Lactobacillus casei*	MRS + C	96%
13MRS + C	*Lactobacillus casei*	MRS + C	94%
11MRS + C	*Lactobacillus* spp.	MRS + C	99%
6KCA + TTC	*Lactobacillus paracasei* subsp. *paracasei*	KCA + TTC	99%
7KCA + TTC	*Lactobacillus paracasei* subsp. *paracasei*	KCA + TTC	98%
3KCA + TTC	*Lactobacillus casei*	KCA + TTC	98%
9KCA + TTC	*Lactobacillus casei*	KCA + TTC	97%
8KCA + TTC	*Lactobacillus paracasei*	KCA + TTC	99%
11KCA + TTC	*Lactobacillus paracasei*	KCA + TTC	97%
12KCA + TTC	*Lactobacillus paracasei*	KCA + TTC	97%
2KCA + TTC	*Mesorhizobium loti*	KCA + TTC	95%
13KCA + TTC	*Mesorhizobium loti*	KCA + TTC	97%
4KCA + TTC	*Acinetobacter radioresistens*	KCA + TTC	99%
5KCA + TTC	*Acinetobacter radioresistens*	KCA + TTC	99%
10KCA + TTC	*Acinetobacter radioresistens*	KCA + TTC	99%
3KCA + V	*Lactobacillus paracasei* subsp. *paracasei*	KCA + V	98%
9KCA + V	*Lactobacillus paracasei* subsp. *paracasei*	KCA + V	97%
10KCA + V	*Lactobacillus paracasei* subsp. *paracasei*	KCA + V	97%
12KCA + V	*Lactobacillus paracasei* subsp. *paracasei*	KCA + V	99%
5KCA + V	*Lactobacillus casei*	KCA + V	97%
1KCA + V	*Lactobacillus paracasei*	KCA + V	98%
4KCA + V	*Lactobacillus paracasei*	KCA + V	98%
6KCA + V	*Lactobacillus paracasei*	KCA + V	99%
7KCA + V	*Lactobacillus paracasei*	KCA + V	97%
11KCA + V	*Lactobacillus paracasei*	KCA + V	99%
13KCA + V	*Lactobacillus paracasei*	KCA + V	98%
2KCA + V	*Lactobacillus* spp.	KCA + V	97%
8KCA + V	*Lactobacillus* spp.	KCA + V	97%
2AAB	*Lactobacillus paracasei* subsp. *paracasei*	MRS + E	98%
3AAB	*Lactobacillus paracasei* subsp. *paracasei*	MRS + E	97%
7AAB	*Lactobacillus paracasei* subsp. *paracasei*	MRS + E	87%
1AAB	*Lactobacillus casei*	MRS + E	99%
5AAB	*Lactobacillus casei*	MRS + E	90%
8AAB	*Lactobacillus casei*	MRS + E	85%
10AAB	*Lactobacillus casei*	MRS + E	97%
4AAB	*Lactobacillus paracasei*	MRS + E	98%
11AAB	*Lactobacillus paracasei*	MRS + E	97%
9AAB	*Lactobacillus paracasei*	MRS + E	83%

[z] Similarity below 95% is normally not sufficient to state a correct identification.

Lactobacillus, including *Lactobacillus casei* and *Lactobacillus paracasei*, as well as the sub-species *Lactobacillus paracasei* subsp. *paracasei*. Three strains isolated from chambiko could only be identified to genus level as *Lactobacillus* spp*.* All the strains isolated from the MRS + C, KCA + V and MRS + E media used for the isolation of lactobacilli, leuconostocs and AAB, respectively were identified as lactobacilli. On the KCA + TTC media used for the isolation of lactococci seven of the twelve strains selected were identified as lactobacilli, along with two strains identified as *Mesorhizobium loti* and three strains as *Acinetobacter radioresistens*. The latter two bacterial strains are most likely microbial contaminants which enter the chambiko from the environment during the production process. *Acinetobacter* spp. have been isolated from human skin and could have entered the chambiko via unhygienic practices. *Acinetobacter* spp*.* are classified as opportunistic pathogens and species from this genera have been responsible for human infections in hospitals (Jawad *et al*., 1998; Krawczyk *et al*., 2002). *Mesorhizobium loti* is mostly found in soil in a symbiotic relationship with plant legumes such as chickpeas (Laranjo *et al*., 2012). This microbe may have entered the production facility through the raw milk that may have been contaminated with soil.

Identification of the microbial strains isolated from the omaere is given in Table 3. LAB present in commercially produced omaere were *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris.* One *Lactococcus lactis* strain that was isolated from the MRS + E medium could not be identified to sub-species level. All the strains isolated from the MRS + C, KCA + TTC and MRS + E media used for the isolation of lactobacilli, lactococci and AAB, respectively were identified as lactococci. No isolations were made from the KCA + V medium as no growth was detected on this media for omaere. *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* are commonly used as a starter culture for the commercial production of fermented milks such as viili produced in Finland and amasi produced in South Africa (Toba *et al*., 1990; McMaster *et al*., 2005).

Identification of isolates from traditional omashikwa, masse and chekapmkaika

Identification of the microbial strains isolated from omashikwa is presented in Table 4. From traditionally prepared omashikwa LAB identified belonged to the genera *Lactobacillus, Lactococcus, Leuconostoc* and *Enterococcus.* The *Lactobacillus* strains isolated were identified as *Lactobacillus helveticus, Lactobacillus kefiri, Lactobacillus casei, Lactobacillus rhamnosus* and *Lactobacillus paracasei.* Two isolated lactobacilli

Table 3 Identification of the microbial strains isolated from the omaere.

