THE INHIBITORY ACTIVITY AND SENSORY PROPERTIES OF KEFIR, TARGETING THE LOW-INCOME AFRICAN CONSUMER MARKET

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DECLARATION

I, the undersigned hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

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ABSTRACT

The rapid urbanisation of the African population has led to the establishment of large low-income communities in and around almost every major town and city in South Africa. Several factors prevent these people from producing or obtaining their traditional fermented milk drink, Maas (*Amasi*), often resulting in the occurrence of malnutrition in low-income urban African communities.

A product with the potential to satisfy the demand for a fermented milk product is Kefir. Kefir, a self-carbonated fermented milk, is commonly manufactured by fermenting unpasteurised or pasteurised milk with re-usable Kefir grains. These Kefir grains consist of a combination of mainly lactic acid bacteria and yeasts. Neither Kefir, nor Kefir grains are as yet marketed in South Africa, thus creating an excellent opportunity to launch these products locally.

It is often difficult for the low-income communities to obtain high quality unpasteurised or pasteurised milk, resulting in a serious health risk. The inhibitory activity of Kefir towards certain spoilage and pathogenic microorganisms was, therefore, studied. Strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Clostridium tyrobutyricum* were inoculated (10^3 – 10^4 cfu.ml^{-1}) into pasteurised milk together with Kefir grains (18 gram per litre) and incubated at 25°C. Uninoculated milk samples and milk samples inoculated only with test organisms served as controls. Growth of all the test organisms were inhibited substantially (≥99.9%) in Kefir over the 30 h incubation period and substantial reductions in microbial log cycles were observed for many of the organisms. This coincided with a steep decrease in pH (6.57 – 4.06) and increase in titratable acidity (0.20 – 0.72%).

If Kefir is eventually marketed to low-income urban African consumers, it will have to compete with Maas and, therefore, comparative sensory testing of Kefir and Maas was conducted. The differences in the sensory properties of Kefir, 'laboratory' Maas (representing traditional Maas) and commercial Maas (containing thickener, colourants and flavourants) were determined by a trained panel. These characteristics were identified as "yeasty" and "cowy" tastes ($p < 0.05$), "effervescence" ($p < 0.01$), as well as "sourness," "creaminess" and "smoothness" ($p < 0.001$). The effect of different incubation temperatures (25°, 30° and 35°C) on the

Kefir sensory properties was studied to simulate the effect of the large temperature variations that would be found in the dwellings of low-income African urbanites. The "sourness" and "creaminess" of the Kefir was found to increase with increase in incubation temperature but no strong off-flavours were found to develop. Sensory preference testing was conducted by consumer panels consisting of panellists of different ages and population groups to indicate whether the specific panels significantly prefer Kefir, commercial Maas or laboratory Maas. It was found that commercial Maas was significantly ($p < 0.001$) preferred to Kefir by young African urbanites. Adult Africans, who presumably still have traditional taste preferences, however, equally ($p > 0.05$) preferred Kefir and laboratory Maas, identifying this segment of the African population as the appropriate starting target market for Kefir. Kefir and laboratory Maas were also tested for preference by a wider panel consisting of people (aged between 18 and 25) representing the different population groups in South Africa. Kefir and laboratory Maas were preferred equally ($p > 0.05$) by all the groups.

Several arguments supporting Kefir marketing to the low-income urban African population of South Africa have been identified. These include: Kefir's ease of preparation; the re-usability of Kefir grains and subsequent affordability; good packaging, distribution and storage possibilities; Kefir's acceptability by lactose-intolerant individuals; high nutritional value; the inhibitory activity of Kefir against potential spoilage and pathogenic organisms and subsequent enhanced safety and keeping ability; and Kefir's acceptable refreshing taste.

UITTREKSEL

Die toenemende verstedeliking van Swart Suid-Afrikaners het gelei tot die vestiging van groot lae-inkomste gemeenskappe in en om die meeste groot dorpe en stede. Verskeie faktore verhoed dat hierdie gemeenskappe hul tradisionele gefermenteerde melk, naamlik Maas (*Amasi*), self kan maak of koop. Dit lei dikwels tot wanvoeding onder lae-inkomste stedelike Swart verbruikers.

Kefir het die potensiaal om te voorsien in die vraag na 'n gefermenteerde melk produk in lae inkomste stedelike Swart gemeenskappe. Kefir is 'n self-gekarboneerde, gefermenteerde melk wat vervaarding word deur die fermentasie van ongepasteuriseerde of gepasteuriseerde melk met herbruikbare Kefirkorrels. Hierdie Kefirkorrels bestaan uit 'n kombinasie van hoofsaaklik melksuurbakterieë en giste. Kefir en Kefirkorrels word glad nie in Suid-Afrika bemark nie, en bied 'n fantastiese geleentheid om hierdie produkte plaaslik bekend te stel.

Dit is dikwels moeilik om hoë kwaliteit ongepasteuriseerde of gepasteuriseerde melk in lae-inkomste gemeenskappe te verkry. Die risiko om siektes deur die verbruik van hierdie melk op te doen, bestaan dus. Om hierdie rede is die inhiberende effek van Kefir teenoor spesifieke bederf- en patogeniese bakterieë bestudeer. Rasse van *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* en *Clostridium tyrobutyricum* is geïnokuleer (10^3 – 10^4 cfu.ml^{-1}) in gepasteuriseerde melk tesame met Kefirkorrels (18 gram per liter) en geïnkubeer by 25°C. Melkmonsters wat slegs geïnokuleer is met die toetsorganismes het as kontroles gedien. Die groei van al die toetsorganismes is substansieël geïnhibeer (≥99.9%) in Kefir gedurende die 30 h inkubasieperiode. Substansiële afnames in logsiklusgetalle is waargeneem vir baie van die organismes. Dit het gepaard gegaan met 'n skerp afname in pH (6.57 – 4.06) en toename in titreerbare suurheid (0.20 – 0.72%) vir die Kefirmonsters gedurende die 30 h inkubasieperiode.

Indien Kefir bemark word aan lae-inkomste stedelike Swart verbruikers sal dit moet kompeteer met Maas. Vergelykende sensoriese toetse is dus uitgevoer. Die verskille in die sensoriese eienskappe van Kefir, 'laboratorium' Maas (verteenwoordigend van tradisionele Maas) en kommersiële Maas (wat verdikker, kleur- en geurmiddels bevat) is bepaal deur 'n opgeleide paneel en geïdentifiseer as

die " "gis-" en "koeismake" ($p < 0.05$), die "gasserigheid" ($p < 0.01$) asook die "suurheid", "romerigheid" en "gladheid" ($p < 0.001$) van die monsters. Die effek van verskillende inkubasietemperature (25°, 30° en 35°C) op die sensoriese eienskappe van Kefir is bestudeer om die effek van die groot temperatuurvariasies wat in lae-inkomste behuising mag voorkom, te simuleer. Daar is bevind dat die "suurheid" en "romerigheid" van Kefir toeneem met verhoging in inkubasietemperatuur terwyl geen afsmake ontwikkel nie.

Sensoriese voorkeurtoetse is deur verbruikerspanele van verskillende ouderdomme en bevolkingsgroepe uitgevoer om te bepaal of die spesifieke panele 'n beduidende voorkeur toon vir Kefir, laboratorium Maas of kommersiële Maas. Daar is bevind dat stedelike Swart jongmense kommersiële Maas beduidend ($p < 0.001$) bo Kefir verkies. Swart volwassenes met verwagte tradisionele smaakvoorkeure het egter Kefir en laboratorium Maas ewe veel verkies ($p > 0.05$). Hierdie segment van die Swart bevolking is dus die geskikte teikenmark vir die bekendstelling van Kefir. Voorkeur vir Kefir en laboratorium Maas is ook getoets deur 'n paneel (ouderdom 18 – 25 jaar) wat bestaan uit mense van verskillende bevolkingsgroepe. Al die groepe het Kefir en Maas ewe veel verkies ($p > 0.05$).

Verskeie argumente ten gunste van die bemarking van Kefir aan lae-inkomste stedelike Swart gemeenskappe in Suid-Afrika is geïdentifiseer. Dit behels die volgende: die gerief van Kefirvervaardiging; die herbruikbaarheid van Kefirkorrels en gevolglike bekostigbaarheid; goeie verpakkings-, verspreidings- en opbergingsmoontlikhede; Kefir se aanvaarbaarheid vir laktose-intolerante individue; Kefir se hoë voedingswaarde; die inhiberende aktiwiteit wat Kefir teenoor potensiële bederf- en patogeniese organismes het en die gevolglike verhoging in veiligheid en rakleeftyd van melk; en Kefir se aanvaarbare verfrissende smaak.

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CONTENTS

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

CHAPTER 1

INTRODUCTION

It has become necessary to expand the local South African food supply as a result of the high food spoilage rate and the rapidly growing population (Myburgh, 1995). As a result of the unnatural mass urbanisation, as well as the limited economic growth and resulting "urban unemployment," communities for which the food consumption behaviour differs significantly from that of the well-to-do Western urban resident can clearly be identified. These are the newly urbanised low-income communities that have evolved in and around almost every town and city in South Africa (Myburgh, 1995).

Commercial dairy products in South Africa have traditionally been developed and produced for sophisticated and affluent consumers. Both the price and the technology (including processing, packaging, storage and distribution) make these products unattainable to the majority of the South African population with their extremely low purchasing power and their specific living conditions.

Fermented milk products can be produced in small dairies with relatively inexpensive equipment, which could even be made in developing countries and, in addition, the fermented product may be sold without having to be retail packaged. Fermented milk products can be sold in bulk without any major hygiene risks and, if it is well suited to local conditions, it can even be sold without refrigeration (Bachmann, 1984).

Due to several limiting factors the low-income urban consumer market is deprived of its traditional fermented milk drink Maas (*Amasi*) which has been produced for many generations by allowing unpasteurised milk to sour (Coetzee, 1982; Keller & Jordaan, 1990). A recent food consumption study conducted in the rural areas of the Eastern Cape Province revealed an average consumption of 1.4 litres Maas per day per adult equivalent (M. Nomakaya, 1999, Department of Agricultural Economics, University of Fort Hare, personal communication). One of the direct results of the urbanisation process is that unpasteurised milk is nowadays

not as freely available as it used to be for the traditional production of Maas (Dr. A.S. Myburgh, 1999, Department of Agricultural Economics, University of Stellenbosch, personal communication). A new law that came into effect in November 1999 stipulates that nobody may sell raw (unpasteurised) milk or raw cream unless it is to be used for further processing (Anon., 1997; Viall, 1999). The production of Maas is not considered as "further processing". Local authorities may apply to be listed to allow the sale of raw milk in their areas if they think they can control the safety of the raw milk (Anon., 1997; Viall, 1999). Commercially manufactured Maas again is too expensive for most members of these low-income communities to purchase, thus distancing them from a traditional and nutritional food product. This situation sets a challenge to the marketer to come up with a product that provides the traditional need for a cheap and easy to produce sour milk. One such a product that could fulfil this need, is Kefir.

Kefir is a self-carbonated, fermented milk that originated in the Caucasian region of the Soviet Union hundreds of years ago. Its flavour is mildly alcoholic, yeasty-sour, with a tangy effervescence (Duitschaever, 1989). Kefir is commonly manufactured by fermenting milk with Kefir grains. The Kefir grains are clusters of microorganisms held together by a matrix of polysaccharides (Garrote *et al.*, 1998). It is formed in the process of making Kefir and, as far as known, only from existing grains (Steinkraus, 1996).

Kefir manufacturing is a low-cost method of preserving milk where *ca.* 18 g of Kefir grains are placed in 1 litre pasteurised full cream milk in a clean container (A. Schoevers, 1999, Department of Food Science, University of Stellenbosch, personal communication). The mixture is then incubated at room temperature for approximately 24 h or until the desired consistency is reached. The Kefir is then strained into a bowl to separate and retrieve the grains, which can immediately be used to ferment the next batch of milk or be stored in a cool place (Marshall, 1993; Saloff-Coste, 1996). Kefir has great nutritional value as well as other health benefits such as a low lactose content, stimulation of digestion and appetite, as well as lowering of blood cholesterol levels (Blanc, 1984; Gurr, 1987; Buttriss, 1997). Kefir has also been reported (Garrote *et al.*, 2000) to possess an inhibitory activity towards certain spoilage and pathogenic microorganisms.

Researchers doing studies on the food and meal pattern of urbanised low-income communities have concluded that insufficient dairy products are consumed by the subjects (Bourne *et al.*, 1994). This is probably mainly due to the unavailability of refrigeration facilities, the unavailability of products that are consumed traditionally (such as Maas) and the high costs of commercially-made traditional products. This presents particularly great opportunities for the marketing of fermented products such as Kefir. If one considers the fact that Africans have a high incidence of lactose intolerance, which means that they prefer fermented milks to other dairy products (Keller & Jordaan, 1990), the marketing of Kefir under the African population presents a promising opportunity. The main factors in favour of Kefir are the fact that it can be produced from pasteurised milk, unlike traditionally-made Maas, and that the starter culture, the Kefir grains, can be used repeatedly (Merin & Rosenthal, 1986; Marshall, 1993; Saloff-Coste, 1996).

If Kefir grains and the technology of Kefir production were more widely known, Kefir consumption would become much more widespread. Since the manufacture of Kefir is simplistic, the general market price of Kefir could be set at just slightly higher than that of milk. Its manufacturing at home is sufficiently easy so that no home would have to be without it once Kefir grains become commercially available. The objectives of this study were to match Kefir's specific characteristics to the needs and requirements of low-income urban African communities in South Africa and to ascertain the feasibility of marketing Kefir and Kefir grains to these communities.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

Milk has been an important food for man since the domestication of cattle and the adoption of a pastoralist agriculture (Kon, 1972). Milk can be defined as the fluid secreted by the mammary glands of mammals for the primary purpose of nourishing their young. It is designed to satisfy the nutrient requirements of the young animal and, therefore, it is generally an excellent source of calories, protein, fat, carbohydrates, vitamins and minerals. Milk is not only very nutritious for humans, it is also an excellent substrate for various microorganisms (Steinkraus, 1996).

In the majority of developing countries, it is rare for large quantities of cooled fresh milk to be collected and distributed efficiently. It is generally recognised that after 5 - 6 h milk will sour or start fermenting (Marshall, 1987). It is not surprising, therefore, that many communities acquired a taste for "sour milk" or that, with time, techniques were developed to ensure that the process of souring followed a particular pattern (Tamime & Robinson, 1988).

Man discovered the preservation effects of fermentation by simply allowing a foodstuff (milk, meat, fish, vegetables, grain or cereal) to sour/decompose/undergo change (Marshall, 1987). Some fermentations were successful, others not. Finding the fermented foods to have a good aroma and pleasant taste and texture, man contrived to repeat the process (Marshall, 1987). Fermentation of milk is an ancient practice and its main aim was to obtain products with characteristic flavour, aroma and consistency which, at the same time, could be stored unspoiled for a longer time than raw milk (Roginski, 1988).

There is archaeological evidence that fermentation of milk in the Middle East dates back as far as 2900 – 2460 BC (Roginski, 1988). There is also a reference to fermentation of milk in the Bible (*Genesis 18.8*). It was not until 1890 that the first defined cultures of lactic acid bacteria were used in Denmark for the production of

cultured butter (Kurmann, 1984). Since then, impressive progress has been made in starter selection and starter improvement techniques (Saloff-Coste, 1996).

Biochemically, fermentation can be defined as the metabolic process in which carbohydrates and related compounds are partially oxidised with the release of energy in the absence of any external electron acceptors (Jay, 1996). The final electron acceptors are organic compounds produced directly from the breakdown of the carbohydrates. Consequently, incomplete oxidation of the parent compound occurs, and only a small amount of energy is released during the process. The products of fermentation consist of organic compounds that are more reduced than others (Jay, 1996).

The most basic reaction occurring during the fermentation of milk is the utilisation of milk sugar (lactose) by lactic acid bacteria to produce lactic acid which sours the milk, lowers the pH, and literally preserves the milk against spoilage. By controlling the conditions of fermentation, the natural microbial population can be altered to produce widely different fermented products (Steinkraus, 1996).

According to the definition proposed in 1963 by the International Dairy Federation, "fermented milks are products prepared from milk, skimmed or not, concentrated or not, with specific cultures; the microflora is kept alive until sale to the consumer and may not contain any pathogenic germ" (Roginski, 1988). Kosikowski added in 1984, that metabolic substances derived from fermentation must be present in fermented milks.

A multitude of fermented milks generally known as "cultured milks" are nowadays commercially produced in large volumes, worldwide. Most of these products reflect long-standing nutritional traditions in different parts of the world, but there are also a number of products successfully developed as a direct consequence of progress in clinical and industrial biotechnology over the last 60 years (Roginski, 1988). A generalised scheme for the classification of fermented milks according to the type of fermentation that occurs, is given in Table 1 (Tamime & Robinson, 1988).

Table 1. Classification and some examples of fermented milk products (Tamime & Robinson, 1988).

Type of fermentation	Traditional name	Country of origin
I. Lactic acid		
A. Mesophilic	Taetmjolk	Scandinavia
	Filmjolk	Scandinavia
	Lattfil	Scandinavia
	Langfil	Scandinavia
	Maziwa lala	Kenya
	Ymer	Denmark
B. Thermophilic	Yogurt	Most countries
	Bulgarian buttermilk	Bulgaria
	Yakult	Japan
	Liquid yogurt	Korea
C. Therapeutic	ACO-yogurt	Switzerland
	A-38 fermented milk	Denmark
	AB-yogurt	Denmark
	Biogarde	Federal Germany
	Bioghurt	Federal Germany
	Bifighurt	Federal Germany
	Mil-Mil E	Japan
	Miru-Miru	Japan
	Yakult	Japan
II. Yeast-lactic acid		
	Kefir	Russia
	Koumiss	Russia
	Acidophilus-yeast milk	Russia
III. Mould-lactic acid		
	Viili	Finland

B. PRESERVATION OF MILK BY FERMENTATION

Microbiology of raw milk

The numbers and types of microorganisms in milk immediately after milking (initial microbial population), reflects directly the level of microbial contamination during this production process. There are three main sources of contamination, namely, from within the udder, from the exterior of the teats and udder and from the milking and storage equipment (Robinson, 1990). The microbial population of the milk when it leaves the farm is determined by the temperatures to which it has been cooled and stored, the time elapsing before collection and the initial microbial population. The bacterial content of raw milk may be increased by the presence of mastitis among the producing animals. Mastitis, or udder inflammation, is usually a consequence of bacterial infection, and is responsible for considerable economic losses to the dairy industry as the milk yield is reduced drastically. *Streptococcus agalactiae* and *Staphylococcus aureus* are most commonly the causes of mastitis, and are spread between udder quarters and cows, primarily during milking, since the major source of the organisms within the herd is the infected udder (Robinson, 1990).

Raw milk held at refrigerator temperatures for several days may invariably exhibit the presence of several or all members of the following genera: *Enterococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Microbacterium*, *Oerskovia*, *Propionibacterium*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Bacillus* and *Listeria*, as well as members of at least one of the coliform group (Jay, 1996). Certain psychrotrophic endosporeformers (*Bacillus* spp.) and mycobacteria (*Mycobacterium* and *Nocardia* spp.) may also be found in raw milk.

Raw milk may contain microorganisms pathogenic to man. Campylobacteriosis and salmonellosis are well established as illnesses that may be contracted from milk and milk products. Listeriosis and hemorrhagic colitis outbreaks have also been traced to milk (Jay, 1996). The most important and serious human diseases disseminated by the consumption of contaminated raw milk are tuberculosis and brucellosis (Robinson, 1990). In both diseases the causative organism, *Mycobacterium bovis* or *M. tuberculosis*, and *Brucella abortus*, *Br. melitensis* or *Br. suis*, may be excreted in the milk from infected animals. Pathogenic bacteria may also be present in raw milk as a direct consequence of udder disease. High numbers

of *Escherichia coli* may be present in milk as a consequence of mastitis, and members of this species is responsible for several different diseases of man of varying severity. Infrequently microorganisms of greater pathogenicity for man produce bovine mastitis and may be present in raw milk. They include *Leptospira* spp., *Listeria monocytogenes*, *Bacillus cereus*, *Pasteurella multocida*, *Clostridium perfringens*, *Nocardia* spp., *Cryptococcus neoformans* and *Actinomyces* subspecies. Additionally, *Coxiella burnetii*, the causative agent of Q-fever, may infect the udder, probably by the haemotogenous route, and contact with, or consumption of, infected milk can lead to human infection (Robinson, 1990). All of these pathogens, with the exception of *Clostridium perfringens* and *Bacillus cereus,* are destroyed by pasteurisation. These two organisms can survive the pasteurisation process because of their ability to sporulate (Robinson, 1990). In Table 2 some of the thermoduric and psychrotrophic microorganisms found in fresh raw milk are listed.

Antimicrobial systems in fermented milks

Milk is literally preserved against spoilage as a result of the process of fermentation (Steinkraus, 1996). As this is one of the primary advantages of milk fermentation, it is worthwhile to consider those characteristics of fermented milks that affect the growth of microorganisms.

Presence and activities of other microorganisms - Some foodborne organisms produce substances that are either inhibitory or lethal to others. Lactic antagonism is often found in fermented milk products and refers to the phenomenon of a lactic acid bacterium inhibiting or killing closely related and food-poisoning or food-spoilage organisms when in mixed culture (Varadaraj *et al.*, 1993; Gupta *et al.*, 1996; Zapico *et al.*, 1998). Evidence exists concerning the inhibition of *Staphylococcus aureus*, *Pseudomonas putrifaciens*, *Escherichia coli*, *Clostridium perfringens*, *Salmonella tennessee*, *Vibrio parahemolyticus*, and other spoilage and pathogenic organisms by lactic cultures, such as *Lactococcus lactis*, *Lactococcus diacetylactis, Leuconostoc cremoris* and the lactobacilli (Shanani & Chandan, 1979). The precise mechanisms are yet unclear. Amongst factors identified are antibiotics, hydrogen peroxide, depressed pH, diacetyl, nutrient depletion and bacteriocins or bacteriocinlike factors (Marshall, 1987; Balasubramanyam & Varadaraj, 1994; Naidu *et al.*, 1999).