Isolate number	Identification	Isolation medium	% Similarity
1MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	98%
2MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	98%
3MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	99%
4MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	99%
5MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	98%
6MRS + C	*Lactococcus lactis* subsp. *cremoris*	MRS + C	98%
7MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	98%
8MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	99%
9MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	98%
10MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	99%
11MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	97%
12MRS + C	*Lactococcus lactis* subsp. *lactis*	MRS + C	98%
1KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	98%
2KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	98%
3KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	98%
4KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	98%
5KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	97%
6KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
7KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	98%
8KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	98%
10KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
11KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
12KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	97%
13KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	98%
1AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	97%
2AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	98%
3AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	98%
4AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	99%
5AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	98%
6AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	99%
9AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	97%
10AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	98%
11AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	99%
12AAB	*Lactococcus lactis* subsp. *lactis*	MRS + E	98%
7AAB	*Lactococcus lactis*	MRS + E	98%

[z] Similarity below 95% is normally not sufficient to state a correct identification.

Table 4 Identification of the microbial strains isolated from the omashikwa.

Isolate number	Identification	Isolation medium	% Similarity
1MRS + C	*Enterococcus* spp.	MRS + C	98%
11MRS + C	*Enterococcus* spp.	MRS + C	99%
2MRS + C	*Enterococcus faecium*	MRS + C	97%
5MRS + C	*Enterococcus faecium*	MRS + C	97%
6MRS + C	*Enterococcus faecium*	MRS + C	99%
9MRS + C	*Enterococcus faecium*	MRS + C	98%
3MRS + C	*Enterococcus durans*	MRS + C	99%
4MRS + C	*Enterococcus durans*	MRS + C	98%
7MRS + C	*Enterococcus durans*	MRS + C	98%
10MRS + C	*Enterococcus durans*	MRS + C	98%
8MRS + C	*Lactobacillus helveticus*	MRS + C	98%
1KCA + TTC	*Enterococcus faecium*	KCA + TTC	98%
8KCA + TTC	*Enterococcus faecium*	KCA + TTC	98%
9KCA + TTC	*Enterococcus faecium*	KCA + TTC	99%
12KCA + TTC	*Enterococcus faecium*	KCA + TTC	97%
3KCA + TTC	*Enterococcus durans*	KCA + TTC	98%
7KCA + TTC	*Enterococcus durans*	KCA + TTC	97%
10KCA + TTC	*Enterococcus durans*	KCA + TTC	99%
2KCA + TTC	*Escherichia coli*	KCA + TTC	99%
5KCA + TTC	*Staphylococcus spp.*	KCA + TTC	98%
6KCA + TTC	*Lactococcus lactis subsp. lactis*	KCA + TTC	96%
11KCA + TTC	*Lactococcus lactis subsp. lactis*	KCA + TTC	98%
1KCA + V	*Leuconostoc pseudomesenteroides*	KCA + V	99%
2KCA + V	*Lactobacillus kefiri*	KCA + V	99%
8KCA + V	*Lactobacillus kefiri*	KCA + V	100%
3KCA + V	*Lactobacillus spp.*	KCA + V	98%
11KCA + V	*Lactobacillus spp.*	KCA + V	97%
4KCA + V	*Lactobacillus casei*	KCA + V	95%
5KCA + V	*Lactobacillus rhamnosus*	KCA + V	97%
6KCA + V	*Lactobacillus rhamnosus*	KCA + V	98%
7KCA + V	*Lactococcus lactis*	KCA + V	99%
9KCA + V	*Lactobacillus paracasei*	KCA + V	97%
12KCA + V	*Lactobacillus paracasei*	KCA + V	97%
10KCA + V	*Acinetobacter johnsonii*	KCA+V	96%
4AAB	*Enterococcus faecium*	MRS + E	99%
6AAB	*Enterococcus faecium*	MRS + E	98%
1AAB	*Lactococcus lactis*	MRS + E	98%
2AAB	*Lactococcus lactis*	MRS + E	98%
3AAB	*Lactococcus lactis*	MRS + E	99%
5AAB	*Lactococcus lactis*	MRS + E	99%
7AAB	*Leuconostoc pseudomesenteroides*	MRS + E	95%
8AAB	*Leuconostoc pseudomesenteroides*	MRS + E	98%
9AAB	*Leuconostoc pseudomesenteroides*	MRS + E	96%
11AAB	*Leuconostoc pseudomesenteroides*	MRS + E	92%

[z] Similarity below 95% is normally not sufficient to state a correct identification.

strains could only be identified to the genus level as *Lactobacillus* spp. Most of the lactobacilli strains were isolated from KCA + V intended for the selection of leuconostocs. Two of the seven *Lactococcus lactis* strains present were identified to sub-species level as *Lactococcus lactis* subsp. *lactis* isolated from KCA + TTC. *Leuconostoc pseudomesenteroides* was the only *Leuconostoc* spp. present in the omashikwa and the majority of these strains were isolated from MRS + E.

Enterococcus strains present were identified as *Enterococcus faecium* and *Enterococcus durans*. Enterococci were mostly isolated from MRS + C and KCA + TTC, although two strains were isolated from MRS + E. No enterococci were isolated from the KCA + V medium and may, therefore, be sensitive to the vancomycin present (Ogier & Serror, 2008). Species of the genus *Enterococcus* is not Generally Recognised as Safe (GRAS) due to their involvement in human infections as opportunistic pathogens. However, enterococci often form part of the inherent food microbiota (Zamfir *et al*., 2006). The presence of these bacteria in fermented milk is an indication of unhygienic conditions during preparation (Gardiner *et al*., 1999; Cortés *et al*., 2006; Foulquié Moreno *et al*., 2006; Zamfir *et al*., 2006; Ogier & Serror, 2008). Enterococci strains isolated must first be identified and characterised to ensure if the product is safe for consumption (Klein, 2003). Other opportunistic pathogens that were identified included *Escherichia coli, Acinetobacter johnsonii* and *Staphylococcus* spp. *Escherichia coli* and *Staphylococcus* spp. can be responsible for food-borne diseases and are not desirable in food products (Krawczyk *et al*., 2002; Akabanda *et al*., 2010).