Table 2. Thermoduric and psychrotrophic microorganisms in fresh raw milk (Robinson, 1990).

Thermoduric genera[a]	Psychrotrophic genera[b]
Microbacterium	*Pseudomonas*
Micrococcus	*Acinetobacter*
Bacillus spores	*Flavobacterium*
Clostridium spores	*Aerobacter*
Alcaligenes	*Alcaligenes*
	Bacillus
	Arthrobacter

[a]Can survive heating at 63°C for 30 min.

[b]Visible growth at 5° - 7°C in 7 - 10 d.

pH - Most microorganisms grow best at pH values around 7.0 (6.6 – 7.5), whereas few grow below 4.0 (Jay, 1996). Bacteria tend to be more fastidious in their tolerance to pH than moulds and yeasts, with the pathogenic bacteria being the most fastidious. Some foods are characterised by inherent acidity while others owe their acidity of pH to the actions of certain microorganisms. The latter type is referred to as biological acidity and is displayed by products such as fermented milks, sauerkraut and pickles (Jay, 1996).

The rapid acidification which occurs during growth of the starter bacteria in fermented milks prevents the growth of many other organisms (Marshall, 1987). This decrease in the luminal pH is due to the production of volatile short-chain fatty acids (SCFA) such as acetic, lactic or propionic acid (Naidu *et al.*, 1999). Adverse pH affects at least two aspects of a respiring microbial cell: the functioning of its enzymes; and the transport of nutrients into the cell (Jay, 1996). When microorganisms are placed in environments below or above neutrality, their ability to proliferate depends on their ability to bring the environmental pH to a more optimum value or range. Among the other effects that are exerted on microorganisms by adverse pH is that of the interaction between H^+ and the enzymes in the cytoplasmic membrane. The morphology of some microorganisms can be affected by the pH. An adverse pH makes cells much more sensitive to toxic agents of a wide variety, and young cells are more susceptible to pH changes than older or resting cells. When microorganisms are grown on either side of their optimum pH range, an increased lag phase results (Jay, 1996).

Lactic acid and volatile acids – Fermentation involving lactic acid bacteria (LAB) results in the accumulation of organic acids, primarily lactic acid as a major end-product of carbohydrate metabolism. The accumulation of lactic acid (and the concomitant reduction in pH of the milieu) results in a broad-spectrum inhibitory activity against Gram-positive and Gram-negative bacteria (Naidu *et al.*, 1999; Garrote *et al.*, 2000).

Lactic and acetic acids are known to inhibit *Staphylococcus aureus*. A synergism between lactic acid and acetic acid in the inhibition of *E. coli* and *Salmonella* has also been reported. The growth of *Bacillus cereus* was blocked in

the presence of LAB due to acetate production and spore germination was inhibited by formate, lactate and acetate (Naidu *et al.*, 1999).

Hydrogen peroxide – In the presence of oxygen, LAB produce hydrogen peroxide (H_2O_2) through electron transport via flavin enzymes (Naidu *et al.*, 1999). In the presence of H_2O_2, superoxide anions form destructive hydroxy radicals. This process may lead to peroxidation of membrane lipids, and increased membrane permeability. The resulting bactericidal effect of these oxygen metabolites has been attributed to their strong oxidizing effect on the bacterial cell as well as destruction of nucleic acids and cell proteins. Also, H_2O_2 could react with other cellular and milieu components to form additional inhibitory substances (Naidu *et al.*, 1999).

Bacteriocins – LAB produce a wide range of antagonistic factors that include metabolic products, antibiotic-like substances, and bactericidal proteins, collectively termed bacteriocins. Bacteriocins vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties (Naidu *et al.*, 1999).

Lactococcus lactis subsp. *cremoris* produces diplococcin which can be antagonistic towards some strains of *Staphylococcus aureus* (Brialy *et al.*, 1995), whilst subsp. *lactis* produces nisin which is primarily active against Gram-positive organisms. *Lactobacillus acidophilus* produces lactocidin, acidophilin, acidolin, lactobacillin, lactocidin and lactolin (Shanani & Chandan, 1979; Marshall, 1987; Varadaraj *et al.*, 1993). *Lactobacillus bulgaricus* produces bulgarican and *L. brevis* produces lactobrevin. Both acidophilin and bulgarican are active against a wide variety of Gram-positive and Gram-negative organisms that include nonpathogens as well as pathogens (Shanani & Chandan, 1979).

Carbon dioxide – Carbon dioxide (CO_2) is a major end-product of hexose fermentation by heterofermentative LAB. It is also produced in substantial volumes during yeast-lactic acid fermentations, such as occur in Kefir. The CO_2 contributes to the antimicrobial activity of LAB. The role of CO_2 in creating an anaerobic environment by replacing existent molecular oxygen, the extra and intracellular capability to decrease pH and the destructive effects on cell membranes, makes CO_2

a potent inhibitory system against a wide variety of microorganisms (Naidu *et al.*, 1999).

Diacetyl and acetaldehyde – Diacetyl is an end product of pyruvate metabolism by citrate-fermenting LAB (Naidu *et al.*, 1999). It elicits a potent antimicrobial activity against various food-borne pathogens and spoilage microorganisms. Diacetyl is more effective against Gram-negative bacteria, yeasts and moulds than against Gram-positive organisms (Naidu *et al.*, 1999).

Acetaldehyde is formed during the carbohydrate metabolism of heterofermentative LAB and has been shown to have antimicrobial activity against several food-borne pathogens e.g. *E. coli, Salmonella typhimurium* and *Staphylococcus aureus* (Naidu *et al.*, 1999).

Antimicrobial constituents in milk – Cow's milk contains several antimicrobial substances, including lactoferrin, conglutinin and the lactoperoxidase system (Jay, 1996). Raw milk has been reported to contain a rotavirus inhibitor that can inhibit up to 10^6 pfu (plaque-forming units).ml^{-1}. This rotavirus inhibitor is destroyed by pasteurisation. Milk casein as well as several free fatty acids have been shown to have antimicrobial activity under certain conditions (Jay, 1996).

C. KEFIR

Kefir is a fermented milk drink that originated in the village of Karatschajeff in the Caucasian Mountains. The history of Kefir is shrouded by legend. Local tribes state that the Kefir grains were given to them by Mohammed (Mohammed grains, "grains du Prophéte") with the command of keeping them secret (Duitschaever, 1989). Kefir is still manufactured in Russia under a variety of names, such as Kephir, Kiaphur, Kefer, Knapon, Kepi, and Kippi (Kwak *et al.*, 1996). It is also popular in Eastern European countries and is produced in small quantities in Czechoslovakia, Poland, Sweden, Finland as well as in Germany, Greece, Austria, Brazil and Israel (Koroleva, 1988b; Libudzisz & Piatkiewicz, 1990). It is currently available in the United States,

primarily as an ethnic drink, and its popularity is growing in Japan (Saloff-Coste, 1996).

Kefir is commonly manufactured by fermenting milk with a mixture of yeast and lactic acid bacteria (Kwak *et al.*, 1996). Two types of Kefir exist: sugary, a fermented sweetened water; and milky, a fermented milk beverage. This review addresses the milky variety, which norm has been established by the International Dairy Federation (Saloff-Coste, 1996).

The traditional method of making Kefir involves grains that are small, irregularly shaped, yellowish and massed, resembling individual florets of a cauliflower (Kwak *et al.*, 1996). Kefir grains are the starter culture used in the production of Kefir by harboring a mixture of lactic acid bacteria and yeast (Kwak *et al.*, 1996), firmly imbedded in a polysaccharide gum called kefiran (Steinkraus, 1996). These grains are generally known to the public in South Africa as a "joghurtplantjie" (yoghurt plant) (Keller & Jordaan, 1990).

General characteristics

Kefir differs from other milk products in that it is not the result of the metabolic activity of a single microbial species or strain. The milk is fermented with a mixed microbial population confined to a matrix of discrete "Kefir grains," which are recovered after fermentation (Garrote *et al.*, 1998). The resulting product is known as Kefir.

Kefir is a self-carbonated, fermented beverage that can be made with any kind of milk (cow, goat, sheep, camel or buffalo). It can also be produced from cooked, homogenised or pasteurised milk. It generally has a pH of about 4.0, ethyl alcohol content from 0.5 – 2.0%, lactic acid from 0.8 - 1.0% as well as formic, succinic and propionic acids, CO_2 (0.08 - 0.2%), trace amounts of isoamyl alcohol, acetone and diacetyl and a fat content depending on the type of milk used (Liu & Moon, 1983; Duitschaever, 1989; Libudzisz & Piatkiewicz, 1990). In Russia, a Kefir range is manufactured using milk with either 3.2, 2.5, 1.0% (m/v) or no fat (Koroleva, 1988b). Kefir is characterised by a homogeneous consistency and specific "biting" taste. The alcohol content and the amount of CO_2 can easily be increased by changing the composition of the starter, the fermentation temperature, the duration of the fermentation and type of packaging. However, the alcohol content of Kefir produced

by modern manufacturing methods does not exceed 0.1% and the amount of CO_2 is also comparatively low (Koroleva, 1988b).

According to Kurmann *et al.* (1992), one millilitre of a good-quality Kefir contains 10^9 lactococci, 10^7 - 10^8 leuconostocs, 10^7 - 10^8 thermophilic lactobacilli, 10^4 - 10^5 yeasts and 10^4 - 10^5 acetic acid bacteria. During the Kefir fermentation some proteolysis occurs in the milk, along with the development of a yeasty aroma. The flavour of Kefir is mildly alcoholic, yeasty-sour, with a tangy effervescence depending on the composition of the Kefir grains (Liu & Moon, 1983). The sharp acid and yeasty flavour, together with the prickly sensation contributed by the carbon dioxide produced by the yeasts, can be considered as the typical Kefir flavour (Duitschaever, 1989). The characteristic flavour, a slight fizziness and low alcohol content is responsible for Kefir's nickname – "the champagne of cultured dairy products" (Merin & Rosenthal, 1986). The shelf-life of Kefir is normally about 36 h at room temperature, but in glass bottles, however, the product can be kept for 8 - 19 d at 3° - 4°C (Koroleva, 1988b; Roginski, 1988).

Nutritional and health aspects of Kefir

The belief that fermented milk product consumption is beneficial to health is ancient and part folklore, having been passed from generation to generation in many parts of Europe (Buttriss, 1997). Many health benefits have been attributed to fermented milk products over the years, some of which are listed in Table 3. For some, like an improved lactose tolerance, a considerable amount of evidence has been amassed, but others remain little more than speculation and are far from well established in scientific terms (Buttriss, 1997). It is thus important to stress the fact that most of these claims still require convincing experimental proof (Roginski, 1988; Buttriss, 1997).

Fermented milk products are a palatable and economical source of a wide range of nutrients (Gurr, 1987). The nutrient composition is similar to that of milk with Kefir containing more vitamin B_1, B_2 and folic acid than milk (Roginski, 1988; Libudzisz & Piatkiewicz, 1990) and concentrations of lactic acid, galactose, free amino acids and fatty acids are increased as a result of the fermentation (Gurr, 1987). Due to the presence of acetic acid bacteria and yeasts, Kefir possesses a high antimicrobial activity against extraneous intestinal microorganisms as compared

Table 3. Health benefits from the consumption of fermented milk products.

Benefit	Reference
Increased proteolysis, digestion and reabsorption of proteins	Blanc, 1984; Gurr, 1987; Koroleva, 1988b; Buttriss, 1997
Reduced allergic reactions to native proteins	Blanc, 1984; Koroleva, 1988b
Heightened lipolysis and release of (volatile) fatty acids	Blanc, 1984
Heightened digestibility and absorption of fats	Blanc, 1984; Gurr, 1987; Roginski, 1988
Reduction of cholesterol	Blanc, 1984; Jay, 1996; Buttriss, 1997
Increased lactic acid content and reduced lactose content, leading to reduced intolerance to lactose due to lactase deficiency	Blanc, 1984; Gurr, 1987; Roginski, 1988; Jay, 1996; Buttriss, 1997
High antibiotic activity against extraneous intestinal microorganisms	Koroleva, 1988b; Buttriss, 1997
Contents of vitamins of group B	Koroleva, 1988b; Roginski, 1988
Inhibition of growth of potentially harmful bacteria in the gut	Koroleva, 1988b; Gurr, 1987; Roginski, 1988; Buttriss, 1997
Increased urine excretion as well as the excretion of other products of nitrogen metabolism	Koroleva, 1988b; Roginski, 1988
Elevated digestive secretions: saliva; bile; gastric; and pancreatic juices	Blanc, 1984; Koroleva, 1988b
Increased speed of gastric evacuation and peristalsis	Blanc, 1984; Koroleva, 1988b; Buttriss, 1997

Table 3. Cont.

Increased P, Ca, Fe retention (compared to milk)	Blanc, 1984
Elevated vitamin enrichment conditions, antimicrobial properties against undesirable bacteria (treatment of diarrhea)	Blanc, 1984; Roginski, 1988; Buttriss, 1997
Enhanced resistance to infections (bacteriostatic properties)	Blanc, 1984; Buttriss, 1997
Enhanced resistance to tumours/Antimutagenic	Blanc, 1984; Gurr, 1987; Jay, 1996; Buttriss, 1997
Increased preservation time of product	Blanc, 1984; Gurr, 1987
Higher organoleptic properties: flavour	Blanc, 1984; Gurr, 1987

to yoghurt or other fermented dairy products (Roginski, 1988). The acetic acid bacteria in Kefir contribute to protein proteolysis and the accumulation of free amino acids and other products of protein hydrolysis in the intestine (Roginski, 1988).

All milk products containing microorganisms are well tolerated, compared to unfermented milk, by individuals who have lactose intolerance (lactose malabsorption, intestinal hypolactemia) (Roginski, 1988). Such lactose intolerant individuals experience gastrointestinal symptoms due to a reduced ability to digest milk lactose into its component sugars – glucose and galactose – which can then be absorbed readily in the small intestine (Buttriss, 1997). Undigested lactose cannot be absorbed and travels to the large bowel (colon) where it is digested by the resident microorganisms, causing excess gas production, intestinal discomfort and diarrhoea.

In humans, lactase levels peak in early infancy to enable digestion of human milk to take place efficiently. In most Europeans, levels remain high during childhood and into adulthood, perhaps stimulated by the readily availability and thus consumption of fresh cow's milk. However, in as many as 70% of the world's population (mainly non-Europeans), activity of the enzyme, and hence ability to digest lactose effectively, declines rapidly after weaning (Roginski, 1988). This has been genetically determined and is considered to be linked to the absence of a tradition for commercial retail distribution of fresh milk in Africa, India and Asia because of their subsistence agriculture and adverse climatic conditions (Roginski, 1988; Buttriss, 1997).

A large number of reports have shown that lactose malabsorbers can consume certain fermented dairy products, of which Kefir is one, without harmful effects; other studies reported no beneficial effects (Roginski, 1988). The most likely explanation for an improved tolerance of lactose when it is consumed as part of Kefir is the presence of microbial ß-galactosidase derived from the bacterial starter cultures used in fermented milk production, which like intestinal lactase, can break down lactose to its component sugars (Buttriss, 1997). Another theory proposed by Gurr (1987) states that cultured products, because of their acidity and the consequent finer dispersion of protein in the stomach, retard the emptying of the stomach's contents into the small intestine. Any capacity to break down lactose, whether it be of microbial or indigenous origin, would then have longer to take effect

and consequently lactose digestion would theoretically be more efficient, even when the specific activity of the enzyme is low (Gurr, 1987).

A high blood cholesterol concentration is considered to be one of the four major risk factors for coronary heart disease (Buttriss, 1997). It has been observed that the Masai tribesmen in East Africa have a low serum cholesterol and a very low incidence of coronary diseases, despite the fact that they consume substantial amounts of meat (Jay, 1996; Buttriss, 1997). This was associated with their common consumption of 4 – 5 litres per day of fermented full-cream milk. A number of studies have since provided support for the hypocholesterolaemic effect of fermented milks (Vujicic *et al.*, 1992; Tamai *et al.*, 1996). The mechanism of this effect has yet to be established (Buttriss, 1997).

There are reports suggesting that fermented milk products may protect the user against certain types of cancer (Buttriss, 1997). Various mechanisms have been suggested including the potential of some strains of lactic acid bacteria to reduce the activity of faecal (bacterial) enzymes known to promote the synthesis of carcinogens from available substrates, or stimulate the host's immune system. However, evidence proving this theory is still inconclusive and much more work is needed before such claims can be made (Buttriss, 1997).

It can be concluded that fermented milks are nourishing without burdening the digestive organs (Blanc, 1984). They offer all nutriments and most of the important compounds found in milk in a form easy to assimilate and attractive due to its diversity. The remarkable organoleptic qualities of the cultured products are part of their value in the diet (Blanc, 1984).

Traditional Kefir production

Historically, Kefir was made using cow's or goat's milk in sacks made from animal hides (Koroleva, 1988b). Occasionally, it was also made in clay pots, wooden buckets or oak vats and in some areas sheep's milk was also used. Usually the Kefir sacks were hung in the sun during the day and during the night returned to the house and hung near the door. Everyone who entered or left the house was expected to prod the sack to mix the contents. As the Kefir was removed, more fresh milk was added, making the fermentation process continuous (Koroleva, 1988b).

Traditionally, Kefir is prepared by culturing milk with Kefir grains representing a natural symbiosis of different microorganisms (Koroleva, 1988a). The grains, if handled properly, can be used repeatedly (Steinkraus, 1996). There are two stages in the manufacturing of traditional Kefir. The first stage is a primary fermentation where milk is inoculated with Kefir grains to provide a mother culture. The second stage is generally described as a fermentation plus ripening process (Marshall, 1993).

The mother culture is prepared from fresh grains by directly adding them to milk that has been pasteurised and cooled to 20° - 25°C (Saloff-Coste, 1996). The ratio of Kefir grains to milk can vary from 0.5 - 10% by weight (Roginski, 1988; Saloff-Coste, 1996). Grain cultivation is carried out at 20° - 25°C for a period lasting around 24 h after which the grains are sieved or filtered through cheesecloth and added to further batches of milk (Marshall, 1993). The flavour and effervescence of this sieved Kefir is improved by incubating it another 24 h either at room temperature or preferably in a refrigerator (Steinkraus, 1996). The Kefir beverage, itself containing live microorganisms from the grain, is then ready for consumption or can be stored at refrigeration temperatures (Roginski, 1988; Marshall, 1993).

The traditional methods produce only small volumes of Kefir and require several steps, each additional step increasing the risk of contamination. Strong pressure from the CO_2 gas can lead to the explosion of the receptacle, unless appropriate containers which can withstand the escaping gas pressure, are used (Saloff-Coste, 1996).

Industrial Kefir production

A number of procedures for the industrial production of Kefir exist and can be divided into two broad categories: those which have been developed by industrialisation of traditional methods (Marshall, 1993; Saloff-Coste, 1996) and those which arise from new starter development technologies (Duitschaever *et al.*, 1987; Marshall, 1993).

Saloff-Coste (1996) described a method (also known as the "Russian method") that permits production of Kefir on a larger scale, and uses a series of fermentations. The first step was to prepare the cultures by incubating pasteurised milk with grains (2 - 3% m/v) in the same way as is done during traditional Kefir

production. The grains are then removed by filtration and the resulting mother culture is added to milk (1 - 3% m/v) which is fermented for 12 – 18 h (Roginski, 1988; Saloff-Coste, 1996). According to Kurmann *et al.* (1992), Kefir culture (mother culture) from which the grains have been removed cannot be used for successive propagation to make an acceptable product as the original balance of the microbial population gets disrupted.

Some producers in Eastern Europe have begun using concentrated lyophilized grain cultures (Libudzisz & Piatkiewicz, 1990; Saloff-Coste, 1996). These mother cultures are then used as bulk starters for direct inoculation of the milk. In the method for Kefir preparation described by Libudzisz & Piatkiewicz (1990), the mother culture is obtained by adding the whole contents of the package (1 g) to 3 litres of milk. More control over the process and fewer steps provide a more consistent quality (Saloff-Coste, 1996). This is an important factor in the successful marketing of Kefir as consumers demand consistency in products.

Some researchers have focused on producing Kefir from pure, defined cultures (Duitschaever *et al.*, 1988; Marshall, 1993; Saloff-Coste, 1996). Marshall (1993) described a method by which two sets of cultures could be prepared, one which is bacterial and the other one containing the Kefir yeasts. Heated milk was inoculated with a starter containing specific lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus kefir*) and incubated at 24° – 27°C for 18 – 20 h. During cooling or after cooling the milk was inoculated with a second culture containing yeasts (*Candida kefir*) as well as *Lactobacillus brevis* (Marshall, 1993). This method allows for better control of the microorganisms involved, an easier production method and leading to a more consistent quality (Saloff-Coste, 1996).

One of the problems encountered during large-scale processing is that of gas production (Marshall, 1993). An authentic Kefir is identified by its yeast content, which results in the production of CO_2 and this leads to problems during the packaging of the Kefir. Fermented milks should be packed in containers impermeable to water and odour, insoluble in water and free from foreign odours (Roginski, 1988). Glass, crown-capped vessels are traditional for Kefir, and these are returned to the dairies in eastern Europe (Marshall, 1993). However, in the disposable culture of the western European countries, alternatives had to be found. Glass, as a packaging material for fermented milks, has now been almost totally

replaced by synthetic materials and plastic-coated paper. The paper can also be silicone-coated and/or impregnated with resins (Roginski, 1988). The foil-capped polystyrene container tends to blow and this is perceived as a defect by consumers. A number of closures have now been patented which allow for gas to escape, thus preventing bulging of the lids, yet retaining the gaseous nature. The design of the aperture also protects against entry of contaminating bacteria and dust (Roginski, 1988; Marshall, 1993). An interesting container specifically designed for Kefir has a lid that consists of three layers that allow the escape of carbon dioxide generated by still viable yeast cells, thus preventing the swelling and bulging of Kefir cups (Roginski, 1988).

In Poland, "Kefir tablets" are produced for the production of Kefir in the home (Libudzisz & Piatkiewicz, 1990). After dissolving one or two tablets in a glass of milk, the container is incubated at 25° - 30°C for 18 - 26 h until curd is formed to produce a starter for the preparation of a larger amount of Kefir. This starter is then used in 4 - 5 tablespoon amounts to inoculate one litre of milk which is left at 20° - 22°C until a curd is formed (usually 14 - 18 h); then it is stored at room temperature for 4 h, cooled and kept in a refrigerator until consumed (Libudzisz & Piatkiewicz, 1990).

D. FACTORS INFLUENCING THE QUALITY OF KEFIR

Starter cultures

Koroleva (1988b) compared the quality of Kefir produced with two different starters. The first starter was derived directly from Kefir grains, while the second was obtained by a re-cultivation of a liquid starter. In the Kefir samples prepared from the first starter, the content of homofermentative and heterofermentative lactic acid streptococci and the yeasts in the product was higher than that in the analogous Kefir samples made with the second starter. In the Kefir samples prepared with the second starter, the number of acetic acid bacteria was similar to the first starter, but the number of thermophilic lactobacilli lead to a too high acidity in the Kefir (Koroleva, 1988b). The composition of Kefir produced by the indirect method was different from the product obtained using the Kefir grains: the ethanol and diacetyl contents were much lower; and the lactose content was higher (3.7 - 3.8% vs. 2.5%) (Roginski,

1988). In conclusion, Koroleva (1988b) found that Kefir grain starter in general improved the quality of Kefir.

Duitschaever *et al.* (1988) compared five procedures for making Kefir that differed mainly in the type of starter culture used. Kefir type 1 was made by using a starter culture consisting of lactic acid bacteria (*Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus salivarius*, *Lactococcus lactis* and *Leuconostoc*) and a non-lactose fermenting yeast (*Saccharomyces cerevisiae*) and sequential fermentation. For Kefir type 2, the milk was inoculated with 1.1% frozen Kefir culture. For Kefir type 3, the milk was inoculated with pure cultures of lactic acid bacteria and yeast. Kefir type 4 was produced in the same way as Kefir type 3 but underwent an additional incubation step. Kefir type 5 was manufactured by using a traditional Kefir grain starter. Sensory evaluation of the different Kefirs showed that Kefir type 1 was more acid than Kefir made from grains. It was also significantly more viscous and effervescent and the score for flavour was considerably higher. For the degree of liking, the tasters gave a significantly higher score for Kefir type 1 than for Kefir type 5. The acidity of Kefir type 1 was significantly higher than the average acidity found for Kefir type 3, but the viscosity was the same. Kefir type 1 was judged to possess a more typical Kefir flavour than either Kefir type 3 or 4, although it had less effervescence. Kefir type 2 differed significantly from the other Kefirs for all sensory characteristics. It had low viscosity and effervescence and a flavour atypical for Kefir, resembling that of buttermilk. In these studies sensory evaluation indicated that Kefir made from pure cultures and a sequential fermentation was superior in quality to the other Kefirs (Duitschaever *et al.*, 1988).

In some instances, the addition of a certain microorganism can improve the quality of Kefir (Liu & Moon, 1983). Some lactic acid bacteria cultivated in milk, whether in pure or mixed culture, consume the vitamins present in the milk (Liu & Moon, 1983; Buttriss, 1997). This has been reported to be especially true for the concentration of B vitamins, including B_{12}, which decreased by as much as 95% during this lactic acid fermentation. Addition of propionibacteria to Kefir resulted in increases, or only small losses of vitamin B_{12} (Cerna & Grabova, 1997). Kefir produced using Kefir grains and *Propionibacterium shermanii* resulted in a product with a high food value, rich in proteins and vitamins, including vitamin B_{12}. The

addition of propionic acid bacteria did not unfavourably affect the sensorial properties of Kefir (Liu & Moon, 1983; Cerna & Grabova,1997).

Preservation method of Kefir grains

Garrote *et al.* (1997) studied the characteristics of the Kefir products obtained with Kefir grains that had been stored under different conditions. Grains were preserved frozen at -20° and -80°C as well as at 4°C. Fermented milks obtained with the grains stored at -20° and -80°C showed the same microbial population, rheological behaviour, acidity and carbon dioxide content as fermented milk obtained with non-stored grains. The product obtained from grains stored at 4°C did not have the acidity and viscosity of the standard product. Later Garrote *et al.* (1998) reported that storage at -20°C is a good method to preserve grains for household manufacture of fermented milk (Garrote *et al.*, 1998).

Season of the year

Bavina & Rozhkova (1973) studied the composition of the microorganisms in Kefir and Kefir grains during the different seasons of the year. The microbiological composition of the starter and the Kefir remained stable over the course of a year. This observation, however, does not apply to pure starter cultures. A small decrease in the content of acetic acid bacteria has been observed in the spring (Bavina & Rozhkova, 1973; Koroleva *et al.*, 1978). It was, therefore, desirable to increase the cultivation temperature of the Kefir grains in order to intensify the growth of the acetic acid bacteria (Koroleva, 1988b).

Heat treatment of milk

Kefir is usually produced from milk which has been subjected to a severe heat treatment aimed firstly at the destruction of bacteria competitive to those present in the Kefir grains and eventually at the denaturation of the milk proteins (Merin & Rosenthal, 1986; Marshall, 1993). In studies (Mann, 1979) on the effects of heat treatment on the quality of Kefir, four different heat treatments of Kefir milk were compared, namely 85° - 87°C for 5 - 10 min, 92° - 95°C for 20 - 30 min, 110°C in an autoclave and double pasteurisation, involving a treatment at 72° - 76°C in a plate pasteuriser, followed by treatment at 85° - 87°C for 20 min in a tubular pasteuriser.

Kefir was then produced by bulk incubation at 20°C with 4% (m/v) starter. The Kefir produced was evaluated for flavour, viscosity and whey separation. The consistency of Kefir was improved by the severity of heating, due to the greater denaturation of the whey proteins and their participation in the formation of the coagulum (Berzhinskas *et al.*, 1978; Mann, 1979; Marshall, 1993). Heating Kefir milk at 92° - 95°C for 20 - 30 min was considered optimal. Double pasteurisation, although uneconomical, had favourable effects on the flavour and consistency of Kefir (Mann, 1979; Marshall, 1993).

Merin & Rosenthal (1986) also studied the suitability of UHT-treated milk as a starting material for the production of Kefir. Kefir produced from 1 to 3% fat content UHT-treated milk yielded a good quality product, the 3% fat product being rated as slightly superior to the 1%. In the studies no sensorial difference were detected between Kefir prepared from UHT milk and that from 95°C for 30 min heat-treated milk (Merin & Rosenthal, 1986).

Starter concentration

In a recent study done in Argentine (Garrote *et al.*, 1998), the effects of changes in the Kefir grain to milk ratio on microbial composition, acidity, apparent viscosity and carbon dioxide content of Kefir were evaluated. Kefir made with different Kefir grain:milk combinations showed large differences in final pH, lactococci concentration, apparent viscosity and CO_2 content. A ratio of 10 g of Kefir grains per litre milk resulted in a viscous and not very acid product. A ratio of 100 g per litre milk gave an acid beverage with low viscosity and a more effervescent taste (Garrote *et al.,* 1998). The ratio of grain to milk recommended in the literature varies from 5 to 200 g per litre, with a ratio of 5% (50 g^{-1}) grains mentioned most often (Marshall & Cole, 1985; Merin & Rosenthal, 1986; Koroleva, 1988b; Roginski, 1988; Tamime & Robinson, 1988).

Fermentation temperature and fermentation time

Koroleva (1988b) studied the influence of temperature on the fermentation of Kefir. At elevated temperatures (25° - 27°C) the required acidity of Kefir was reached in 6 - 8 h. At these temperatures the heterofermentative lactic acid streptococci and yeasts had no time to develop and, as a result, the Kefir taste became atypical.

According to Koroleva (1988b), the optimum fermentation time at 20° - 22°C is 10 - 12 h. If the coagulum is subsequently cooled to 8° - 10°C, the heterofermentative lactic acid streptococci and yeasts do not develop and the taste of the product also becomes atypical. If the coagulum is, however, cooled slowly during a 10 - 12 h period these microorganisms have the chance to grow, and the characteristic taste and aroma develops in the Kefir (Koroleva, 1988b).

Liu & Moon (1983) reported that, with an incubation temperature in the 15° - 22°C range, a product high in acid and low in alcohol and carbon dioxide is obtained. This is the result of a lactic acid fermentation and some inhibition of the yeasts. In contrast, a Kefir product high in alcohol and carbon dioxide production is obtained at temperatures from 4° - 15°C. This temperature range naturally leads to the selection of the yeasts with a resulting inhibition of the lactic acid bacteria. In conclusion, they recommended incubation at 25°C to give maximum production of ethanol and volatile acids as well as good specific flavour and consistency. They also found that temperature influences the microbial interactions, which can alter the formation of volatile substances in determining the flavour of Kefir (Liu & Moon, 1983).

Fat content of milk

Kefir can be manufactured with milk containing either 3.2, 2.5, 1.0% (m/v) or no fat (Koroleva, 1988b). In an experiment by Merin & Rosenthal (1986), Kefirs produced from 1% fat and 3% fat UHT-treated milk were compared sensorial. The 3% fat product was rated as only slightly superior to the 1% product (Merin & Rosenthal, 1986).

Type of milk used

Kneifel & Mayer (1991) studied the vitamin profiles of Kefirs made from different milks. An increase in the vitamin concentration was observed for thiamin (only in ewe's milk Kefir), pyridoxine (Kefir from ewe's, goat's and mare's milk) and folic acid (Kefir from all milk sources except mare's milk). Orotic, nicotinic and pantothenic acids showed unchanged or reduced concentrations with variation in milk type (Kneifel & Mayer, 1991). The composition and flavour of Kefir varied significantly, depending on the source – cows, ewes, goats or mares - of the milk used (Saloff-Coste, 1996).

E. KEFIR GRAINS

General characteristics

Kefir grains (Kefir starters) have a structure similar to tiny florets of cauliflower, which vary in size from 0.3 to 3.5 cm diameter (Garrote *et al.*, 1998). The grains are white to yellow in colour and have a specific characteristic smell (Libudzisz & Piatkiewicz, 1990). They are insoluble in water and common solvents, but when added to milk, they swell and turn white (Liu & Moon, 1983). They are composed mostly of proteins and polysaccharides in which the complex microbial community is enclosed. The average chemical composition of Kefir grains is 890 - 900 $g.kg^{-1}$ water, 2 $g.kg^{-1}$ lipids, 30 $g.kg^{-1}$ protein, 60 $g.kg^{-1}$ sugars and 7 $g.kg^{-1}$ ash (Garrote *et al.*, 1998; Libudzisz & Piatkiewicz, 1990).

The activity of the grains depends on the viability of the microbial community (Garrote *et al.*, 1998). Active Kefir grains float on the milk surface (Roginski, 1988). The microbial composition of grains depends on the origin and the milk type used (Garrote *et al.*, 1998). Yeast and lactic acid bacteria (LAB) co-exist in a symbiotic association and are responsible for an acid-alcoholic fermentation. The microbial community in the grains is held together by a matrix of fibrillar material composed largely of polysaccharides often referred to as "Kefiran", the capsular material from certain *Lactobacillus* species (Duitschaever, 1989; Pintado *et al.*, 1996).

Kefir grains are a symbiotic system of lactococci and lactobacilli in concentrations of 10^8 - 10^9 and yeasts at approximately $10^8.g^{-1}$ (Libudzisz & Piatkiewicz, 1990). Generally, lactobacilli (homo- and heterofermentative, meso- or thermophilic) constitute about 65 - 80% of the microbial content. The remaining 20% are lactococci (souring and aroma forming) and different species of lactose fermenting and non-lactose fermenting yeasts – about 5% (Koroleva, 1988a). Non-lactose fermenting yeasts are found in the deeper layers of the Kefir grains while lactose fermenting yeasts are in the peripheral layers (Libudzisz & Piatkiewicz, 1990). In addition, Kefir grains can contain acetic acid bacteria (Garrote *et al.*, 1998). The numerous microbial species that have been reported to be associated with Kefir and Kefir grains, are listed in Table 4.

Table 4. Microorganisms associated with Kefir and Kefir grains.

Microorganism	Reference
Lactic acid bacteria	
Enterococcus durans	Marshall, 1993
Lactobacillus acidophilus	Marshall, 1987; Libudzisz & Piatkiewicz, 1990; Kwak *et al.*, 1996
Lactobacillus brevis	Marshall, 1987; Libudzisz & Piatkiewicz, 1990; Kwak *et al.*, 1996;
Lactobacillus casei	Kurmann *et al.*, 1992; Marshall, 1993; Kwak *et al.*, 1996
ssp. *alactosus*	Libudzisz & Piatkiewicz, 1990; Marshall, 1993
ssp. *rhamnosus*	Koroleva, 1988a; Libudzisz & Piatkiewicz, 1990; Marshall, 1993
Lactobacillus cellobiosus	Libudzisz & Piatkiewicz, 1990; Marshall, 1993; Kwak *et al.*, 1996
Lactobacillus delbrueckii	
ssp. *bulgaricus*	Koroleva, 1988a; Marshall, 1993; Kwak *et al.*, 1996
ssp. *lactis*	Libudzisz & Piatkiewicz, 1990; Marshall, 1993
Lactobacillus helveticus	
ssp. *jugurti*	Koroleva, 1988a; Libudzisz & Piatkiewicz, 1990; Kwak *et al.*, 1996
ssp. *lactis*	Marshall, 1993
Lactobacillus kefir	Marshall, 1987; Marshall, 1993; Kwak *et al.*, 1996; Pintado *et al.*, 1996
Lactobacillus kefiranofaciens	Marshall, 1993; Kwak *et al.*, 1996; Pintado *et al.*, 1996
Lactobacillus kefis	Pintado *et al.*, 1996
Lactobacillus lactis ssp. *lactis*	Kwak *et al.*, 1996
Lactobacillus plantarum	Kwak *et al.*, 1996
Lactococcus filant	Kwak *et al.*, 1996
Lactococcus lactis	Koroleva, 1988a; Libudzisz & Piatkiewicz, 1990;
ssp. *cremoris*	Koroleva, 1988a; Marshall, 1993; Libudzisz & Piatkiewicz, 1990; Kwak *et al.*, 1996
ssp. *lactis*	Marshall, 1993; Kwak *et al.*, 1996; Pintado *et al.*, 1996
ssp. *lactis* biovar *diacetylactis*	Libudzisz & Piatkiewicz, 1990; Marshall, 1993; Kwak *et al.*, 1996
Leuconostoc kefir	Kwak *et al.*, 1996

Table 4. Cont.

Leuconostoc mesenteriodes	
ssp. *cremoris*	Libudzisz & Piatkiewicz, 1990; Marshall, 1993
ssp. *dextranicum*	Koroleva, 1988a; Libudzisz & Piatkiewicz, 1990; Kwak *et al.*, 1996
ssp. *mesenteriodes*	Marshall, 1993; Kwak *et al.*, 1996
Streptococcus durans	Marshall, 1987; Libudzisz & Piatkiewicz, 1990; Kwak *et al.*, 1996
Streptococcus filant	Libudzisz & Piatkiewicz, 1990
Streptococcus salivarius	
ssp. *thermophilus*	Libudzisz & Piatkiewicz, 1990; Marshall, 1993; Kwak *et al.*, 1996
Acetic acid bacteria	
Acetobacter aceti	Koroleva, 1988a; Kurmann *et al.*, 1992; Marshall, 1993;
Acetobacter rasens	Koroleva, 1988a; Marshall, 1993
Yeasts	
Candida holmii	Marshall, 1993; Brialy *et al.*, 1995
Candida kefir	Marshall, 1987; Libudzisz & Piatkiewicz, 1990; Marshall, 1993
Candida pseudotropicalis	Libudzisz & Piatkiewicz, 1990; Marshall, 1993; Pintado *et al.*, 1996
Candida tenuis	Pintado *et al.*, 1996
Kluyveromyces bulgaricus	Libudzisz & Piatkiewicz, 1990; Marshall, 1993
Kluyveromyces lactis	Libudzisz & Piatkiewicz, 1990; Marshall, 1993; Kwak *et al.*, 1996
Kluyveromyces marxianus	Libudzisz & Piatkiewicz, 1990; Marshall, 1993; Kwak *et al.*, 1996
ssp. *marxianus*	Koroleva, 1988a; Marshall, 1993; Kwak *et al.*, 1996
Saccharomyces carlsbergensis	Libudzisz & Piatkiewicz, 1990; Kwak *et al.*, 1996; Pintado *et al.*, 1996
Saccharomyces cerevisiae	Marshall, 1987; Koroleva, 1988a; Kwak *et al.*, 1996
Saccharomyces florentinus	Libudzisz & Piatkiewicz, 1990; Brialy *et al.*, 1995

Table 4. Cont.

Saccharomyces globosus	Libudzisz & Piatkiewicz, 1990
Saccharomyces kefir	Kosikowski, 1977; Kwak *et al.*, 1996
Saccharomyces lactis	Pintado *et al.*, 1996
Saccharomyces unispores	Libudzisz & Piatkiewicz, 1990
Torula Kefir	Kosikowski, 1977; Kwak *et al.*, 1996
Torulaspora delbrueckii	Marshall, 1987; Koroleva, 1988a; Libudzisz & Piatkiewicz, 1990
Moulds	
Geotrichum candidum	Marshall, 1987; Roginski, 1988

Mass cultivation of Kefir grains

Kefir grains grow in size during usage as the microorganisms multiply and Kefiran accumulates (Marshall & Cole, 1985; Saloff-Coste, 1996). The grains start out very small and increase in size during fermentation of milk, but as far as known, they can only grow from pre-existing grains (Steinkraus, 1996). Whole milk, skimmed milk or neutralised whey can be used to grow Kefir grains (Steinkraus, 1996).

In Poland, modern production of grains is based on a continuous cultivation in milk, resulting in a biomass increase of 5 – 7% per day (Libudzisz & Piatkiewicz, 1990). As soon as the fermentation is completed, the grains are removed from the milk by sieving and then directly introduced into fresh milk. These authors recommended that the grains be rinsed once a week with sterile, cooled water and that stainless steel or glass vessels be used for cultivation. According to Steinkraus (1996), 500 g wet weight Kefir grains can double their weight in 7 – 10 d if they are transferred to 500 ml fresh milk six times a week. Growth is, however, greatly retarded if the grains are rinsed with water after each sieving (Steinkraus, 1996).

Schoevers (2000) studied the influence of different factors on the growth rate of Kefir grains with the aim of obtaining optimal conditions for maximum biomass increase of Kefir grains. Different incubation temperatures (18°, 22°, 25° and 30°C) were evaluated as well as the effect of the addition of nutrients like tryptose and yeast extract to the milk medium. The influence of the volume of milk used during cultivation as well as the effect of shaking during grain propagation was also studied. Schoevers (2000) concluded that optimum mass cultivation of Kefir grains is obtained by using more than 1% active Kefir grains as starter and then cultivating the grains at 25ºC in milk containing added urea (1.5%) and yeast extract (2%), as well as agitating the cultivation vessel and replacing all the fermented milk daily (Schoevers, 2000).

Preservation of Kefir grains

Literature on the preservation of Kefir grains is scarce and contradictory. According to Vedamuthu (1982), Kefir grains can be stored in cold water. The grains may also be dried in a warm oven and stored in foil pouches (Vedamuthu, 1982). Grains stored in water are active for 8 – 10 d in contrast to properly dried grains that are active for 12 – 18 months (Vedamuthu, 1982). According to Libudzisz &

Piatkiewicz (1990), in Poland cultures for Kefir production are produced in the form of fresh Kefir grains suspended in a sterile solution of 0.9% NaCl or in a lyophilized culture made from Kefir grains, standardised to 10% of the whole microflora by the addition of yeasts isolated from these Kefir grains.

Steinkraus (1996) reported that Kefir grains could not be dehydrated with heat and survive but that they might survive freeze-drying. He proposed that the best method of maintaining viable Kefir grains is by transferring them periodically into milk and holding at refrigerator temperature (4° - 7°C) (Steinkraus, 1996). In contrast, Saloff-Coste (1996) found that grains can be dried at room temperature and kept at cold temperature (4°C). For a longer preservation period, they can be lyophilized (freeze-dried) or frozen (Libudzisz & Piatkiewicz, 1990; Saloff-Coste, 1996).

F. SENSORY EVALUATION

Background

Sensory evaluation has been defined as "a scientific discipline used to evoke, measure, analyse and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing" (Sensory Evaluation Division of the Institute of Food Technologists, 1981). The most commonly occurring industrial applications are: new product development; product matching; process change; cost reduction and/or selection of a new source of supply; storage stability; product grading or rating; consumer acceptance and/or opinions; consumer preference; panelist selection and training; and correlation of sensory with chemical and physical measurements (Sensory Evaluation Division of the Institute of Food Technologists, 1981).

In new product development, product developers need information of the sensory quality and relative acceptability of experimental prototype samples as input for marketability (Sensory Evaluation Division of the Institute of Food Technologists, 1981). Sensory evaluation of a new product may involve the characterisation of product prototype samples to determine uniqueness or a "point of differentiation" from related established products. This can be done by descriptive testing. Descriptive tests attempt to identify sensory characteristics and quantify them.

Panelists are thus selected on their ability to perceive differences between test products and verbalise perceptions. An example of a descriptive test that can be performed, is category scaling. In this test coded samples are presented simultaneously or sequentially in a balanced order which differs among the individual panel members. Category scales, consisting of a series of word phrases structured in ascending or descending order of intensity, are used to measure a specified attribute. An alternate scaling procedure is an unstructured vertical or horizontal line with verbal anchors at each end to describe or limit the attribute. For analysis purposes, successive digits are later assigned to each point represented on the scale, usually beginning at the end representing zero intensity. A statistical analysis (e.g., analysis of variance) of the mean intensity scores for each sample, is used to determine significant differences among the mean scores for the samples represented (Sensory Evaluation Division of the Institute of Food Technologists, 1981).