Identification of the microbial strains isolated from masse is given in Table 5. LAB strains identified from traditionally prepared masse belong to the genera *Lactococcus, Leuconostoc* and *Enterococcus. Lactococcus* strains present were all identified as *Lactococcus lactis* subsp. *lactis*, except for one lactococci strain that could only be identified to genus level. All the lactococci were isolated on KCA + TTC specific for their selection. From masse *Leuconostoc* spp. were identified that included *Leuconostoc pseudomesenteroides, Leuconostoc lactis* and *Leuconostoc garlicum.* All the isolates obtained from MRS + C and MRS + E were leuconostocs. The majority of strains isolated on KCA + V medium specific for the selection of *Leuconostoc* spp. were identified as *Leuconostoc lactis*. Two of the enterococci strains present in the masse were identified as *Enterococcus durans.*

Microbial contaminants identified from masse on KCA + TTC medium included *Kluyvera georgiana* and *Klebsiella* oxytoca, as well as two isolates identified as *Enterobacter* spp. Microbial contaminants including *Klebsiella pneumonia*

Table 5 Identification of the microbial strains isolated from the masse.

Isolate number	Identification	Isolation medium	% Similarity
1MRS + C	*Leuconostoc pseudomesenteroides*	MRS + C	98%
2MRS + C	*Leuconostoc lactis*	MRS + C	99%
3MRS + C	*Leuconostoc lactis*	MRS + C	99%
4MRS + C	*Leuconostoc lactis*	MRS + C	99%
5MRS + C	*Leuconostoc lactis*	MRS + C	99%
6MRS + C	*Leuconostoc lactis*	MRS + C	99%
7MRS + C	*Leuconostoc lactis*	MRS + C	98%
8MRS + C	*Leuconostoc lactis*	MRS + C	98%
9MRS + C	*Leuconostoc lactis*	MRS + C	98%
10MRS + C	*Leuconostoc lactis*	MRS + C	99%
11MRS + C	*Leuconostoc lactis*	MRS + C	99%
12MRS + C	*Leuconostoc lactis*	MRS + C	99%
13MRS + C	*Leuconostoc lactis*	MRS + C	99%
1KCA + TTC	*Kluyvera georgiana*	KCA + TTC	98%
2KCA + TTC	*Enterobacter* spp.	KCA + TTC	99%
6KCA + TTC	*Enterobacter* spp.	KCA + TTC	99%
4KCA + TTC	*Lactococcus lactis*	KCA + TTC	99%
3KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	97%
5KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
8KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
10KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
11KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
13KCA + TTC	*Lactococcus lactis* subsp. *lactis*	KCA + TTC	99%
7KCA + TTC	*Enterococcus durans*	KCA + TTC	99%
9KCA + TTC	*Enterococcus* spp.	KCA + TTC	100%
12KCA + TTC	*Klebsiella oxytoca*	KCA + TTC	98%
3KCA + V	*Leuconostoc lactis*	KCA + V	99%
4KCA + V	*Leuconostoc lactis*	KCA + V	99%
5KCA + V	*Leuconostoc lactis*	KCA + V	99%
6KCA + V	*Leuconostoc lactis*	KCA + V	98%
7KCA + V	*Leuconostoc lactis*	KCA + V	98%
9KCA + V	*Leuconostoc lactis*	KCA + V	99%
10KCA + V	*Leuconostoc lactis*	KCA + V	98%
11KCA + V	*Leuconostoc lactis*	KCA + V	98%
12KCA + V	*Leuconostoc lactis*	KCA + V	98%
1KCA + V	*Klebsiella* spp.	KCA + V	98%
2KCA + V	*Klebsiella pneumoniae*	KCA + V	98%
8KCA + V	*Klebsiella pneumoniae*	KCA + V	97%
1AAB	*Leuconostoc lactis*	MRS + E	99%
2AAB	*Leuconostoc lactis*	MRS + E	99%
4AAB	*Leuconostoc lactis*	MRS + E	99%
5AAB	*Leuconostoc lactis*	MRS + E	99%
6AAB	*Leuconostoc lactis*	MRS + E	99%
7AAB	*Leuconostoc lactis*	MRS + E	99%
8AAB	*Leuconostoc lactis*	MRS + E	99%
9AAB	*Leuconostoc lactis*	MRS + E	99%
11AAB	*Leuconostoc lactis*	MRS + E	99%
12AAB	*Leuconostoc lactis*	MRS + E	99%

Table 5 (continued)

10AAB	*Leuconostoc garlicum*	MRS + E	98%
3AAB	*Leuconostoc garlicum*	MRS + E	99%

[z] Similarity below 95% is normally not sufficient to state a correct identification.

and a *Klebsiella* spp. were isolated on KCA + V medium. It may be possible that the *Klebsiella* spp. present in the masse is resistant to vancomycin, although further testing should be done to confirm this possibility (Donabedian & Andriole, 1977). *Klebsiella pneumonia*, *Klebsiella oxytoca* and *Kluyvera* spp. are often isolated from hospital environments, although these bacteria are rarely responsible for human infections (Hartstein *et al*., 1993; Chen *et al*., 2006; Ohlasova *et al*., 2007). *Enterobacter* spp. are classified as opportunistic pathogens and species such as *Enterobacter sakazakii* present in infant formula milk have been responsible for food-borne diseases (Shaker *et al*., 2007). Food infections due to the presence of these bacteria in other food products are unknown (Friedemann, 2007).