In new product development, it is also important to determine whether the prototype samples meet the acceptability requirements established for the product (Sensory Evaluation Division of the Institute of Food Technologists, 1981). Affective tests are used to evaluate preference and/or acceptance of products (Ellis, 1969). Generally, a large number of respondents are required for such evaluations. These panelists are not trained, but are selected at large to represent target or potential target populations. Hedonic rating scales are used to measure the level of liking for food products by a population. The method relies on test subjects' capabilities to report, directly and reliably, their feelings of like and dislike. The test subject is asked to evaluate each sample and mark the scales accordingly. Instructions must not influence the subject's response. Hedonic scale ratings are converted to numerical scores, and statistical analysis is applied to determine difference in degree of liking between or among samples (Sensory Evaluation Division of the Institute of Food Technologists, 1981).

Sensory evaluation techniques for evaluation of cultured products

A common scorecard applicable to all types of cultured milk products is not feasible since the products differ widely in their sensory characteristics (Bodyfelt *et al.*, 1988). In Fig. 1 a typical scorecard that can be used specifically for the sensory

Panellist:..... **Date:** ..... **Sample:**

A. FLAVOUR

1. **Sourness**

none — extreme

2. **Sweetness**

none — extreme

3. **Yoghurt (green apple)**

none — extreme

4. **Buttery (caramel)**

none — extreme

5. **Yeasty (cheesy)**

none — extreme

6. **Cowy (barny)**

none — extreme

B. BODY (TEXTURE)

7. **Creaminess**

watery — extremely creamy

8. **Effervescence (gassiness)**

none — extremely gassy

9. **Smoothness**

gritty — extremely smooth

Figure 1. Sensory evaluation form for Kefir (J. Calefato, Department of Food Science, University of Stellenbosch, personal communication).

evaluation of Kefir is depicted (J. Calefato, 1997, Department of Food Science, University of Stellenbosch, personal communication). The scorecard is based on the pattern of other dairy product scorecards (Marshall & Cole, 1985; Duitschaever *et al.*, 1987).

To facilitate the evaluation of cultured products with some degree of procedural uniformity, certain precautions need to be observed (Bodyfelt *et al.*, 1988). Temperatures of samples presented for flavour assessment should be reasonably uniform from day to day. When a sample is poured into a container, it should be evaluated immediately since product contact with air (oxygen) for only a brief period of time may alter the perceived flavour (Bodyfelt *et al.*, 1988).

Sensory evaluation conducted by a panel with a seemingly low level of literacy

It may sometimes happen that a sensory evaluation has to be conducted by a panel with a seemingly low level of literacy (De Bruin & Minnaar, 1994). The population of South Africa consists of people of diverse literacy levels. There are two ways to regard literacy, which may seem contradictory. Firstly, as the ability to read and write. This is the common dictionary definition and one that is generally reflected in statistics on literacy. Secondly, literacy can be viewed as the ability to think and reason like a literate person. Here, the focus is not just on the reading and writing abilities, but also on the thinking that accompanies it. An illiterate person may think or reason for himself, and, thus be suitable to assess the acceptability of food products. In South Africa people with a lower qualification than Standard 5 can be regarded as illiterates (De Bruin & Minnaar, 1994).

De Bruin & Minnaar (1994) conducted a study with the objective to design and test a method for determining the acceptability of a food product with consumers with a seemingly low level of literacy. They proposed that a five-point hedonic scale, as is shown in Fig. 2, gives satisfactory results. De Bruin & Minnaar (1994) concluded that further research is still needed into how to conduct sensory studies with consumer groups with a seemingly low level of literacy.

Sensory characteristics of Kefir

Kemp (1984) described the sensory characteristics of high quality Kefir as follows: "It has a pH of about 4.0; a clean, pleasant acid taste without any bitterness

Example: **How acceptable is the *taste* of this product?**

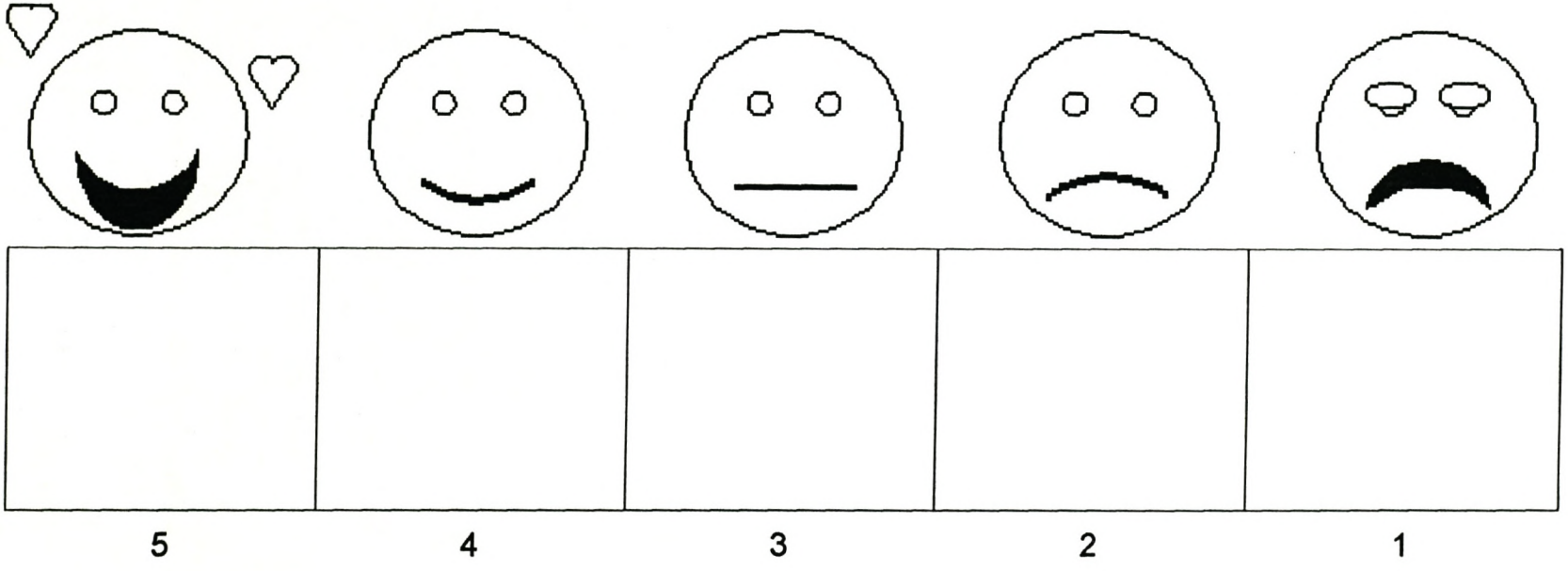

Figure 2. Sensory evaluation using a five-point hedonic facial scale (De Bruin & Minnaar, 1994).

and a smooth texture; altogether a very refreshing beverage." Kosikowski (1977) described the compositional properties of typical or high-quality Kefir in terms of major end-products of fermentation: approximate pH of 4.4; 0.8% lactic acid; about 0.5% to 1% ethyl alcohol; and sufficient CO_2 content to make the beverage "fizz and foam like a beer." Furthermore, Kosikowski (1977) emphasised that Kefir's typical flavour is due mainly to an optimum ratio (3:1) of diacetyl (approximately 3 $mg.l^{-1}$) to acetaldehyde (approximately 1 $mg.l^{-1}$).

A variety of flavours and off-flavours can occur in Kefir which will subsequently influence the sensory characteristics (Bodyfelt *et al.*, 1988). Typical examples found are:

- a "yoghurt" or "green apple" off-flavour that may occur due to the formation of relatively high concentrations of acetaldehyde by the starter culture. The flavour may remind one of the flavour of "green apples" or "plain yoghurt". To some tasters, this off-flavour may suggest high acid or a somewhat astringent character. This aromatic defect is readily noted upon either 'whiffing' or tasting the sample. Causes of the "green" off-flavour may simply be selection of the wrong culture, incubation at incorrect temperatures, and/or over-incubation;
- a "buttery" or "caramel-like" off-flavour that may occur due to a too high diacetyl concentration in the Kefir;
- a "cheesy" or "yeasty" off-flavour which is quite uncommon and is more often associated with products which have been stored for some time. A yeasty flavour is as undesirable as overacidification (Marshall, 1982). The flavour characteristics of cheesy are: a lack of typical culture flavour; a definite proteolytic flavour note; and sometimes a slightly bitter aftertaste. The aroma is somewhat suggestive of alcohol or acetic acid; and
- "cowy" or "barny" or "unclean off-flavours" which are those objectionable off-flavours that are associated with unsanitary farm conditions, inadequate milk cooling or foul-smelling stable areas. It is the result of an abnormally high acetone content and is characterised by an unpleasant and lingering after taste (Bodyfelt *et al.*, 1988).

A cultured product of high quality tends to have a "glossy" to a "semi-glossy", "velvety" and "uniform" (homogenous) appearance (Bodyfelt *et al.*, 1988). In reflected light, the surface of the product should appear to have a definite "sheen".

G. THE COMMERCIALISATION OF KEFIR

Commercialisation entails the decision to market a product (Lamb *et al.*, 1998). The marketing process includes understanding the organisation's mission and the role marketing plays in fulfilling that mission, setting marketing objectives, scanning the environment, developing a marketing strategy by selecting a target market strategy, developing and implementing a marketing mix, implementing the strategy, designing performance measures, evaluating marketing efforts and making changes if needed. The marketing mix combines product, distribution, promotion and pricing strategies in a way that creates exchanges satisfying to individual and organisational objectives (Lamb *et al.*, 1998). The key to marketing is the marketing plan (Cohen, 1998). A marketing plan is essential for every business operation and for efficient and effective marketing of any product or service (Cohen, 1998). A marketing plan is a written document that acts as a guidebook of marketing activities for the marketing manager. In Fig. 3, an outline of the different elements of a marketing plan is given (Cohen, 1998).

Neither Kefir nor Kefir grains are currently marketed in South Africa. If the Kefir grains and knowledge of the methods of producing Kefir were more widely distributed, there is no question that Kefir utilisation would become much more widespread (Steinkraus, 1996).

One of the major benefits of Kefir lies in its nutritional value and other health aspects. In a study done by Bourne *et al.* (1994) on the food and meal pattern of the urban African population in the Cape Peninsula, it was concluded that insufficient dairy products were consumed by the subjects. If one considers the fact that Africans have a high incidence of lactose intolerance (Buttriss, 1997), which means that they prefer fermented milks to other dairy products (Keller & Jordaan, 1990), the marketing of Kefir under the African population in South Africa presents a great opportunity. The addition of Kefir grains to milk results in a rapid lowering of the pH

MARKETING PLAN FOR XXX

Executive Summary *(overview of entire plan)*

I. Introduction

What is the product or service and how does it fit into the market?

II. Situational Analysis

A. The Situational Environment

1. Demand and demand trends.

What is the forecast demand for the product? Who is the decision maker and purchase agent? How, when, where, and why do they buy?

2. Social and cultural factors.
3. Demographics
4. Economic and business conditions for this product at this time and in the geographical area selected.
5. State of technology for this class of product.
6. Politics.

Are politics in any way affecting the situation for marketing this product?

7. Laws and regulations.

What laws and regulations are applicable here?

B. The Neutral Environment

1. Financial environment

How does the availability or unavailability of funds affect the situation?

2. Government environment.
3. Media environment.

Does current publicity favour this project?

4. Special interest environment.

Are any other influential groups likely to affect marketing of this product or service?

C. The Competitor Environment

D. The Company Environment

III. The Target Market

IV. Problems and Opportunities

V. Marketing Objectives and Goals

VI. Marketing Strategy

VII. Marketing Tactics

VIII. Implementation and Control

IX. Summary

X. Appendices

Figure 3. Marketing plan outline (Cohen, 1998).

of the milk (Jay, 1996). This leads to a preservational effect on the milk - a further quality of Kefir that makes it attractive to the African market. Kefir thus offers a means of providing wholesome foods to low-income populations wherever milk is available. Where animal milk is unavailable, soybean milk can provide a nutritious substitute.

Since the manufacturing of Kefir is simple and Kefir grains reusable, the price of Kefir on the market can be only slightly higher than that of milk (Steinkraus, 1996). Its manufacture in the home is sufficiently easy so that no home would have to be without it as soon as the Kefir grains become readily available.

A drawback for marketing of traditionally produced Kefir is that secondary alcohol fermentation tends to occur at the distribution stage (Kwak *et al.,* 1996), resulting not only in substantial changes in flavour and taste because of the continued formation of ethanol and carbon dioxide gas, but also in bulging containers and leakage of contents because of the internal pressure created by carbon dioxide produced (Kwak *et al.*, 1996). This is one of the reasons why it might be more practicable to market Kefir grains and not Kefir itself.

H. DISCUSSION

The method of manufacturing Kefir is quite simple and is a low-cost method of preserving milk. Unfortunately the different process parameters that are described in the literature differ substantially. It is thus necessary to standardise the process by determining the optimum parameters for Kefir production. The preservation techniques of Kefir grains also present an area for future research. This is because literature on this subject is scarce and contradictory. Stored Kefir grains have to be activated for a period of three days before they can be used and it is thus important to find a way of preserving Kefir grains without inactivating them. In order to market Kefir grains, it is essential to develop a suitable container for packaging. The type of container will depend on the type of preservation method used to preserve the Kefir grains.

It is clear that Kefir has definite benefits for the health and well being of man. Kefir is an attractive sour milk with excellent keeping qualities. The production of

fermented milks, especially Kefir should be encouraged and the South African market should be made aware of it.

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CHAPTER 3

INHIBITORY ACTIVITY OF KEFIR AGAINST POTENTIAL SPOILAGE AND PATHOGENIC ORGANISMS

Abstract

The inhibitory activity of Kefir towards certain spoilage and pathogenic organisms was evaluated. Strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Clostridium tyrobutyricum* were individually inoculated (10^3 - 10^4 cfu.ml^{-1}) in pasteurised milk together with Kefir grains (18 g grains per litre milk) and incubated at 25°C for 30 h. Pasteurised milk containing no test organisms, Kefir containing no test organisms and pasteurised milk containing test organisms (10^3 - 10^4 cfu.ml^{-1}) but no Kefir grains, served as controls. The viable counts, pH and titratable acidity (TA) were determined for each sample and control at selected time-intervals. Each trial was done in triplicate.

Substantial inhibition (%) and reductions in log cycles were observed for all test organisms when compared to their growth in milk. This coincided with a steep decrease in pH and increase in TA observed for the Kefir samples over the 30 h incubation period.

The data obtained in this study clearly shows that Kefir does possess an inhibitory activity towards the test organisms. The possibility of using Kefir as a probiotic exists and as Kefir inhibits spoilage microorganisms, it is an excellent way of preserving milk.

Introduction

Milk is highly nutritious but susceptible to spoilage, which will not only limit the shelf-life, but also lead to health risks and substantial economic losses. Contamination of milk and milk products with spoilage and/or pathogenic organisms during processing,

is a recurring problem (Gupta *et al.*, 1996). Strains of the genera *Escherichia*, *Bacillus*, *Staphylococcus*, *Listeria* and *Clostridium* are but a few of the pathogens and potential pathogens that have been implicated in dairy-related foodborne human illnesses (Robinson, 1990; Prescott *et al.*, 1993; Rusul & Yaacob, 1995; Muriana, 1996; Dineen *et al.*, 1998; Zapico *et al.*, 1998).

The presence of bacterial foodborne pathogens have been identified by the public as the most critical food-related risk factor affecting consumers (Muriana, 1996). Because of this, increased attention is being given to the use of foods containing metabolites produced by lactic acid bacteria (LAB) as they are known to have a strong inhibitory effect on the growth and survival of spoilage and potentially pathogenic bacteria (Varadaraj *et al.*, 1993).

Kefir is a traditional fermented milk that originated in the Caucasian Mountains (Duitschaever, 1989) and is commonly manufactured by fermenting milk with Kefir grains (Kwak *et al.*, 1996). Yeasts as well as lactic and acetic acid bacteria are generally found as constituents of the Kefir microbial population (Garrote *et al.*, 2000). Many health benefits have been attributed to fermented milk products, including Kefir, some of which are still speculative (Blanc, 1984; Buttriss, 1997). Of great significance is the antimicrobial activity that Kefir possesses *in vitro* against a wide variety of Gram-positive and negative bacteria, as well as some fungi (Saloff-Coste, 1996; Garrote *et al.*, 2000).

It has been reported that coliforms are actively inhibited by natural Kefir microorganisms, and pathogenic bacteria like *Shigella* and *Salmonella* do not grow when they are introduced to Kefir (Koroleva, 1988; Dineen *et al.*, 1998; Garrote *et al.*, 2000). Studies have also indicated that yeasts (*Torulaspora*), when separated from Kefir, possess pronounced antimicrobial activity against coliforms (Robinson, 1990). Of all the Kefir starter microbial components, the microphilic homofermentative lactococci and the acetic acid bacteria are the most active against coliforms. This antagonism has both bacteriostatic and bactericidal characteristics (Koroleva, 1988; Robinson, 1990; Naidu *et al.*, 1999; Garrote *et al.*, 2000).

From the studies on factors affecting the viability of pathogens in fermented milks, it was reported that at the beginning of fermentation, the decrease in growth of pathogens is probably due to antimicrobial compounds, peroxide and decrease in redox potential. As the fermentation process progresses, the lower pH, higher lactic

acid and shorter chain fatty acid concentrations and perhaps the presence of diacetyl may contribute to the inhibition of pathogens present in fermented diary products (Khedkar *et al.*, 1991; Varadaraj *et al.*, 1993; Gupta *et al.*, 1996; Garrote *et al.*, 2000).

There are also several reports on the inhibitory activity of *Lactobacillus acidophilus* and other Kefir organisms against several Gram-positive and negative organisms (Gupta *et al.*, 1996; Garrote *et al.*, 2000). However, information on the antagonistic activity of Kefir organisms in milk is limited.

The aim of this study was to determine the inhibitory activity of Kefir on the survival of some common spoilage organisms and foodborne pathogens. The organisms that were evaluated included strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Clostridium tyrobutyricum*.

Materials and methods

Milk pasteurisation

Fresh pasteurised milk was purchased at local supermarkets and given a further heat treatment in a temperature-controlled waterbath at 83° - 85°C for 20 min and then cooled to 4°C. This was done to ensure the destruction of pathogenic and competing spoilage microorganisms that might have survived the commercial pasteurisation.

Grain activation

Frozen Kefir grains (-18°C) were allowed to defrost at room temperature, added to fresh pasteurised milk at 25°C (18 g Kefir grains to 500 ml milk) and incubated at 25°C for 24 h. The grains were then retrieved using a sterilised stainless steel sieve (1.25% Milton solution for 30 min, then rinsed with sterile distilled water) and placed directly into fresh pasteurised milk at 25°C for 24 h. This procedure was repeated for three consecutive days before the grains were used to produce Kefir (Schoevers, 2000).

Bacterial strains and growth conditions

Escherichia coli strain ATCC 11775, *Staphylococcus aureus* strains B21N and ATCC 12600, *Bacillus cereus* strains B31T and DSM 31, *Listeria monocytogenes* strains ATCC 15313 and NTCC 7973 and *Clostridium tyrobutyricum* strain BZ15, were obtained from the culture collection of the Department of Food Science, University of Stellenbosch, South Africa. Stock cultures of *E. coli*, *S. aureus* and *B. cereus* were maintained at 4°C on Plate Count Agar (Biolab). The *Listeria monocytogenes* strains were maintained on PALCAM-Listeria Selective Agar (Merck) and the *Cl. tyrobutyricum* strain in Reinforced Clostridial Medium (Biolab) under anaerobic conditions.

Preparation of inoculum

To construct growth profiles for the test organisms, cultures of the *E. coli*, *S. aureus*, *B. cereus* and *L. monocytogenes* strains were grown in a special light-coloured broth containing ($g.l^{-1}$): yeast extract 4; peptone 5; glucose 2; and NaCl 8 (pH 7.0 ± 0.2) for 18 h at 35°C. The *Cl. tyrobutyricum* strain was grown in Reinforced Clostridial Medium (Biolab). The respective bacterial counts were determined by plating on Violet Red Bile Agar (Biolab) for *E. coli*, Baird-Parker Agar (Biolab) for *S. aureus*, Cereus Selective Agar (Merck) for *B. cereus,* PALCAM-Listeria Selective Agar (Merck) for *L. monocytogenes* and Reinforced Clostridial Agar for *Cl. tyrobutyricum*. A growth profile of colony forming units (cfu).ml^{-1} against absorbance at 540 nm was constructed for each strain using a spectrophotometer (Spectronic® 20, Genesys™). These profiles were used to standardise the inoculum to a size of 10^3 - 10^4 cfu.ml^{-1}.

Sample Preparation

The different test organisms (*E. coli*, *S. aureus*, *B. cereus, L. monocytogenes* and *Cl. tyrobutyricum*), were individually inoculated (10^3 - 10^4 cfu.ml^{-1}) into 500 ml sterile screw-cap glass containers containing 500 ml pasteurised milk at 25°C. Activated Kefir grains (9 g) were simultaneously inoculated into each bottle. The containers were incubated at 25°C for 30 h and the increase/decrease in numbers of the test organism (cfu.ml^{-1}), the pH and titratable acidity were determined at 0, 4, 8, 12, 16, 20, 24 and 30 h. Each trial was done in triplicate.

Controls

The following controls were included in the study: pasteurised milk containing no test organisms; Kefir containing no test organisms and only Kefir grains; and pasteurised milk containing $10^3 - 10^4$ cfu.ml^{-1} of each test organism but no Kefir grains. All the controls were also incubated at 25°C. Each trial was done in triplicate.

Microbial enumeration

At the selected time-intervals, 1 ml samples were taken from each of the containers and a ten-fold dilution series made using a sterile saline solution (0.85% (w/v) NaCl). Suitable dilutions were vortexed and pipetted in duplicate into petri dishes and then 15 ml of the specific melted agar was added to these plates. The selective agars used were: Violet Red Bile Agar for *E. coli*; Baird-Parker Agar base + 1% sterile Potassium Tellurite Solution + 50% sterile Egg Yolk Emulsion for *S. aureus*; Cereus Selective Agar base + 50% Egg Yolk Emulsion + Polymixin-B-sulfate for *B. cereus*; and PALCAM Listeria Selective Agar base + PALCAM Listeria Selective Supplement for *L. monocytogenes*. The plates were incubated at 35°C for 24 – 72 h, depending on the growth of the test organisms. *Clostridium tyrobutyricum* was enumerated in Reinforced Clostridial Agar. After the Reinforced Clostridial Agar had set, a thin layer of pure Agar (Biolab) was poured onto the plates to enhance anaerobic growth. These plates were incubated at 30°C under anaerobic conditions.

Inhibition (%) of each of the test organisms at selected time-intervals were estimated according to the method recommended by Gilliland & Speck (1977):

$$\% \text{ Inhibition} = \frac{(\text{cfu.ml}^{-1} \text{ in control sample}) - (\text{cfu.ml}^{-1} \text{ in experimental sample})}{(\text{cfu.ml}^{-1} \text{ in control sample})} \times 100$$

The log cycle reductions of each of the test organisms at selected time-intervals were estimated as follows:

$$\text{Log cycle reduction} = (\log \text{cfu.ml}^{-1} \text{ in control sample}) - (\log \text{cfu.ml}^{-1} \text{ in experimental sample})$$

Determination of pH and titratable acidity

The pH of the inoculated milk samples and controls was determined at the selected time-intervals using a Knick pH-meter (pHB-4) according to the AOAC (1990) method. The titratable acidity (TA) of the inoculated milk samples and controls was determined according to the method recommended by James (1995). According to this method 10 ml aliquots of a thoroughly mixed milk sample were pipetted into conical flasks. One millilitre of a 0.5% phenolphthalein solution was added and the sample titrated to a faint pink colour with 0.11M sodium hydroxide solution. The TA of the milk sample was calculated, given that:

TA (as % lactic acid) = (ml 0.11M NaOH used)/10

Statistical analyses

Means and standard deviations of the bacterial enumeration data, pH and TA values were calculated using the SigmaPlot 2000 program (Version 6, SPSS Inc.).

Results and discussion

This study was done to determine if Kefir has an inhibitory activity towards certain spoilage microorganisms and potential pathogens. The growth of the different test organisms in milk and Kefir, as well as the changes in pH and TA over time, are shown in Fig. 1 – 8. The data in Fig. 9 shows the percentage inhibition of the different test organisms when grown in milk inoculated with Kefir grains in comparison to their growth in milk at selected time-intervals. In Fig. 10 the different log cycle reductions for the test organisms in Kefir, when compared to their growth in milk, are illustrated against incubation time.

Controls

The data showed that for the total study there were no viable microbial counts on any of the specific media when used for evaluation purposes on the control milks (without test organisms) and Kefir (without test organisms) samples. The pH for milk (without test organisms) was found to drop slightly over the 30 h incubation period

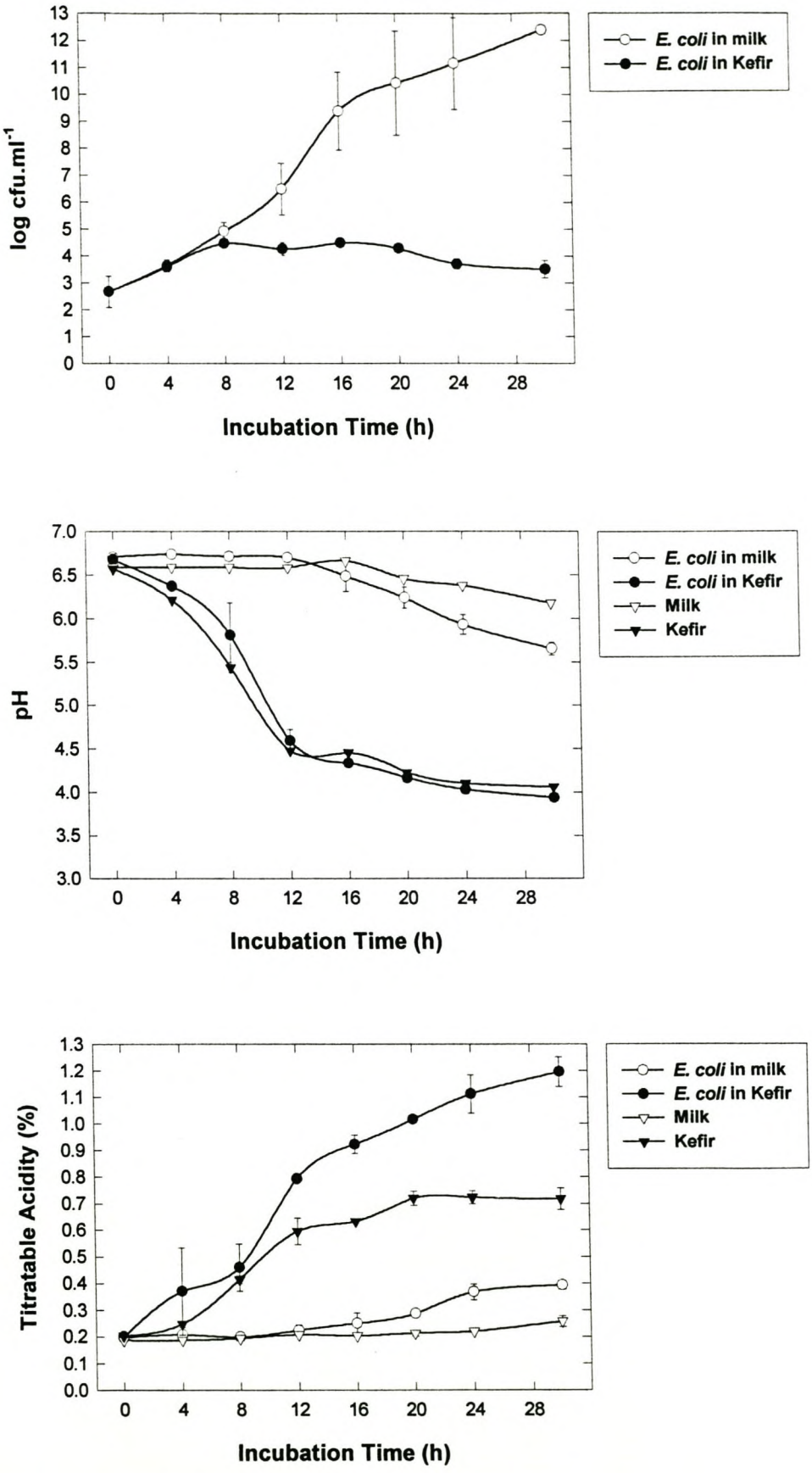

Figure 1. Changes in the *E. coli* strain ATCC 11775 viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

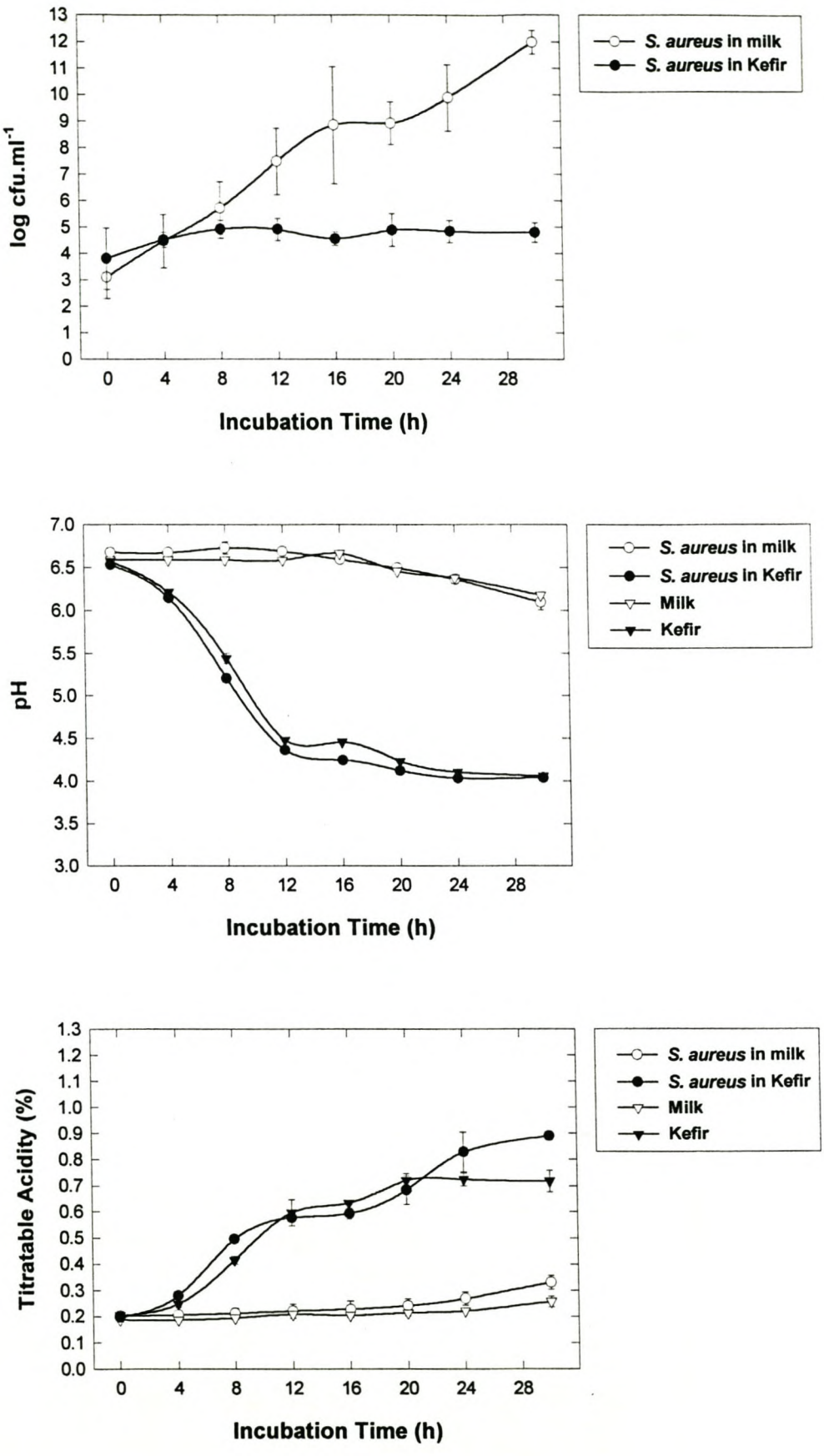

Figure 2. Changes in the *S. aureus* strain B21N viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

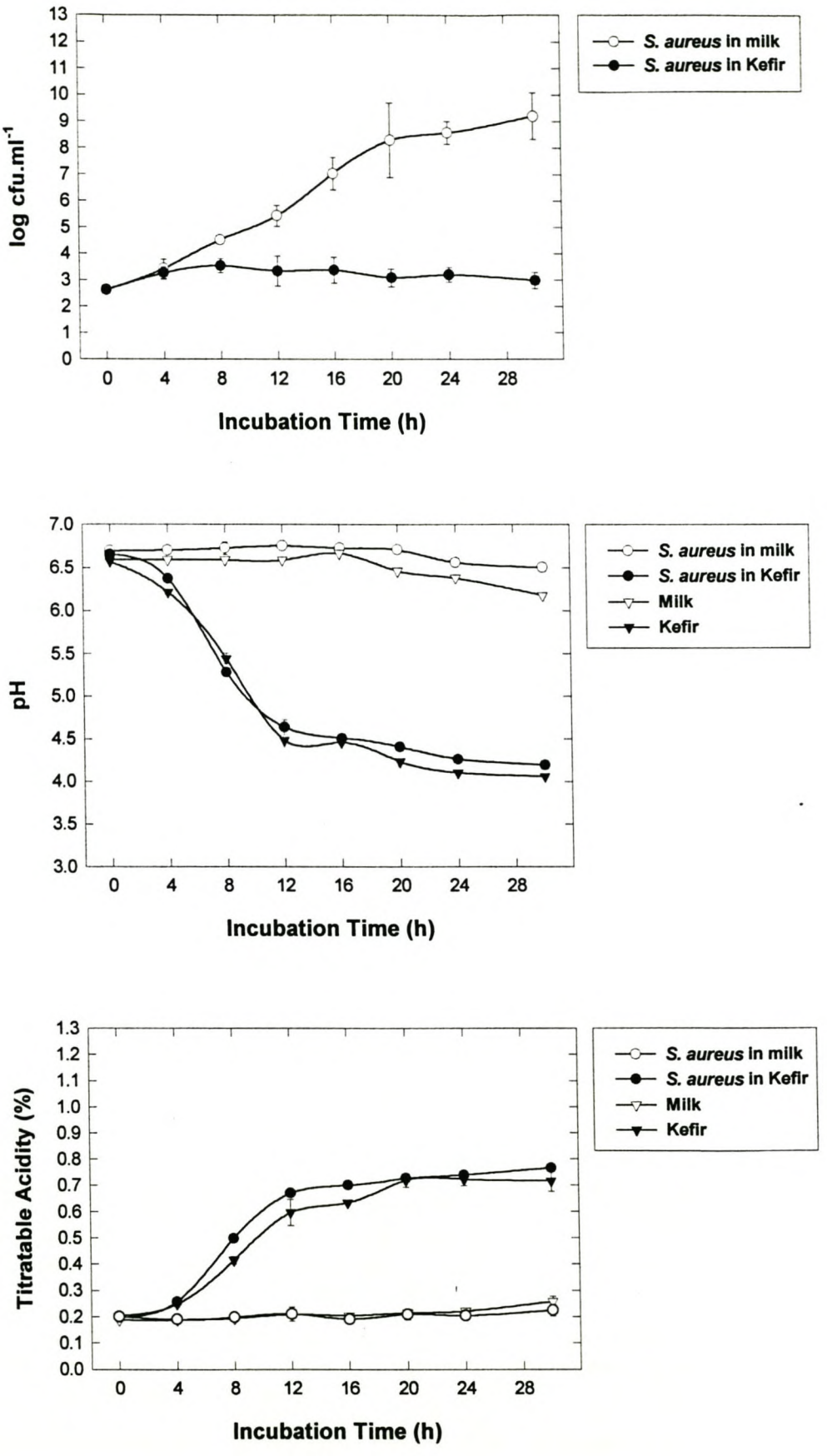

Figure 3. Changes in the *S. aureus* strain ATCC 12600 viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

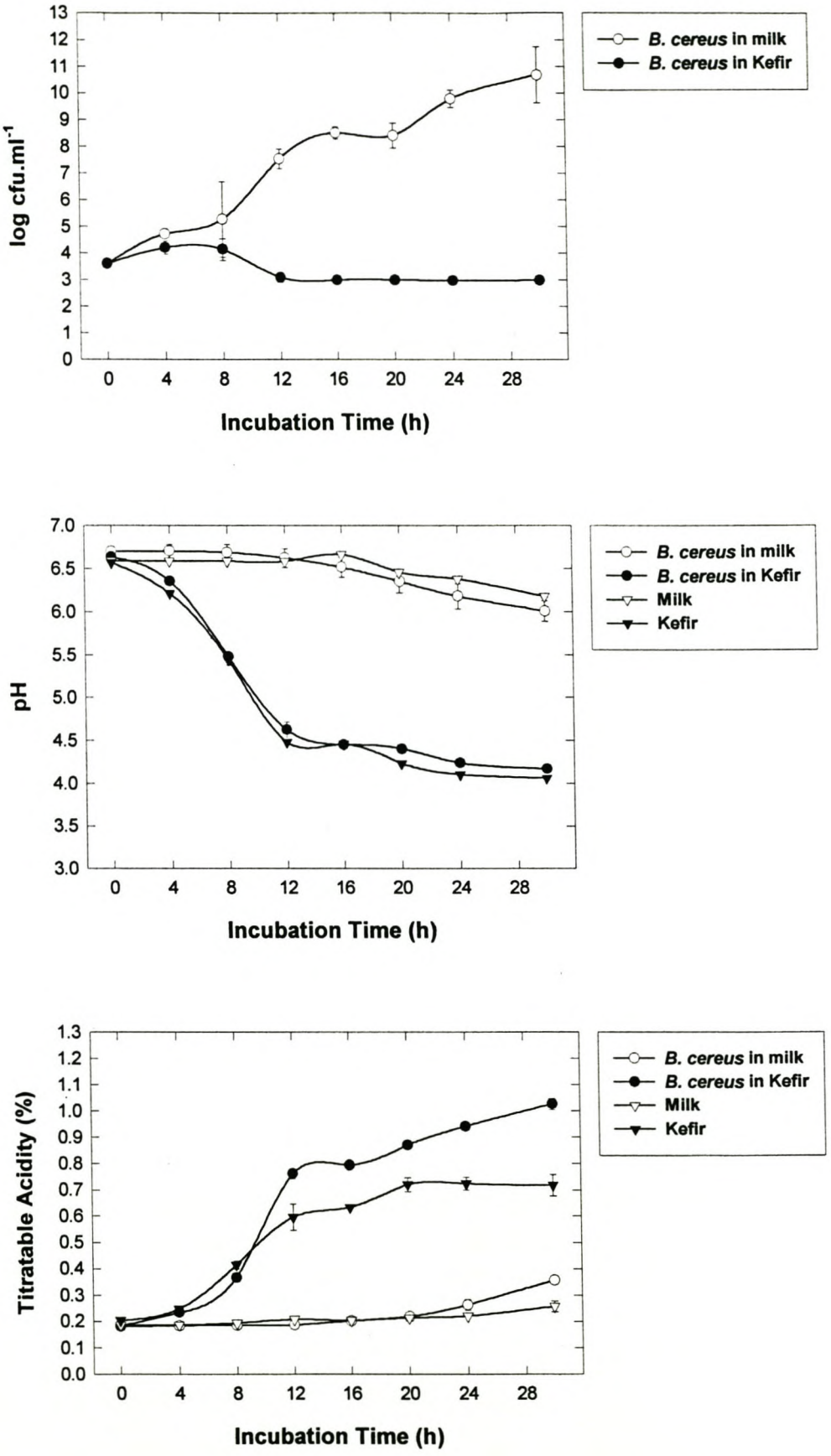

Figure 4. Changes in the *B. cereus* strain B31T viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

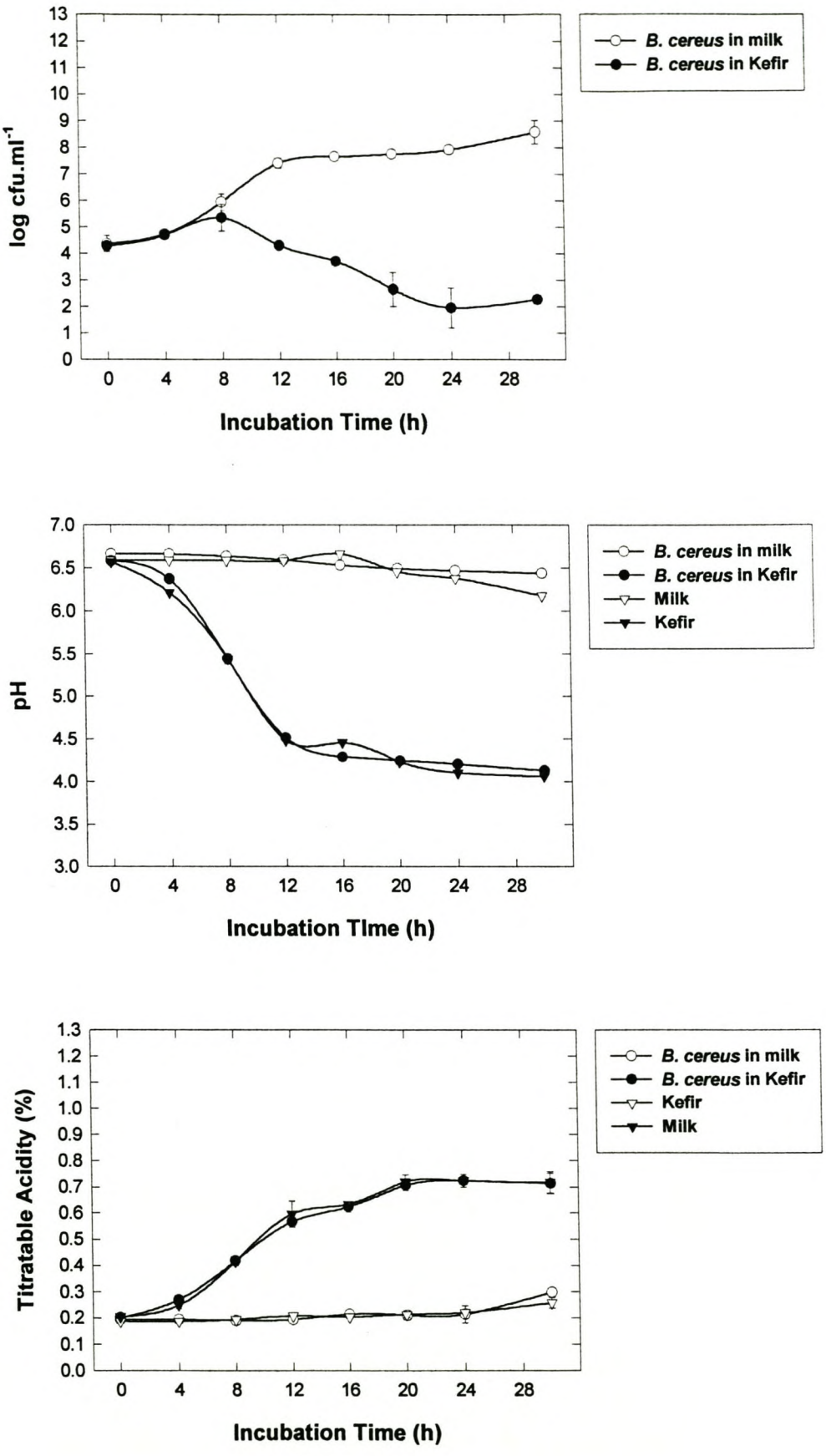

Figure 5. Changes in the *B. cereus* strain DSM 31 viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