Identification of the microbial strains isolated from chekapmkaika is presented in Table 6. *Lactobacillus helveticus* was the only LAB strain isolated from chekapmkaika. All the lactobacilli strains were isolated on MRS + C medium. No isolates were obtained from KCA + V and KCA + TTC media, because no growth was detected on these media for the chekapmkaika. *Bacillus cereus* and *Bacillus thuringiensis* were identified from the MRS + E medium along with ten isolates that were identified to genus level as *Bacillus* spp. *Bacillus* spp. are microbial contaminants frequently found in dairy products (Slaghuis *et al*., 1997). *Bacillus cereus* is a pathogenic bacterium and is often responsible for food poisoning (Røssland *et al*., 2003). These bacteria can be sensitive to the antibiotic cycloheximide (Ha *et al*., 1995) and this may explain why *Bacillus* spp. was not detected on MRS + C. *Bacillus* spp. are often isolated from raw, as well as pasteurised milk because they form spores which can survive milk pasteurisation (Salustiano *et al*., 2009). Another microbial contaminant present in the chekapmkaika was identified as *Staphylococcus warneri*. *Staphylococcus warneri* is a pathogenic bacterium that has been isolated from raw milk and is responsible for human and animal infections (Barigye *et al.*, 2007).

The media used for the isolation of the LAB (MRS + C, KCA + TTC and KCA + V media) and AAB (MRS + E medium) in this study were expected to be more selective. Specific microbial species were present on multiple selective media, for example *Leuconostoc* spp. were isolated on MRS + C, MRS + E and KCA + V, *Lactobacillus* spp., *Enterococcus* spp. and *Lactococcus* spp. were isolated on MRS + C, KCA + TTC, KCA + V and MRS + E. Microbial contaminants were also detected on the four different selective media, including *Mesorhizobium loti, Acinetobacter radioresistens, Kluyvera georgiana, Escherichia coli, Enterobacter* spp., *Staphylococcus* spp. and *Klebsiella oxytoca* on KCA + TTC*, Acinetobacter johnsonii* and *Klebsiella pneumonia* on KCA + V,

Table 6 Identification of the microbial strains isolated from the chekapmkaika.

Isolate number	Identification	Isolation medium	% Similarity
1MRS + C	*Lactobacillus helveticus*	MRS + C	98%
2MRS + C	*Lactobacillus helveticus*	MRS + C	97%
3MRS + C	*Lactobacillus helveticus*	MRS + C	97%
4MRS + C	*Lactobacillus helveticus*	MRS + C	97%
5MRS + C	*Lactobacillus helveticus*	MRS + C	98%
6MRS + C	*Lactobacillus helveticus*	MRS + C	98%
8MRS + C	*Lactobacillus helveticus*	MRS + C	97%
9MRS + C	*Lactobacillus helveticus*	MRS + C	98%
10MRS + C	*Lactobacillus helveticus*	MRS + C	99%
11MRS + C	*Lactobacillus helveticus*	MRS + C	99%
12MRS + C	*Lactobacillus helveticus*	MRS + C	97%
13MRS + C	*Lactobacillus helveticus*	MRS + C	98%
7MRS + C	*Staphylococcus warneri*	MRS + C	99%
1AAB	*Bacillus* spp.	MRS + E	92%
3AAB	*Bacillus* spp.	MRS + E	99%
4AAB	*Bacillus* spp.	MRS + E	99%
5AAB	*Bacillus* spp.	MRS + E	99%
6AAB	*Bacillus* spp.	MRS + E	99%
9AAB	*Bacillus* spp.	MRS + E	99%
10AAB	*Bacillus* spp.	MRS + E	99%
11AAB	*Bacillus* spp.	MRS + E	96%
13AAB	*Bacillus* spp.	MRS + E	99%
14AAB	*Bacillus* spp.	MRS + E	98%
2AAB	*Bacillus cereus*	MRS + E	99%
7AAB	*Bacillus cereus*	MRS + E	99%
8AAB	*Bacillus thuringiensis*	MRS + E	99%
12AAB	*Bacillus thuringiensis*	MRS + E	98%

[z] Similarity below 95% is normally not sufficient to state a correct identification.

Staphylococcus warneri on MRS + C and *Bacillus* spp. on MRS + E. The low selectivity of the media used may be because the isolated strains are resistant to the antibiotics used or that the concentration of the antibiotics present is too low to have a measurable lethal effect. This clearly shows that it is vital to take medium selectivity into account when conclusions are made on the microbial groups present when only looking at the enumeration values obtained (Witthuhn *et al*. 2005).