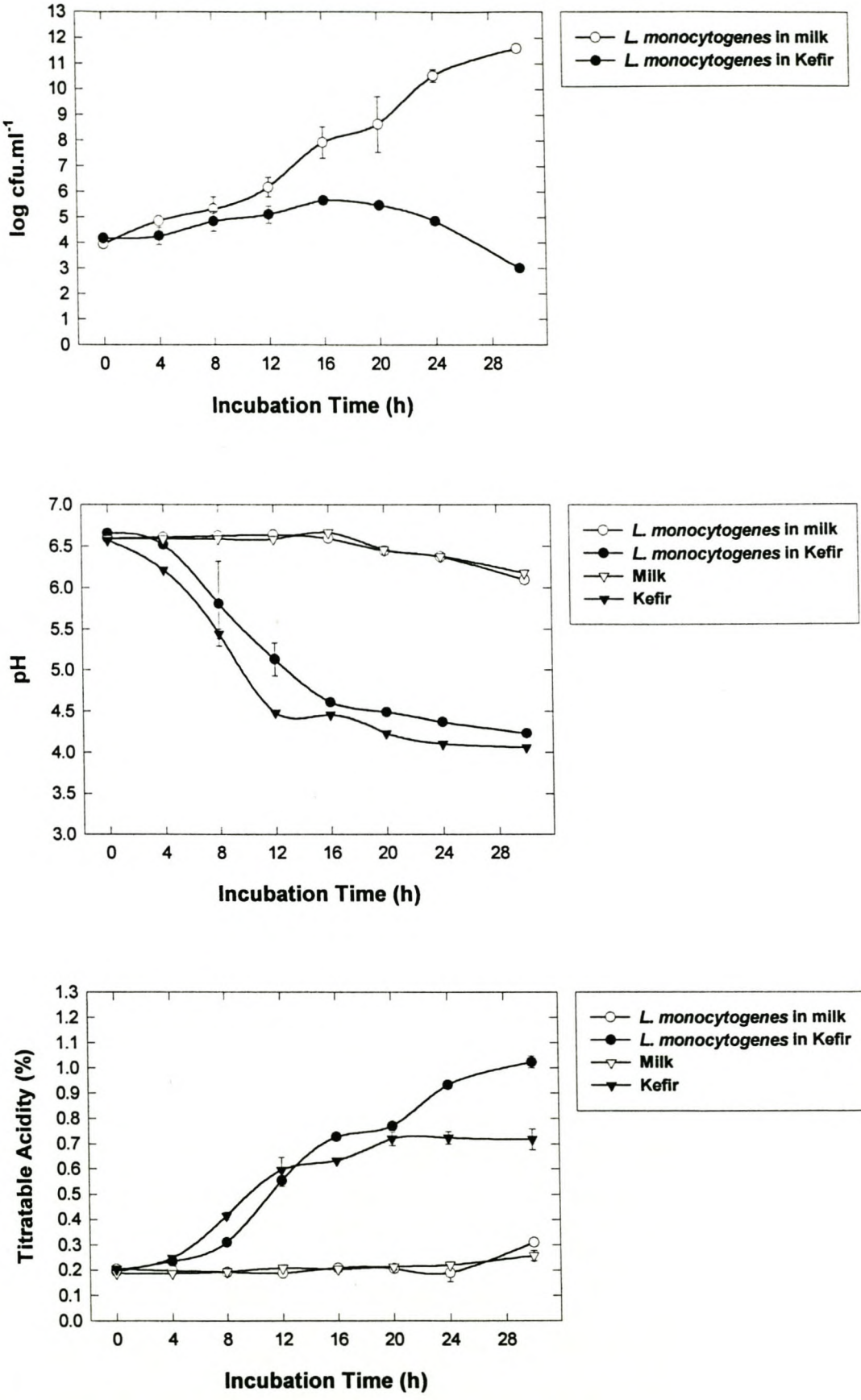

Figure 6. Changes in the *L. monocytogenes* strain ATCC 15313 viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

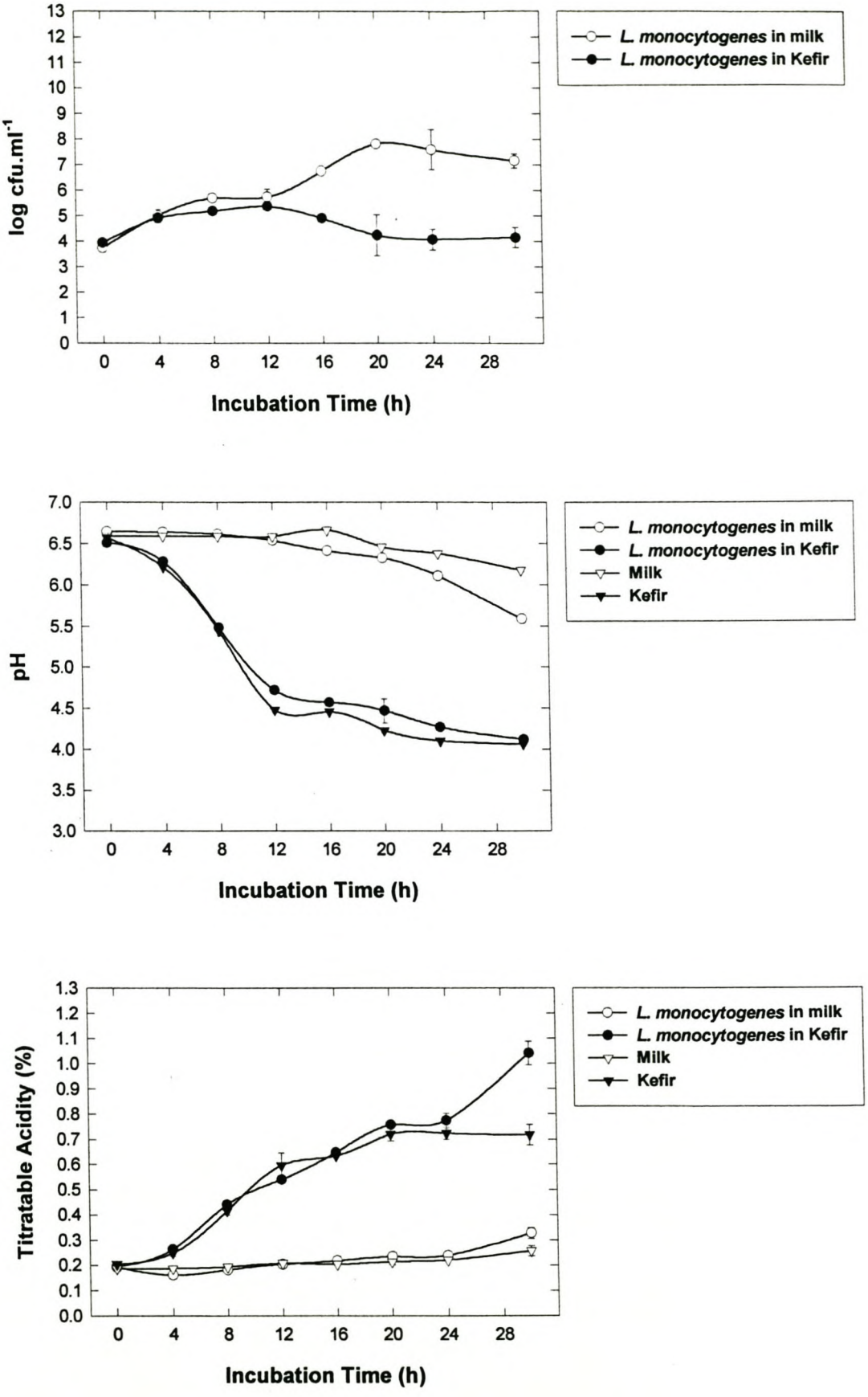

Figure 7. Changes in the *L. monocytogenes* strain NTCC 7973 viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

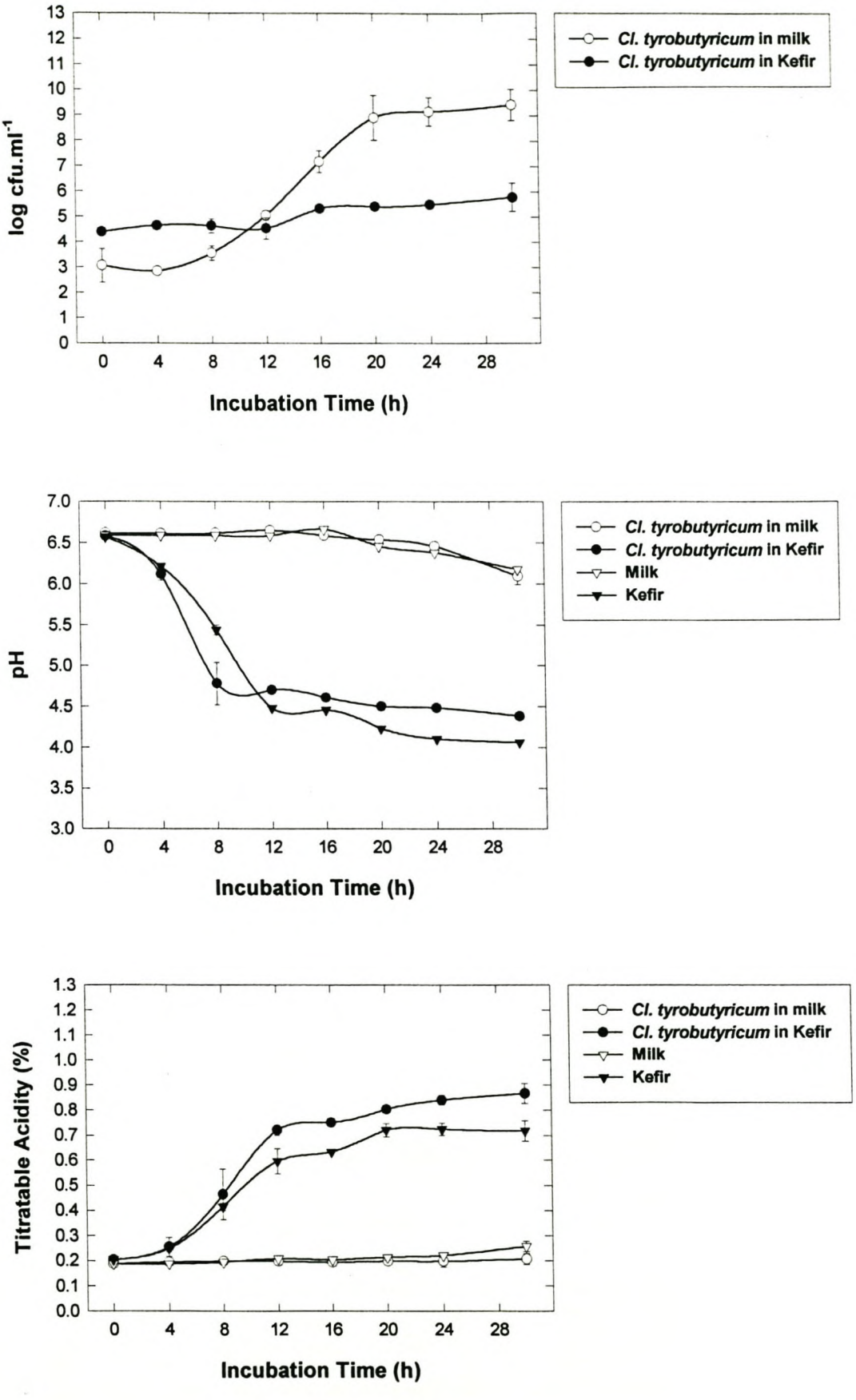

Figure 8. Changes in the *Cl. tyrobutyricum* BZ15 viable counts, pH and TA during the production of Kefir. The standard deviation was used as the error bar length.

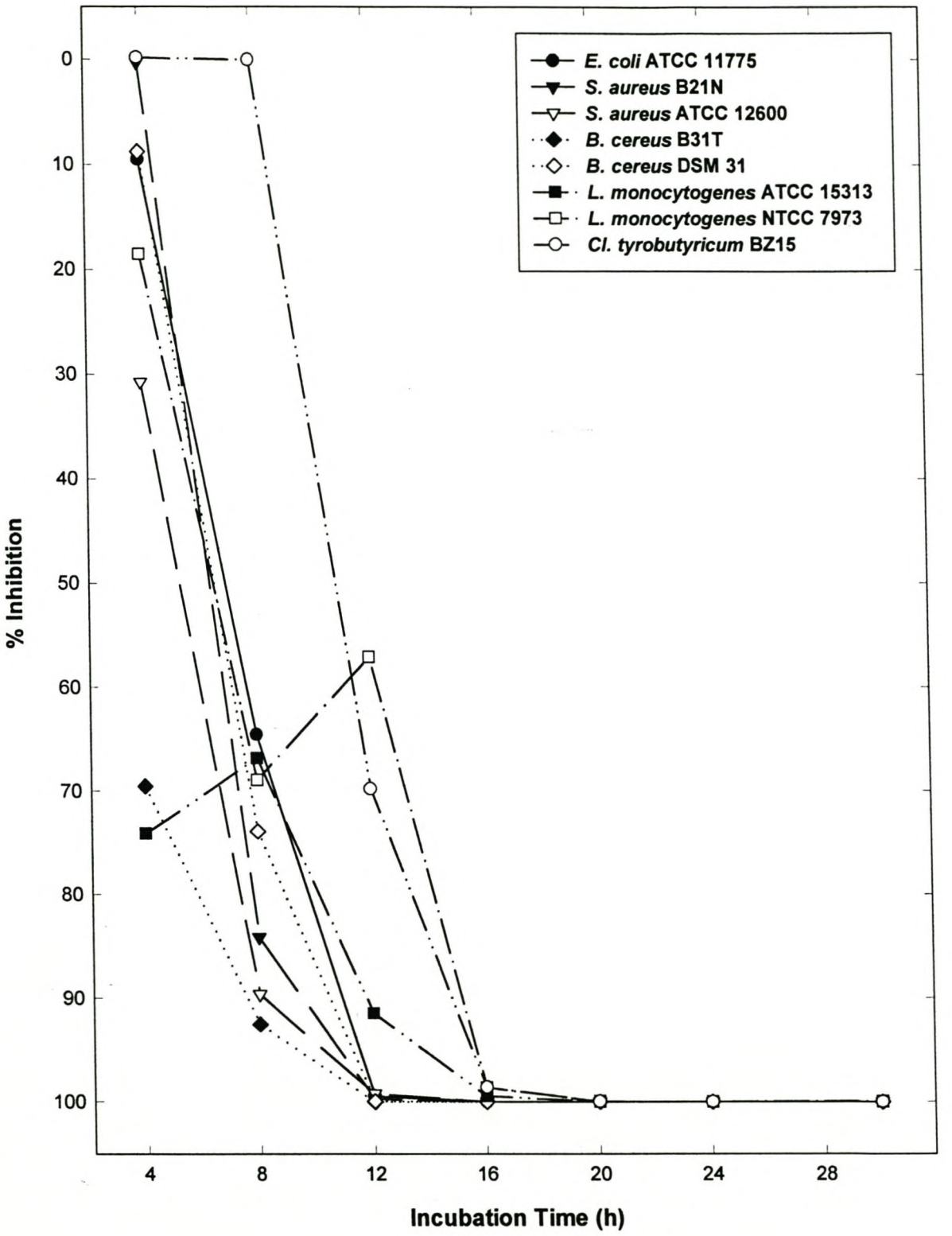

Figure 9. Inhibition of the different test organisms at the different time intervals while growing in Kefir in comparison to their growth in the milk controls.

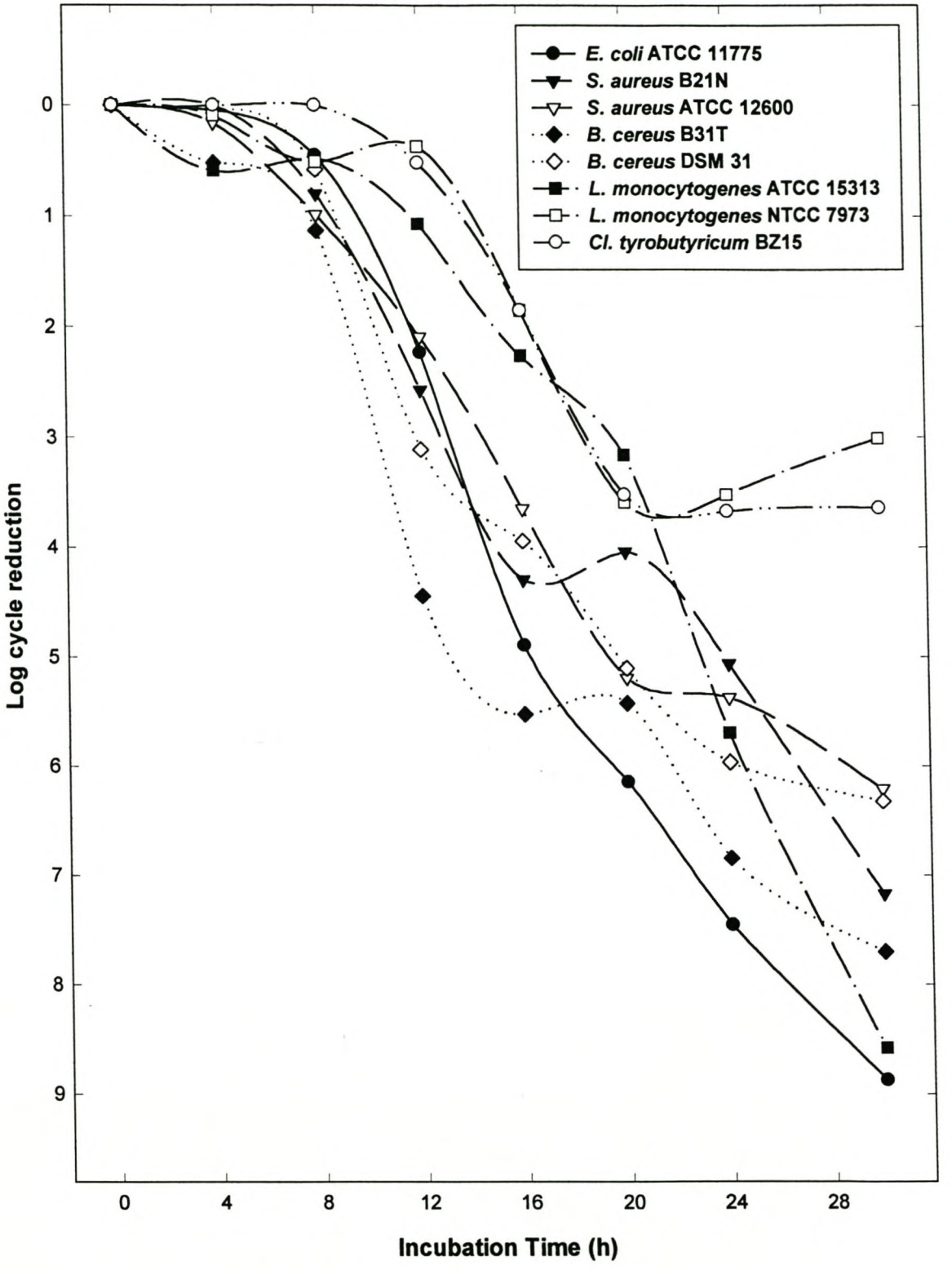

Figure 10. Log cycle reductions of the different test organisms at the different time intervals while growing in Kefir in comparison to their growth in the milk controls.

from 6.59 to 6.18, while the pH of Kefir without test organisms, decreased substantially from 6.57 to 4.06 during the 30 h incubation period.

Titratable acidity measurements of the samples and controls were done at the various time-intervals to determine the concentration of organic acids, primarily lactic acid, formed by the LAB present in the Kefir grains during the Kefir fermentation. The TA of milk (without test organisms) did not vary strongly (0.19 to 0.26%) over the 30 h incubation period. However, the TA for the Kefir without test organisms exhibited a steep incline from 0.2 to 0.72% during the incubation period.

Escherichia coli

It can be seen from the data in Fig. 1 that the *E. coli* population increased in both the milk and Kefir samples in an almost identical gradient for the first 8 h of incubation and thereafter, for the Kefir samples, there was a gradual decline of the viable counts. This coincided with a steep drop in the pH of the Kefir samples after 8 h. The TA levels for the Kefir samples inoculated with *E. coli* were substantially higher than those of the control (Kefir without *E. coli*). This phenomenon can be attributed to the fact that *E. coli* produces acid during its natural carbohydrate catabolism (Holt *et al.,* 1994). After 16 h incubation, the *E. coli* in the Kefir samples showed an inhibition of >99.9%, when compared to the growth of *E. coli* in the milk samples (Fig. 9). The *E. coli* strain exhibited a log cycle reduction of 8.87 units after 30 h incubation in Kefir in comparison to its viable counts in milk (Fig. 10).

Garrote *et al.* (2000) also examined milk fermented with Kefir grains for inhibitory activity towards *E. coli.* He reported a bacteriostatic effect on *E. coli* when the organism was inoculated in milk at 10^6 cfu.ml^{-1} with Kefir grains (2 g.100 ml^{-1}). A log cycle reduction of 2.6 units was found after 24 h of incubation in Kefir at 20°C when compared to the growth of *E. coli* in Nutrient Broth. Over the same time period it was found in this study that a much higher log cycle reduction of 7.45 took place when compared to the growth of *E. coli* in milk (Fig. 10). This difference could be explained by the difference in initial *E. coli* concentrations between the studies as well as in the difference in controls used (Nutrient Broth vs. milk).

Acid production by lactic acid bacteria is one of the oldest methods used to influence the growth of Gram-negative bacteria (Helander *et al.*, 1997). Various reports recount the inhibition of *E. coli* by lactic (Presser *et al.*, 1997; Garrote *et al.*,

2000) as well as by acetic acid (Shafic & Musleh, 1988). Fermentations with LAB usually result in the accumulation of organic acids, primarily lactic acid, as a major end-product of carbohydrate metabolism. As acetic acid bacteria also occur in Kefir grains, acetic acid is another end-product of Kefir fermentation. A synergism between lactic and acetic acid in the inhibition of *E. coli* has been reported in previous studies (Naidu *et al.*, 1999). The results obtained by Garrote *et al.* (2000) showed that the effect of Kefir on *E. coli* was bacteriostatic and mainly due to organic acids produced during the fermentation process.