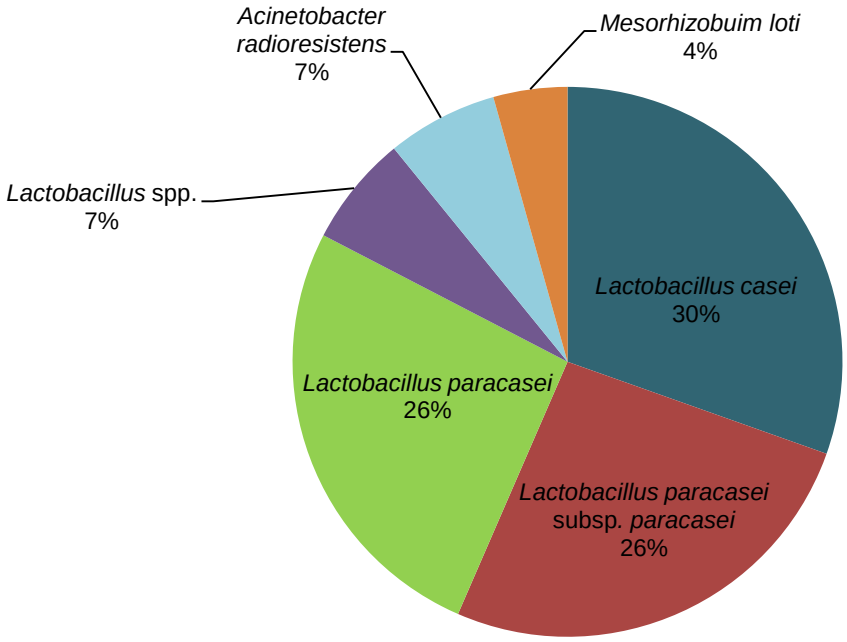
Distribution frequencies

The distribution frequencies of the microbes present in the commercially fermented milk chambiko are shown in Fig. 1. The largest group of lactobacilli present in the chambiko are *Lactobacillus casei* (30%), followed by *Lactobacillus paracasei* (26%) and *Lactobacillus paracasei* subsp*. paracasei* (26%). Bacterial strains *Acinetobacter radioresistens* (7%) and *Mesorhizobium loti* (4%) are microbial contaminants and were present in lower concentrations.

The distribution frequencies of the microbes present in the commercially fermented milk omaere are shown in Fig. 2. The predominant microbial species present in the omaere was *Lactococcus lactis* subsp. *lactis* (94%), followed by *Lactococcus lactis* subsp*. cremoris* (3%). Therefore, only one LAB genus was present in both the chambiko and omaere. The standardised production process followed during the production of omaere is most likely better controlled than the production process followed for the chambiko, because no microbial contaminants were detected in the omaere.

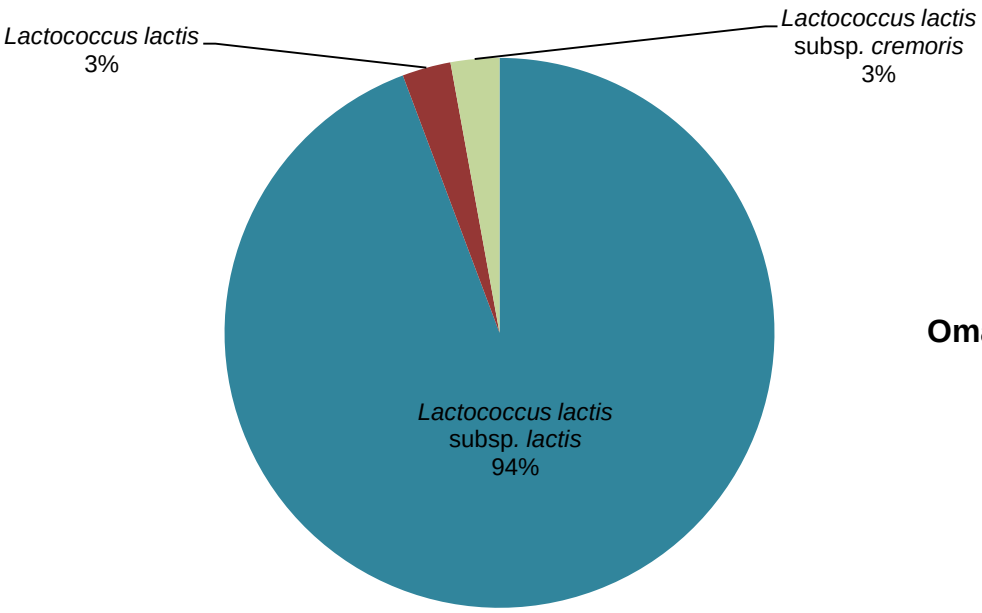
The distribution frequencies of the prevalent microbes isolated from the traditionally fermented milk omashikwa are shown in Fig. 3. The largest microbial group present was enterococci (43%), where 23% was *Enterococcus faecium,* 16% *Enterococcus durans* and 2% *Enterococcus* spp*.* The other LAB groups present included lactobacilli (24%), lactococci (16%) and *Leuconostoc pseudomesenteroides* (11%). Except for the enterococci, a large diversity of microbial contaminants were also present in low numbers (2%). Enterococci are possible microbial contaminants present in traditionally fermented milk. The traditional starter methods used for the preparation of traditional omashikwa resulted in a product with a variety of microbes present. It is clear that the traditional omashikwa was made under poor hygienic conditions due to the large variety of microbial contaminants identified.

The distribution frequencies of the prevalent microbes isolated from the traditionally fermented milk masse are shown in Fig. 4. The largest microbial group present was



Chambiko

Figure 1 Distribution frequency of the prevalent microbial species in commercial chambiko.



Omaere

Figure 2 Distribution frequency of the prevalent microbial species in commercial omaere.