Khedkar *et al.* (1991) studied the inhibition of the growth of *E. coli* during production and storage of acidophilus milk. *Escherichia coli* (10^3 cfu.ml^{-1}) was grown in milk in the presence of *Lb. acidophilus* at 37°C and it was found that the growth of *E. coli* was restricted after 60 h. When these findings are compared to those of the present study the assumption can be made that mixed lactic cultures (as can be found in Kefir grains) have a much more pronounced inhibitory effect on the growth of *E. coli*, than just a single strain of *Lb. acidophilus*. In a similar study by Gupta *et al.* (1996), *E. coli* ($3.0 – 6.0 \times 10^8$ cfu.ml^{-1}) was grown in milk as a mixed culture with a *Lb. acidophilus* strain at 37°C. In their studies the controls consisted of sterilised milk inoculated with the test pathogen in the same manner as in the experimental samples. The acidified milk samples were prepared by inoculating milk with the test pathogen and adjusting the pH equivalent to that of experimental samples at 2 h intervals using sterilised lactic acid. *Escherichia coli* showed a much higher rate of inhibition in mixed cultured milk than in acidified milk. Therefore, it was concluded that the antagonistic effect produced by *L. acidophilus* towards different pathogens is not due to acid production alone. After 4 and 8 h in the mixed cultured milk, *E. coli* was inhibited by, respectively, 91 and 92%. The rate of inhibition was thus higher than that found in the study by Khedkar *et al.* (1991) and that of the present study for *E. coli* in Kefir. These differences may be attributed to strain variation as well as differences in inoculum concentration and experimental conditions.

Balasubramanyam & Varadaraj (1994) studied the effect of culture filtrates of natural isolates of lactic acid bacteria from *Dahi* (a popular Indian fermented milk product) against a few important foodborne pathogenic and spoilage bacteria. *Escherichia coli* was not inhibited by any of the LAB cultures. This indicates that the inhibition found for *E. coli* in Kefir is either due to the activity of very specific LAB or a

combination of other environmental factors, such as a low pH or high organic acid concentration.

Small reductions (0.8 – 2 log units) in numbers of *E. coli* O157:H7 have been shown during preparation and storage of yoghurt and cheddar cheese. Similar rates of inactivation were seen in commercial products, including sour cream and buttermilk that had been inoculated with *E. coli* O157:H7 (McClure & Hall, 2000). No other fermented dairy products, however, showed the same high rate of inhibition of growth of *E. coli*, than was found in this study using Kefir as growth medium.

Staphylococcus aureus

The two strains of *S. aureus* used as test organisms in this study showed very similar growth patterns in milk and Kefir (Fig. 2 and 3). A variation in the inoculum size was found for *S. aureus* strain B21N in the milk and Kefir samples. This was probably due to the fact that *S. aureus* cells occur in irregular clusters, making it difficult to standardise the inoculum size (Holt *et al.*, 1994).

A sharp increase in cell numbers was observed for both strains when grown in milk over 30 h. The numbers of *S. aureus*, however, varied only slightly in Kefir after inoculation. No notable decline in cell numbers was observed for both *S. aureus* strains when grown in Kefir. The pH and TA measurements of the samples and the corresponding controls were also very similar. *Staphylococcus aureus* strains B21N and ATCC 12600 showed >99.9% inhibition after, respectively, 24 and 20 h of incubation in Kefir when compared to growth in the control milk samples (Fig. 9). The growth of the *S. aureus* strain B21N in Kefir compared to the growth in milk resulted in a 7.17 log cycle reduction after 30 h. The corresponding value for *S. aureus* strain ATCC 12600 was 6.21 log cycles (Fig. 10).

Khedkar *et al.* (1991) studied the inhibition of the growth of *S. aureus* during production and storage of acidophilus milk. *Staphylococcus aureus* (10^3 cfu.ml^{-1}) was grown in milk in the presence of *Lb. acidophilus* at 37°C. *Lactobacillus acidophilus* restricted the growth of *S. aureus* after 16 h, whilst with *E. coli* 60 h was needed to show the same inhibition. *Staphylococcus aureus* is thus probably more susceptible to 'lactic' antagonism than *E. coli*. Naidu *et al.* (1999) reported that lactic and acetic acids, such as are produced during Kefir fermentation, have an inhibitory activity towards especially *S. aureus*. This was confirmed in a study by Gupta *et al.*

(1996) where *S. aureus* also showed a much higher inhibition than *E. coli* in mixed cultures with *Lb. acidophilus.* Gupta *et al.* (1996) also found that the antagonistic effect of *Lb. acidophilus* towards different pathogens was not due to the formation of organic acids alone. *Lactobacillus acidophilus* has been reported to produce bacteriocins such as acidolin, acidophilin and lactocidin. The inhibition of *S. aureus* observed in the above-mentioned study, as well as in the present study, might also be attributed to the activity of such compounds produced by *Lb. acidophilus* and other LAB.

Bacillus cereus

A steep increase in cell counts was observed for both *B. cereus* strains when grown in milk over 30 h (Fig. 4 and 5). It can be observed from Fig. 4 that the *B. cereus* strain B31T numbers gradually increased up to 4 h of incubation in Kefir. This was followed by a decrease in cell counts up to 12 h where after the population stayed more or less constant for the remainder of the incubation period. The same pattern was observed for *B. cereus* strain DSM 31 (Fig. 5) for the first 8 h of incubation in Kefir, which was followed by a sudden drop, leading to a substantial decrease (approximately 4 log cycles) in numbers of this strain after 24 h.

The pH for the samples and the corresponding controls were very similar. The growth of *B. cereus B31T* in Kefir resulted in a higher TA than that of Kefir without the organism. This might be due to acid formation by the organism during its fermentative metabolism. *Bacillus cereus* strains B31T and DSM 31 showed a >99.9% inhibition after, respectively, 12 and 20 h of incubation in Kefir, when compared to growth in the control milk samples (Fig. 9). After 30 h incubation in Kefir, *B. cereus* strain B31T exhibited a log cycle reduction of 7.70 units and strain DSM 31 of 6.32 units, when compared to growth in milk without Kefir grains (Fig. 10).

Balasubramanyam & Varadaraj (1994) studied the effect of culture filtrates of natural isolates of lactic acid bacteria from *Dahi* against a few important foodborne pathogenic and spoilage bacteria. Among the 50 LAB isolates obtained, sterilised culture filtrates of 14 isolates exhibited either strong or moderate degree of inhibition of *B. cereus.* The inhibitory activity was lost in the sterilised culture filtrate of antagonistic LAB isolates treated with trypsin, thereby indicating the proteinaceous nature of the antimicrobial compound. Furthermore, the reaction of catalase with the

culture filtrate showed a reduced inhibition, indicating the role of hydrogen peroxide. The inhibitory activity of the 14 isolates was found to be more pronounced against *B. cereus* than against *E. coli* and *S. aureus*.

Naidu *et al.* (1999), however, reported that the growth of *B. cereus* is blocked in the presence of LAB due to acetate production and that spore germination is inhibited by formate, lactate and acetate (all products of the LAB metabolism).

Listeria monocytogenes

An increase in cell counts was observed for the *L. monocytogenes* strain ATCC 15313 when grown in milk over 30 h (Fig. 6). The *L. monocytogenes* strain NTCC 7973 exhibited an increase in cell counts for the first 20 h of incubation in milk followed by a gradual decline (Fig. 7). This was probably due to the growth reaching a death phase because of detrimental environmental changes like nutrient deprivation and the build up of toxic wastes. It can be observed from the data in Fig. 6 that there was an increase in counts for *L. monocytogenes* strain ATCC 15313 up to 16 h in Kefir and, thereafter, there was a sharp decline in the counts (approximately 2.5 log cycles) up to 30 h of incubation. *Listeria monocytogenes* strain NTCC 7973 also exhibited an increase in counts in Kefir up to 12 h of incubation followed by a gradual decline (Fig. 7).

The pH for the test samples and the corresponding controls were very similar. The growth of both *L. monocytogenes* strains resulted in a higher TA than that for samples without the added organism. This can be attributed to the fact that *Listeria* exerts a carbohydrate metabolism that yields mainly L-lactic acid (Holt *et al.*, 1994). Therefore, the levels of lactic acid, and subsequently the TA measurements in the samples, might have increased due to the presence of the *Listeria* strains. The presence of Kefir organisms might also have further stimulated the *Listeria* strains into producing more lactic acid or *vice versa*. Strain ATCC 15313 exhibited a >99.9% inhibition after 24 h incubation in Kefir, while *L. monocytogenes* strain NTCC 7973 was inhibited by 99.9% after 30 h of incubation in Kefir, when compared to growth in the control milk samples (Fig. 9). The strains showed, respectively, log cycle reductions of 8.58 and 3.01 units after 30 h incubation in Kefir, in comparison to their growth in the control milk samples (Fig. 10).

In a study on the prevalence of *Listeria* species in South African dairy products, no listeriae were detected in cultured buttermilk, yoghurt and Maas samples, in contrast to incidences in pasteurised milk (0.7%), pasteurised flavoured milk (6.7%), ice cream (7.5%), butter (2.2%), cream (4.3%) and cheese (3.9%) (Wnorowski & Bergman, 1993). This implies that the fermentation processes, in which cultured buttermilk, yoghurt and Maas were involved, may have prevented the growth/occurrence of *Listeria* species.

Muriana (1996) reported that the bacteriocins of *Lactobacillus acidophilus* (acidocin A), *Lb. plantarum* (plantaricin), *Lactococcus lactis* (nisin) and *Leuconostoc mesenteroides* (mesenterocin) are especially active against *Listeria.* All these bacteriocin-producers have been isolated from Kefir grains (Kwak *et al.*, 1996; Libudzisz & Piatkiewicz, 1990). Zapico *et al.* (1998) reported that nisin added at 10 or 100 $IU.ml^{-1}$ to UHT skimmed milk had no effect on counts of *L. monocytogenes* after 24 h at 30°C, whereas addition of lactoperoxidase resulted in counts of viable cells 3 log units lower that those of control milk after 24 h at 30°C. The addition of nisin and activation of the lactoperoxidase system showed a synergistic effect and resulted in counts of up to 5.6 log units lower than that of the control milk. As *Lactococcus lactis* subsp. *lactis* (the producer of nisin) is usually part of the Kefir grain population (Kwak *et al.*, 1996) the same synergistic mechanism for inhibition of *L. monocytogenes* in milk may also apply in Kefir.

Clostridium tyrobutyricum

A variation in the inoculum size was found for the *Cl. tyrobutyricum* strain in the milk and Kefir samples (Fig. 8). This was probably due to the fact that the cells of this *Clostridium* strain occur in pairs or short chains, making it difficult to standardise the inoculum size (Holt *et al.*, 1994). An increase in numbers for *Cl. tyrobutyricum* was observed during the first 20 h of growth in the control milk samples. Thereafter the counts in the milk increased only minimally and this was probably due to the growth reaching a stationary phase. The counts for the *Cl. tyrobutyricum* strain varied only slightly in Kefir during the 30 h incubation period. Up until 12 h of incubation no growth was observed for the *Cl. tyrobutyricum* strain, which is probably because there may have been a competition for nutrients between Kefir microorganisms and *Cl. tyrobutyricum* under these experimental conditions. The lack

of positive growth could also probably have been caused by the presence of inhibitory compounds that were already present at early stages in the milk fermentation. After 12 h a slight increase was observed in the *Cl. tyrobutyricum* population in the Kefir. This can probably be attributed to the germination of clostridial endospores in the Kefir samples.

The pH and TA measurements for the samples and the corresponding controls were very similar, although slightly higher TA measurements were observed for the Kefir samples containing *Clostridium*. This was probably due to the formation of butyric and acetic acid as major metabolic products by this organism that had been stimulated in the presence of the competing Kefir microorganisms (Klijn *et al.*, 1995). *Clostridium tyrobutyricum* showed a 99.9% inhibition after 30 h of incubation in Kefir in comparison to its growth in milk (Fig. 9). Incubation in Kefir reduced this population by 3.64 log cycles when compared to the clostridial population in milk after 30 h incubation (Fig. 10). Lacticin, a bacteriocin produced by *Lb. acidophilus*, has been shown to have an inhibitory activity against *Cl. tyrobutyricum*, which might be one of the reasons for the inhibitory activity found in Kefir (Naidu *et al.*, 1999).

Inhibition and log cycle reductions

The data in Fig. 9 clearly shows that no inhibition was found for *S. aureus* strain B21N up to 4 h of incubation in Kefir and none for the *Cl. tyrobutyricum* strain up to 8 h of incubation in Kefir. In contrast, all the other test organisms showed inhibition when grown in Kefir after only 4 h of incubation. *Bacillus cereus* strain B31T exhibited the highest rate of inhibition with a >99.9% inhibition reached after only 12 h of incubation in Kefir, followed by *E. coli* with a >99.9% inhibition after 16 h of incubation in Kefir. An unexpected decrease in the level of inhibition is observed for the *L. monocytogenes* strain NTCC 7973 after 12 h of incubation in Kefir. This is probably due to the phenomenon of unbalanced growth occurring in the control milk samples where the rates of synthesis of cell components may vary relative to one another until a new balanced state is reached (Prescott *et al.*, 1993). This response is readily observed in any 'shift-up' experiment in which bacteria are transferred from a nutritionally poor medium to a richer one which might have resulted when the organism was transferred from the special broth used for the preparation of the inoculum to pasteurised milk. The cells first construct new ribosomes to enhance

their capacity for protein syntheses. This is followed by increases in protein and DNA synthesis. Finally, the expected rise in reproductive rate takes place. This was observed for the *L. monocytogenes* strain NTCC 7973 after 16 h of incubation in milk (Fig. 7). The decrease in inhibition of the *L. monocytogenes* strain NTCC 7973 at 12 h of incubation may also have been caused by a process known as feedback inhibition (Prescott *et al.*, 1993). If the end-product in a metabolic pathway becomes too concentrated, it inhibits the regulatory enzyme and slows its own synthesis. As the end-product concentration decreases, pathway activity again increases and more product is formed. This might result in sudden decreases and increases in cell growth.

Listeria monocytogenes strain NTCC 7973 showed the lowest level of inhibition in Kefir after 30 h of incubation, followed by the *Cl. tyrobutyricum* strain. *Clostridium tyrobutyricum* took the longest period to show a high level of inhibition. This is probably due to the endospore-forming ability of this organism. After the 30 h incubation period all the test organisms showed substantial levels of inhibition (≥99.9 %) in Kefir in comparison to their growth in milk.

The standard incubation period for Kefir production is 18 h at 25°C, followed by a 6 h ripening period at 22°C (Schoevers, 2000). It can be seen in Fig. 9 that all the test organisms showed substantial levels of inhibition (99.7 – 99.9%) after 18 h of incubation at 25°C.

After 30 h incubation in Kefir, the *E. coli* strain showed the highest log cycle reduction (Fig. 10), followed by *L. monocytogenes* strain ATCC 15313. The *Listeria monocytogenes* NTCC 7973 population exhibited the lowest reduction in log cycles after 30 h followed by *Cl. tyrobutyricum*. The difference in the results obtained for the two *L. monocytogenes* strains suggests a substantial variation in the characteristics of these two strains.

Bacillus cereus B31T showed the highest rate of log cycle reduction in the shortest time. The *B. cereus* DSM 31 strain exhibited a similar behaviour but showed a much lower level of log cycle reduction. This may imply that the mechanism by which inhibition was achieved is strain specific. It can be concluded that the *B. cereus* strain B31T was the most sensitive to incubation in Kefir. This confirms the results obtained by Balasubramanyam & Varadaraj (1994) who reported that *B. cereus* was inhibited more effectively by culture filtrates of natural isolates of lactic

acid bacteria from *Dahi*, when compared to *E. coli* and *S. aureus*. When compared to the other test organisms, *Cl. tyrobutyricum* showed the most resistance against inhibition by Kefir, although it was also severely inhibited (Fig. 9) after 30 h incubation in Kefir.

Conclusions

It is well known that many metabolic products from lactic acid bacterial fermentations have strong inhibitory activity towards the growth of saprophytic and pathogenic bacteria. This antagonistic activity may involve different mechanisms, such as competition for available nutrients and production of inhibitory metabolites (hydrogen peroxide, organic acids, diacetyl and bacteriocins) (Garrote *et al.*, 2000).

There are only a few reports in the literature on the inhibitory potential of Kefir (Koroleva, 1988; Brialy *et al.*, 1995; Dineen *et al.*, 1998; Garrote *et al.*, 2000). It has been reported that pathogenic bacteria like *Shigella* and *Salmonella* do not grow when they are introduced to Kefir. Garrote *et al.* (2000) reported that for an agar diffusion assay performed to determine the inhibitory power of Kefir supernatant, the inhibitory zone diameter was greater for Gram-positive than for Gram-negative microorganisms. The present work shows the ability of milk fermented with Kefir grains to inhibit the growth of several Gram-positive bacteria, as well as a Gram-negative bacterium, *E. coli*.

It is generally known that raw milk may contain microorganisms pathogenic to man (Robinson, 1990). In South Africa it is not unusual for the population to consume unpasteurised or raw milk, especially in the rural areas. The possibility of contracting diseases through milk thus exists and is a reason for concern. This health risk is, however, not obliterated by pasteurisation. Pasteurised milk may still contain pathogens due to post-processing contamination resulting from inadequate handling practices. "Die Burger" (Friday, 29 September 2000) reported that a study conducted by the Cape Metropolitan Council Health Department revealed that the viable bacterial counts of 76% of pasteurised bulk milk samples and 36% of packaged milk sold in 1999 exceeded the legal limit of 5×10^4 cfu.ml^{-1} (Brümmer,

2000). If pathogens are present, this situation can result in a serious threat to the health of all consumers.

This study showed that Kefir possesses an inhibitory activity towards certain spoilage organisms and potential pathogens that may occur in either raw or pasteurised milk. Growth of all the test organisms were inhibited substantially (≥99.9%) in Kefir over a 30 h incubation period, when growth was compared to that in pasteurised milk. Substantial reductions in log cycles were observed for many of the organisms. If one considers, for instance, the fact that the South African legal limits for *E. coli* (as well as for pathogenic organisms), in both unpasteurised and pasteurised milk sold for consumption, are no *E. coli* in 1.0 ml, the inoculum size of 10^3 cfu.ml^{-1} was an exaggerated value (Anon., 1997). The chances for the occurrence of such high numbers of potential pathogens in milk meant for consumption are highly unlikely and it is probable that when pathogens occur in concentrations lower than that used as inoculum in this study, the possibility exists that they will be totally eliminated. The use of milk in the production of Kefir, therefore, has the ability to make milk safer.

After 16 h, the *E. coli* in the Kefir samples showed total growth inhibition, when compared to the growth of *E. coli* in the milk control samples. Considering the inhibitory effect of Kefir towards *E. coli*, a Gram-negative bacterium usually isolated from faeces, Kefir could be potentially considered as a probiotic. If gut colonisation with Kefir grain organisms could be proved, resulting acid production could inhibit colonisation with pathogenic microorganisms (Garrote *et al.*, 2000). Furthermore, as a home-made product, Kefir presents a low risk of contamination due to its ability to inhibit spoilage microorganisms. Kefir is thus an excellent way of preserving milk.

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CHAPTER 4

COMPARISON OF THE SENSORY PROFILES AND PREFERENCES OF KEFIR AND MAAS

Abstract

Kefir is a traditional-fermented milk commonly manufactured using Kefir grains. In this study Kefir, commercial Maas and laboratory Maas were evaluated for their sensory properties by a trained panel using descriptive analysis with scaling and the data analysed by analysis of variance (ANOVA) for significance. The main differences between the three products were found in their "sourness", "creaminess" and "smoothness" ($p < 0.001$), "effervescence" ($p < 0.01$), as well as "yeasty" and "cowy" tastes ($p < 0.05$). The differences found were ascribed to the unique yeast-lactic acid fermentation that occurs during Kefir production, in contrast to the lactic acid fermentation that occurs during Maas production, as well as to the added flavourants, colourants and other food additives in commercial Maas.

The effect of different incubation temperatures (25°, 30° and 35°C) on the sensorial properties of Kefir was also investigated using a trained panel and descriptive analysis with scaling. It was found that "sourness" and "creaminess" of the Kefir increased with increase in incubation temperature. The different incubation temperatures did not affect the sensory properties of Kefir unfavourably as no strong off-flavours developed.

Kefir and commercial Maas were tested for preference by a panel consisting of 50 young African urbanites and it was found that commercial Maas was significantly preferred to Kefir. Kefir and laboratory Maas were tested for preference by a panel consisting of 89 adult Africans and in this case no significant preference was found. This suggests that adult Africans, whose traditions are still preserved, might be the appropriate starting target market for Kefir.

Kefir and laboratory Maas were also tested for preference by a wider panel consisting of 371 people representing the different population groups. There was no

significant preference as well as no significant difference between the preference of Kefir and laboratory Maas between the different population groups. A statistical probability chart for two-tailed tests was used to determine significance in all paired preference tests. Although this study did not indicate if White and Coloured panellists actually/specifically "liked" Kefir or Maas, the possibility for the marketing of Kefir as a "natural" product with numerous health benefits, does exist.

Introduction

Kefir is a traditional fermented milk that originated in the Caucasian Mountains (Duitschaever, 1989) and is commonly manufactured by fermenting milk with Kefir grains (Kwak *et al.*, 1996). These grains have a structure similar to tiny florets of cauliflower, which may vary in size from 0.3 to 3.5 cm diameter and contain several organisms that co-exist in a symbiotic association. These organisms are responsible for a lactic acid-alcoholic fermentation which gives Kefir its typical flavour (Liu & Moon, 1983; Pintado *et al.*, 1996; Garrote *et al.*, 1998).

Kemp (1984) described the sensory characteristics of high quality Kefir as follows: "It has a pH of about 4.0; a clean, pleasant acid taste without any bitterness (aftertaste); prickling and sparkling of CO_2; a slight taste and aroma of yeast; a smooth texture; altogether a very refreshing beverage". In contrast, Kosikowski (1977) rather described the compositional properties of typical or high-quality Kefir in terms of major fermentation end-products: "approximate pH of 4.4; 0.8% lactic acid; about 0.5 to 1% ethyl alcohol; and sufficient CO_2 content to make the beverage 'fizz and foam' like a beer". Furthermore, Kosikowski (1977) emphasised that Kefir's typical flavour is due mainly to an optimum ratio of 3:1 of diacetyl (*ca.* 3 $mg.l^{-1}$) to acetaldehyde (*ca.* 1 $mg.l^{-1}$).