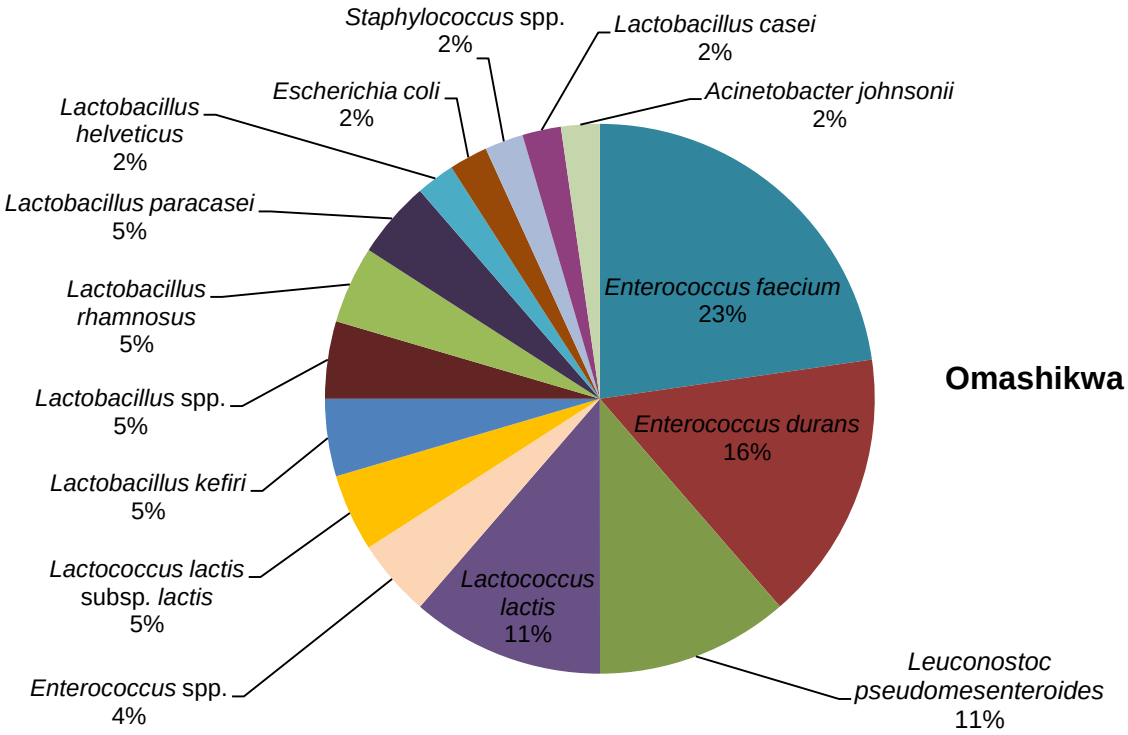


Figure 3 Distribution frequency of the prevalent microbial species in traditional omashikwa.

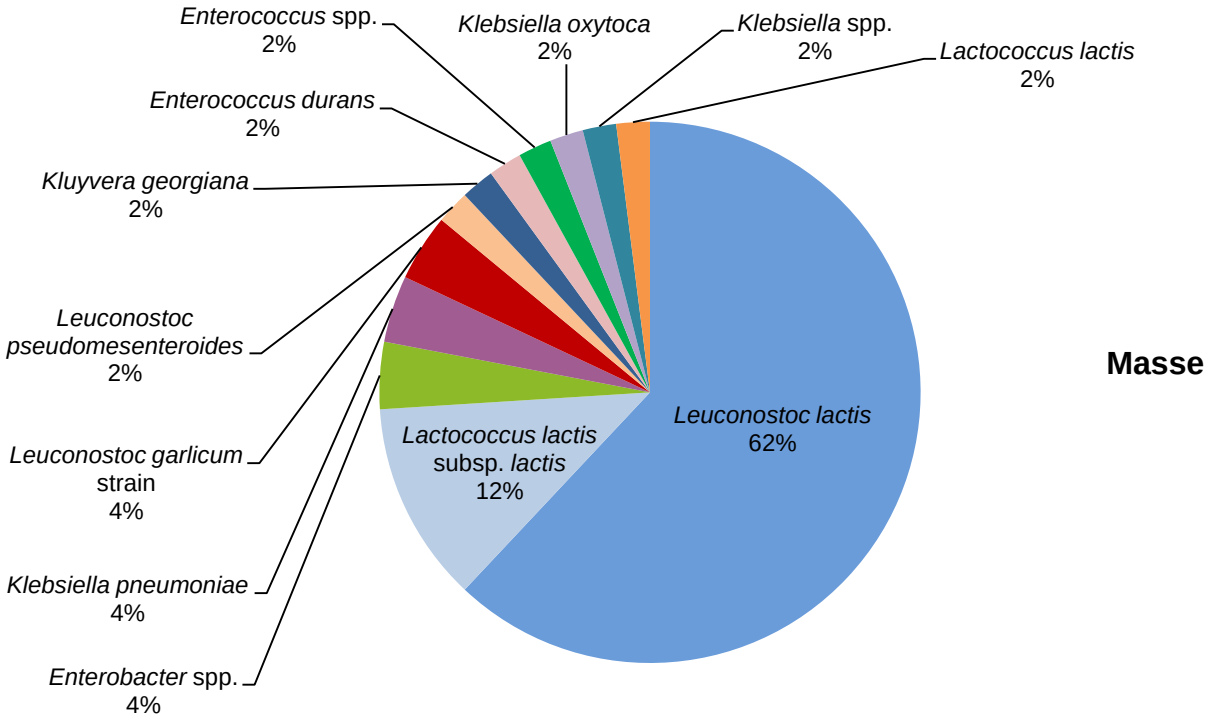


Figure 4 Distribution frequency of the prevalent microbial species in traditional masse.

leuconostocs (68%). If one combines the percentages of all the microbial contaminants present, including the enterococci isolates these microbes form the second largest microbial group (18%). The only other LAB group present except for the leuconostocs and enterococci were lactococci (14%). A smaller LAB diversity is observed in the masse than in the omashikwa. It is clear that the traditional masse was made under poor hygienic conditions due to the large variety and percentages of microbial contaminants identified.

The distribution frequencies of the prevalent microbes isolated from the traditionally fermented milk chekapmkaika are shown in Fig. 5. The most dominant microbial group present in the chekapmkaika consisted of three different *Bacillus* spp. (51%). The only LAB group present was lactobacilli consisting of only one *Lactobacillus helveticus* (45%) strain. *Staphylococcus warneri* (4%) was the only other microbial contaminant present.

A smaller LAB diversity is observed in the chekapmkaika than in the omashikwa and masse. Severe contamination of the product was observed due to the large number of *Bacillus* spp. identified. This product was, therefore, probably made from raw milk that was contaminated with *Bacillus* spp. and possible further contamination of these microbes could have taken place during production.

Conclusions

The results obtained in this study clearly show that the LAB diversity and the number of each LAB group present in the traditionally fermented milks omashikwa, masse and chekapmkaika differed from one another. The LAB diversity in the chekapmkaika was lower than in the omashikwa and masse, since only one LAB group was present in chekapmkaika and multiple LAB groups in the other traditional milks. The LAB diversity in traditionally fermented milk was generally more diverse than observed in the commercially fermented milk. Only one LAB group was present in each of the commercially fermented milks, chambiko and omaere and no more than three strains belonging to each group were identified. Starters used for these commercial fermented milks, therefore, consist of one to three LAB strains.

The data also clearly indicate that microbial contaminants were frequently present in the traditionally fermented milk. The masse had a more diverse number of microbial contaminants than what was seen in the omashikwa. Although, only one group of microbial contaminants was isolated from chekapmkaika it was the largest microbial

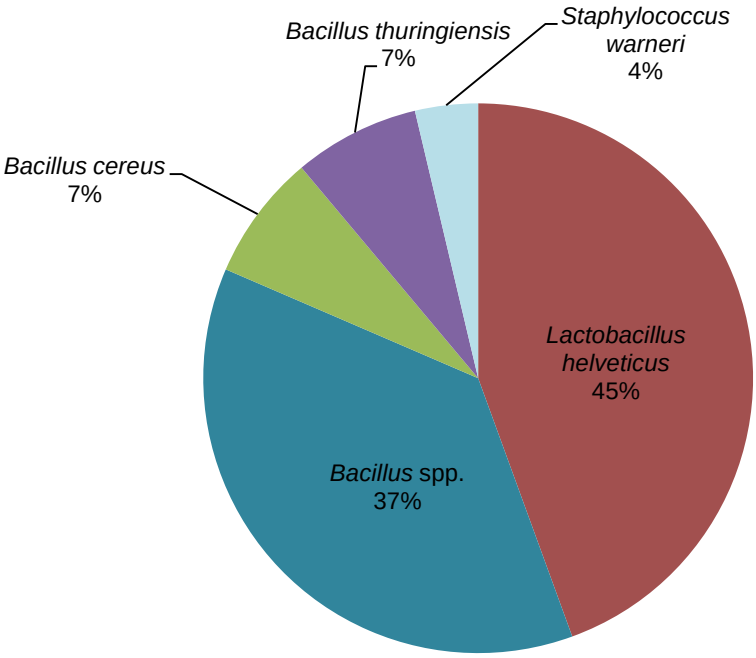


Figure 5 Distribution frequency of the prevalent microbial species in traditional chekapmkaika.

group present. Poor hygienic standards during the preparation of traditionally fermented milk can explain the extensive amounts of microbial contaminants present. The hygienic standards followed during traditional preparations must be improved for the purpose of product safety and quality.

The LAB strains isolated from the traditionally fermented milks can be used to develop new commercial starters. As a result new and original fermented dairy products with unique tastes, aromas and characteristics can be produced on an industrial scale.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Background

The preparation and consumption of traditionally fermented milk is popular among various communities (Mathara *et al*., 2004; McMaster *et al*., 2005). Traditionally fermented milk relies on spontaneous fermentation of raw milk or on backslopping of previously fermented milk (Oyewole, 1997). The microbial consortium and product characteristics of traditionally fermented milk are influenced by the milk type, quality, the heat-treatment applied to the milk before fermentation, the ambient temperatures, cultural starter traditions, container vessels used and the duration of the fermentation process (Holzapfel, 2002, Wouters *et al*., 2002, Savadogo *et al*., 2004; Kebede *et al*., 2007; Akabanda *et al*., 2010). Poor hygienic practices often occur during the production of these milks and microbial contaminants are frequently isolated (Mensah, 1997; Motarjemi, 2002).

The aim of this study was to enumerate and identify the microbial consortium present in each of three traditionally fermented milks from Sub-Saharan Africa, namely omashikwa from Namibia, masse from Mozambique and chekapmkaika from Uganda and two commercially fermented milks, namely chambiko from Malawi and omaere from Namibia. After enumeration, colonies were selected by means of the Harrison Disk method from the media used for the selection of lactic acid bacteria (LAB) and acetic acid bacteria (AAB). The selected microbes were identified using the polymerase chain reaction (PCR) and DNA sequencing of part of the 16S ribosomal RNA (rRNA) gene.

Enumeration of the commercial and traditional Sub-Sahara African fermented milks

In this study the microbial consortium present in three traditionally fermented milks and two commercially fermented milks were enumerated on six different selective media. No significant differences were found between the enumeration values obtained for the three chambiko samples, as well as for the two omaere samples on each of the six media. Similarities between samples were also observed after measuring the pH values, since the same pH values were obtained between the samples. The low variances between these two sample sets in terms of microbial counts and pH values

could be due to the production of commercially fermented milks according to standardised production processes under optimally controlled environmental conditions.

The average enumeration values obtained on the media used for the isolation of LAB for the chambiko and omaere indicated that the highest microbial counts were observed on the KCA + V medium in the chambiko (1.8×10^5 cfu.ml^{-1}) used for the isolation of leuconostocs and on the KCA + TTC medium in omaere (2.3×10^6 cfu.ml^{-1}) used for the isolation of lactococci. Therefore, it may be possible that the predominant microbes present in the starters used for the chambiko and omaere could be strains from the genus *Leuconostoc and Lactococcus*, respectively. A comparison of the average enumeration values obtained for the chambiko and omaere on the MRS + C, KCA + TTC, KCA + V and MRS + E revealed that the average enumeration values obtained for the omaere were significantly larger. The higher microbial counts present in the omaere could reflect a higher concentration of microbes present in the starter used for its production.

All the enumeration values obtained for the three traditional omashikwa samples differed significantly from one another on all selective media except on the MRS +C, showing large variances within the sample set. For omashikwa, samples 2 and 3 were more comparable to each other in terms of their microbial count and pH values. Since no average enumeration values could be determined due to these large sample variances, the highest enumeration values for samples 2 and 3 obtained on KCA + TTC medium (1.0×10^6 and 1.4×10^6 cfu.ml^{-1}, respectively) were taken as an indication of the media with the highest microbial growth. Therefore the main dominant microbial group present in omashikwa may be lactococci.

Significant differences between enumeration values of three traditional masse samples were found for the media MRS + C, KCA + TTC, KCA + V and MRS + E. Significant differences between enumeration values of the two traditional chekapmkaika samples were only found on two media (MRS + C and MRS + E).

Variances between the samples may be a result of the inconsistent preparation methods of traditionally fermented milk and the exposure of different batches to varying environmental conditions. However, little sample variance found between the masse and chekapmkaika may indicate that traditional fermentations can be of consistent quality.

Based on the average enumeration values obtained on the media used for the isolation of LAB, the highest counts for both the masse and chekapmkaika were observed on MRS + C medium (6.2×10^6 and 2.0×10^3 cfu.ml^{-1}, respectively). It is,

therefore, possible that the predominant LAB group present in these two milks are lactobacilli. The average enumeration values obtained on all six selective media for the masse were significantly larger than the average enumeration values on these media obtained for the chambiko. Significant differences were also found in microbial counts detected on each of the six media when comparing the average enumeration values between the commercial and traditionally fermented milks.

Selection and identification of the commercial and traditional Sub-Sahara African fermented milks

In this study the microbial consortium present in each of the three traditionally fermented milks and two commercially fermented milks were identified. This was done by selecting bacterial isolates obtained on MRS + C (lactobacilli), KCA + TTC (lactococci), KCA + V (leuconostocs) and MRS + E (AAB) media by means of the Harrison Disk method. The selected microbes were identified by using PCR and DNA sequencing.

All the LAB strains isolated from commercial chambiko were identified as lactobacilli. *Lactobacillus casei* (30%) was the largest lactobacilli group, followed by *Lactobacillus paracasei* and *Lactobacillus paracasei* subsp. *paracasei.* Lactobacilli were isolated from MRS + C, KCA + V, KCA + TTC and MRS + E. Microbial contaminants present in the chambiko selected from KCA + TTC were identified as *Mesorhizobium loti* and *Acinetobacter radioresistens*.

All the LAB strains isolated from commercial omaere were identified as lactococci. The largest group present was *Lactococcus lactis* subsp. *lactis* (94%), followed by *Lactococcus lactis* subsp. *cremoris.* Lactococci were isolated from MRS + C, KCA + TTC and MRS + E. Only one LAB group was present in both the commercially fermented milks. Starters used for these commercial fermented milks, therefore, consist of only a few LAB strains. Since no microbial contaminants were found in the omaere, it may be possible to say that the standardised production process followed during the production of omaere is most likely better controlled.

LAB strains identified from traditional omashikwa belonged to the genera *Enterococcus* (43%), *Lactobacillus* (24%), *Lactococcus* (16%) and *Leuconostoc* (11%). Leuconostocs, lactococci and lactobacilli strains were selected from KCA + TTC, KCA + V and MRS + E. The two main enterococci strains representing the enterococci group were *Enterococcus faecium* and *Enterococcus durans.* Due to the involvement of

enterococci in human infections the role of enterococci in foods are controversial. Other opportunistic pathogens present were *Escherichia coli, Acinetobacter johnsonii* and *Staphylococcus* spp.

LAB strains identified from traditionally prepared masse belong to the genera *Leuconostoc* (68%), *Lactococcus* (14%) and *Enterococcus* (4%). Most of the lactococci strains were identified as *Lactococcus lactic* subsp*. lactis* and isolated from KCA + TTC. Leuconostocs present were *Leuconostoc pseudomesenteroides, Leuconostoc lactis* and *Leuconostoc garlicum. Leuconostoc* spp. were selected from all the media, except KCA + TTC. Other contaminating microbes that were isolated included *Kluyvera georgiana, Klebsiella oxytoca*, *Enterobacter* spp. and *Klebsiella pneumonia.*

Lactobacillus helveticus (45%) was the only LAB strain isolated from chekapmkaika. All the lactobacilli strains were isolated on MRS + C medium. *Bacillus* spp*.*, including *Bacillus cereus* and *Bacillus thuringiensis* were identified from MRS + E medium and was the largest group present (51%). *Bacillus* spp. are bacteria that forms endospores and are frequently found in dairy products.

In this study it was found that the media used for the isolation of the LAB (MRS + C, KCA + TTC and KCA + V media) and AAB (MRS + E medium) was not highly selective and microbial strains were present on more than one of the selective media. Therefore, it is vital to take medium selectivity into account when conclusions are made on the microbial groups present when only looking at the enumeration values obtained.

Concluding remarks

From the data obtained in this study it is clear that fermented milks from Sub-Saharan Africa vary significantly from each other in terms of microbial loads and dominant microbial groups present. Identification of microbial isolates after enumeration was important before the microbial consortium present in each milk could be described as the selective media used were not highly selective for specific microbes.

The microbes isolated and identified from traditionally fermented milks were more diverse than the microbes present in the commercially fermented milks. The microbial diversity of the traditionally fermented milks also varied significantly from one another. A wide variety of contaminating microbes were detected in the traditionally fermented milks and this may indicate that hygienic practices were not followed during these preparations.

Little information is currently available on the microbial consortiums present in fermented milk from Sub-Saharan Africa. Furthermore, the cultural starter traditions are poorly understood. LAB strains isolated from these traditionally fermented milk products can be used to develop new commercial starter cultures and as a result new dairy products can be produced with unique organoleptic properties and characteristics.

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