Maas (*Amasi*) is a traditional fermented milk beverage of the African population of Southern Africa and has been made for many generations by letting raw milk sour (Coetzee, 1982; Keller & Jordaan, 1990). A recent food consumption study conducted in the rural Eastern Cape revealed an average consumption of 1.4 litres of Amasi per day per adult equivalent (M. Nomakaya, 1999, Department of Agricultural Economics, University of Fort Hare, personal communication). One of the direct

results of the urbanisation process is that unpasteurised milk is not as freely available for use in the traditional production of Maas (Dr A.S. Myburgh, 1999, Department of Agricultural Economics, University of Stellenbosch, personal communication). Legislation now stipulates that raw (unpasteurised) milk or raw cream may not be sold unless it is to be used for further processing (Anon., 1997). The production of Maas is not considered as "further processing" (Viall, 1999). Local authorities may apply to be listed to allow the sale of raw milk in their areas if they think they can control the safety of the raw milk. In many cases this is highly unlikely (Anon., 1997; Viall, 1999).

Commercially manufactured Maas is too expensive for most members of low-income communities, to purchase. This results in a situation where urban, low-income African consumers are distanced from a highly nutritional traditional food product. The food industry is thus challenged to produce a product that provides the traditional need for a fermented milk product which is cheap and, especially important, easy to produce. One such a product that could fit this description, is Kefir, since the manufacture is easy and the Kefir grains are re-usable. Thus, the price of Kefir on the market would or should only be slightly higher than that of milk (Steinkraus, 1996).

The aims of this study were, firstly, to compare the sensory properties of Kefir and Maas, to indicate how these products differ according to their specific sensory characteristics. This was done using descriptive analyses with scaling. Secondly, the sensory properties of Kefir produced at different incubation temperatures will be investigated to determine if temperature changes in the standard method of Kefir preparation would result in detectable changes in sensory properties. This was considered important in case consumers' preference indicated that changes in the standard sensory properties of Kefir were necessary to make the product more acceptable. Finally, consumer preference testing will be done to determine if there is a market for Kefir relative to that of the existing commercial product, Maas.

Materials and methods

Milk pasteurisation

Fresh pasteurised milk purchased at local supermarkets was given a further heat treatment in a temperature-controlled waterbath at 83° - 85°C for 20 min and the milk cooled to 4°C. This was done to ensure the destruction of pathogenic and competing spoilage microorganisms that might have survived the commercial pasteurisation.

Grain activation

Frozen Kefir grains (-18°C) were allowed to defrost at room temperature, added to fresh pasteurised milk at 25°C (18 g Kefir grains to 500 ml milk) and incubated at 25°C for 24 h. The grains were then retrieved using a sterilised stainless steel sieve (soaked in 1.25% Milton solution for 30 min, then rinsed with sterile distilled water) and placed directly into fresh pasteurised milk at 25°C for 24 h. This procedure was repeated for three consecutive days before the grains were used to produce Kefir (Schoevers, 2000).

Standard preparation of Kefir

One litre of double pasteurised full cream milk was inoculated with 18 g activated Kefir grains and incubated at 25°C for 18 h. The grains were removed from the Kefir with the sterilised sieve. The fermented Kefir was incubated at 22°C for a further 6 h and cooled to 4°C before sensory evaluation (Schoevers, 2000).

Preparation of Maas starter culture

One litre of double pasteurised full cream milk was inoculated with 0.6 g lypholised commercial starter culture (minimum cell concentration of 5×10^{10} cfu.g^{-1}) containing *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris* (1 – 10%) and *Lactococcus lactis* subsp. *diacetylactis* (5 - 30%) (Darleon, 1997). The starter culture was activated for 8 h at 22°C (Human, 1998).

Standard preparation of laboratory Maas

One litre of double pasteurised full cream milk was inoculated with 10 ml (1%) activated Maas starter culture and incubated at 22°C for 13 h. The finished Maas was cooled to 4°C before sensory evaluation was undertaken.

Commercial Maas

Commercial Maas was purchased from local supermarkets and kept at 4°C for no longer than 2 days before being used in sensory testing. The label on the product indicated that it contained added preservatives, colourants and thickeners.

Experimental Study 1 - Comparison of the sensory profiles of Kefir, commercial Maas and laboratory Maas.

Sensory evaluation method - A panel of eight assessors, experienced and trained in profiling a wide range of foods and beverages, rated the samples. The sensory tests were conducted in a fluorescent-lighted room. Sensory evaluation was done using descriptive analysis with scaling (Larmond, 1982). During preliminary sessions the sensory properties of the product were identified by the trained panel. Samples were prepared to illustrate the different properties so that the panel could agree on the meaning of each term used. During these training sessions the panellists worked together as a group and discussion was encouraged.

The panellists assessed the samples individually. The samples were evaluated for flavour and body (texture). The scorecard that was used is depicted in Fig. 1 (Calefato, 1997, personal communication). A line scale of 100 mm (1 mm = 1 unit) on which only the lowest and highest values for each attribute were indicated, was used. Panellists recorded their evaluation by making a vertical line across the horizontal line at the point that best reflected his/her perception of the magnitude of a specified property. It was assumed that each word phrase on the scale had the same meaning to each panellist.

Panellists were instructed to cleanse their palates with a plain biscuit and tap water before profiling each sample in the order presented (Larmond, 1982). The sensory evaluation was repeated on three separate days.

Panellist:..... **Date:** ..... **Sample:**

A. FLAVOUR

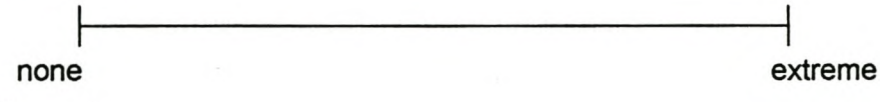
1. **Sourness**

2. **Sweetness**

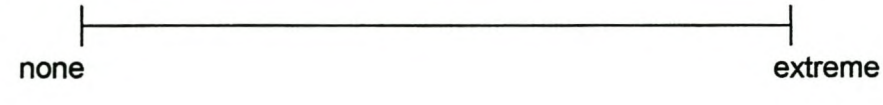
3. **Yoghurt (green apple)**

4. **Buttery (caramel)**

5. **Yeasty (cheesy)**



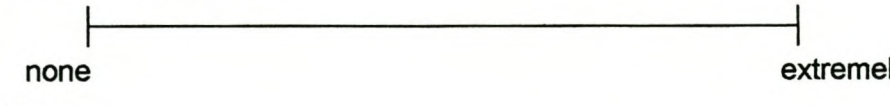
6. **Cowy (barny)**



B. BODY (TEXTURE)

7. **Creaminess**

8. **Effervescence (gassiness)**



9. **Smoothness**

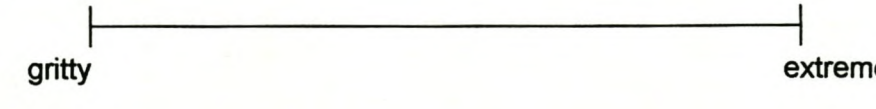


Figure 1. Sensory evaluation form used in descriptive analysis with scaling (J. Calefato, Department of Food Science, University of Stellenbosch, personal communication).

Preparation and sample serving - Three samples were served to the panellists: Kefir made by the standard method (Schoevers, 2000); commercial Maas purchased from a local retailer; and Maas made by the standard method (Human, 1998). The samples were presented to the panellists in random order as 60 ml in small polystyrene cups marked with three-digit random codes. The samples were cooled and served at a temperature of 4°C (Bodyfelt *et al.*, 1988).

Data analysis - The data was analysed by ANOVA to indicate significant differences between the products (Larmond, 1982).

Experimental Study 2 - The effect of different incubation temperatures on the sensory attributes of Kefir.

Sensory evaluation method – The evaluation was done as described in Experimental Study 1.

Preparation and sample serving – Three Kefir samples were prepared using the standard method (Schoevers, 2000) but the incubation temperature was varied and included: Kefir incubated at 22°C for 24 h; Kefir incubated at 25°C for 24 h; and Kefir incubated at 30°C for 24 h. The samples were presented to the panellists in random order as 60 ml in small polystyrene cups marked with three-digit random codes. The samples were cooled and served at a temperature of 4°C (Bodyfelt *et al.*, 1988).

Data analysis - The data was analysed by ANOVA to indicate significant differences between the different treatments (Larmond, 1984).

Experimental Study 3 - Paired preference testing to determine if Kefir or commercial Maas is preferred by young African urbanites.

Sensory evaluation method - A consumer panel of 50 Black pupils (24 males and 26 females) from a local high school, was used. Their ages ranged from 15 – 20 years. They live in "townships" and represent the modern generation Black South African (Morris, 1992). They do not follow the traditional diet of the African people and are,

therefore, not very familiar with traditional Maas. The testing was conducted in a classroom at the local high school.

Sensory evaluation was done using a simple paired preference test (Larmond, 1982). The panellists assessed the samples individually. They were presented with two samples and asked which one they preferred. The scorecard that was used, is depicted in Fig. 2. The instructions were given in English, as well as in their mother language, Xhosa (De Bruin & Minnaar, 1994). The panellists were rewarded with a sweet after the evaluation.

Preparation and sample serving - Two samples were served to the panellists and included Kefir made by the standard method and commercial Maas purchased from a local retailer. Commercial Maas was selected because the panellists were fairly familiar with this form of Maas.

The samples were presented in random order as 60 ml in small polystyrene cups marked with three-digit random codes. The samples were cooled and served at a temperature of 4°C (Bodyfelt *et al.*, 1988).

Data analysis – A statistical probability chart for two-tailed tests was used to determine whether or not a significant number of assessors preferred one product (Meilgaard *et al.*, 1988).

Experimental Study 4 - Paired preference testing to determine if Kefir or Maas is preferred by adult Africans.

Sensory evaluation method - A consumer panel consisting of 89 Africans (78 males and 11 females) from a local farming community, was used. Their ages ranged from 20 - 60 years. They were all seasonal workers who still have homes in the former homelands and follow the traditional diet of the African people (Morris, 1992) and are, therefore, familiar with traditional Maas. The panel had a seemingly low level of literacy which means that most of them were unable to read or write (De Bruin & Minnaar, 1994). The testing was conducted at Rhodes Fruit Farms, Groot Drakenstein.

Age: _____

Gender: Male / Female

Does your family buy amasi? Yes / No

_____ _____

Please taste the two samples in the order presented, from left to right.
Circle the number of the sample that you prefer.
Khawuve la masi aphambi kwakho ukusuka ekhohlo ukuya ekunene. Yenza isangqa kuloo masi uwathanda kakhulu.

Comment: .....

.....

Figure 2. Sensory evaluation form used in simple paired preference testing with young Africans.

Sensory evaluation was done using a simple paired preference test (Larmond, 1982). The panellists assessed the samples individually. The panellists were presented with two samples and asked which one they preferred. No scorecard was used. Interpreters were used to instruct them in their mother language, Xhosa, and to generally assist them (De Bruin & Minnaar, 1994). The consumer panel members indicated their favourite by marking the cup with a provided sticker. The panellists were rewarded with a sweet after the evaluation.

Preparation and sample serving - Two samples were served to panellists: Kefir and Maas made by the standard methods. Laboratory Maas was selected because it does not differ substantially from traditional Maas in comparison to commercial Maas that contains preservatives, thickeners and colourants. The panellists were familiar with traditional Maas (Coetzee, 1982).

The samples were presented to the panellists in random order as 60 ml in small polystyrene cups marked with three-digit random codes. The samples were cooled and served at a temperature of 4°C (Bodyfelt *et al.*, 1988).

Data analysis – A statistical probability chart for two-tailed tests was used to determine whether or not a significant number of assessors preferred one product (Meilgaard *et al.*, 1988).

Experimental Study 5 - Paired preference testing to determine if Kefir or Maas is preferred by different population groups.

Sensory evaluation method - A consumer panel consisting of 371 subjects of different population groups (149 White, 179 Coloured and 43 Black) was used. Their ages ranged from 16 – 50 years. The majority of the panellists were younger than 25. All the panellists were able to read and write English and had reached at least the final school level. The testing was conducted at an open day for students and their parents at the local university.

Sensory evaluation was done using a simple paired preference test (Larmond, 1982) and the panellists assessed the samples individually. The panellists were presented with two samples and asked which one they preferred. The scorecard that

was used, is similar to the one depicted in Fig. 2. The instructions were given only in English and the panellists were rewarded with a sweet after the evaluation.

Preparation and sample serving - Two samples were served to the panellists: Kefir and Maas made by the standard methods. Laboratory Maas was again selected because it does not differ substantially from traditional Maas. It contained no preservatives, thickeners or colourants.

The samples were presented to the panellists in random order as 60 ml in small polystyrene cups marked with three-digit random codes. The samples were cooled and served at a temperature of 4°C (Bodyfelt *et al.*, 1988).

Data analysis – A statistical probability chart for two-tailed tests was used to determine whether or not a significant number of assessors preferred one product (Meilgaard *et al.*, 1988).

Results and discussion

Experimental Study 1 - Comparison of the sensory profiles of Kefir, commercial Maas and laboratory Maas.

In this study Kefir, commercial Maas and laboratory Maas were compared as to their sensory characteristics. This was done to characterise the main differences between these products. A trained panel rated the samples.

The key differences between the products are illustrated by the star charts shown in Fig. 3 and the ANOVA data obtained, is given in Table 1. Of the nine variables tested, the ANOVA showed significant differences in the "yeasty (cheesy)" and "cowy (barny)" tastes at $p < 0.05$ (5% level), "effervescence" at $p < 0.01$ (1% level) and "sourness", "creaminess" and "smoothness" of the products at $p < 0.001$ (0.1% level). The panellists perceived no significant differences ($p > 0.05$) in the "sweetness", "green apple (yoghurt)" and "buttery (caramel)" tastes between the products. The panellists showed no significant difference in their mean scores, except for "sourness" ($p < 0.01$).

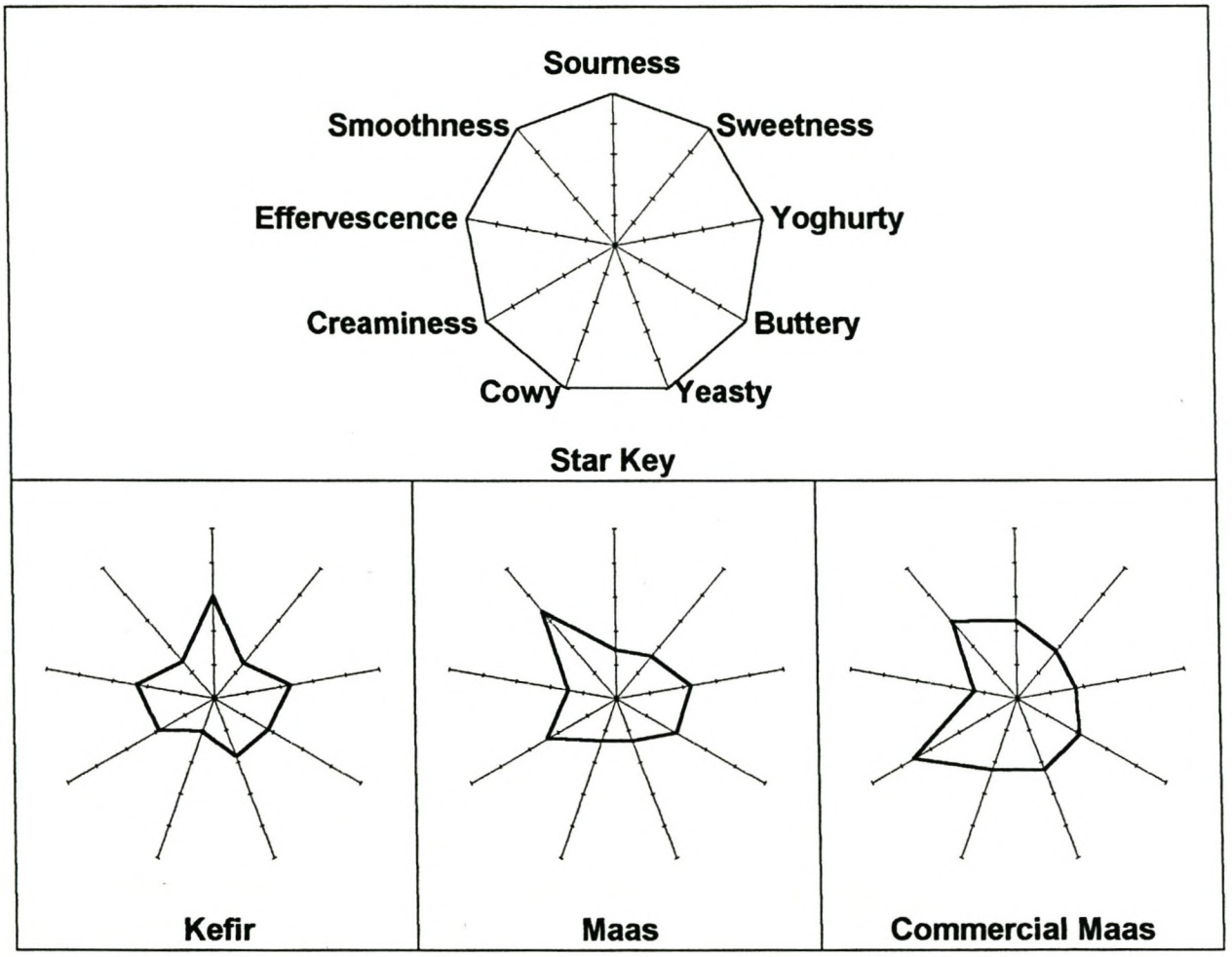


Figure. 3 Star charts of the different categories of fermented milks.

Table 1. Analyses of variance for sensory characteristics of Kefir, commercial Maas and laboratory Maas (8 panellists).

Source of variability	SS[a]	df[a]	MS[a]	F[a]	At 5% level		At 1% level		At 0.1% level	
					P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]
Sourness										
Panellists	2957.21	7	422.46	4.76	0.006411	2.76	0.006411*	4.28	0.006411	7.08
Products	4026.89	2	2013.44	22.67	0.000041	3.74	0.000041	6.51	0.000041	11.78*
Error	1243.42	14	88.82							
Total	8227.51	23								
Sweetness										
Panellists	1189.47	7	169.92	1.98	0.13	2.76	0.13	4.28	0.13	7.08
Products	353.50	2	176.75	2.06	0.16	3.74	0.16	6.51	0.16	11.78
Error	1198.98	14	85.64							
Total	2741.944	23								
Yoghurt (green apple)										
Panellists	585.98	7	83.71	0.52	0.81	2.76	0.81	4.28	0.81	7.08
Products	547.60	2	273.80	1.70	0.22	3.74	0.22	6.51	0.22	11.78
Error	2252.90	14	160.92							
Total	3386.48	23								

*level of significant difference

[a]SS = sum of squares, df = degrees of freedom, MS = mean square, F = variance ratio, F_{crit} = the critical value of F, P-value = significance level

Table 1. Cont.

Source of variability	SS[a]	df[a]	MS[a]	F[a]	At 5% level		At 1% level		At 0.1% level	
					P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]
Buttery (caramel)										
Panellists	2378.55	7	339.79	1.25	0.34	2.76	0.34	4.28	0.34	7.08
Products	120.00	2	60.01	0.22	0.80	3.74	0.80	6.51	0.80	11.78
Error	3796.61	14	271.19							
Total	6295.16	23								
Yeasty (cheesy)										
Panellists	1071.36	7	153.05	0.96	0.49	2.76	0.49	4.28	0.49	7.08
Products	1312.17	2	656.09	4.13	0.04*	3.74	0.04	6.51	0.04	11.78
Error	2221.52	14	158.68							
Total	4605.05	23								
Cowy (barny)										
Panellists	2155.46	7	307.92	0.95	0.501	2.76	0.501	4.28	0.501	7.08
Products	2556.38	2	1278.19	3.95	0.044*	3.74	0.044	6.51	0.044	11.78
Error	4525.80	14	323.27							
Total	9237.63	23								

*level of significant difference

[a]SS = sum of squares, df = degrees of freedom, MS = mean square, F = variance ratio, F_{crit} = the critical value of F, P-value = significance level

Table 1. Cont.

Source of variability	SS[a]	df[a]	MS[a]	F[a]	At 5% level		At 1% level		At 0.1% level	
					P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]
Creaminess										
Panellists	1518.11	7	216.87	1.41	0.28730	2.76	0.28730	4.28	0.28730	7.08
Products	4912.56	2	2456.28	15.93	0.00025	3.74	0.00025	6.51	0.00025*	11.78
Error	2158.11	14	154.15							
Total	8588.77	23								
Effervescence (gassiness)										
Panellists	1943.83	7	277.69	1.91	0.1429	2.76	0.1429	4.28	0.1429	7.08
Products	1902.18	2	951.09	6.55	0.0098	3.74	0.0098*	6.51	0.0098	11.78
Error	2032.32	14	145.17							
Total	5878.33	23								
Smoothness										
Panellists	2481.86	7	354.55	1.75	0.17682	2.76	0.17682	4.28	0.17682	7.08
Products	6772.92	2	3386.46	16.70	0.00020	3.74	0.00020	6.51	0.00020*	11.78
Error	2838.43	14	202.74							
Total	12093.21	23								

*level of significant difference

[a]SS = sum of squares, df = degrees of freedom, MS = mean square, F = variance ratio, F_{crit} = the critical value of F, P-value = significance level

Kefir was judged as more acid (sour) than laboratory and commercial Maas (Fig. 3). The "yeasty" and "cowy" tastes of commercial Maas were more pronounced than that of Kefir and laboratory Maas. Kefir was the most effervescent of the three products and this was ascribed to the yeast-lactic acid fermentation that takes place in Kefir. Maas was judged as smoother than Kefir while commercial Maas was judged as the creamiest of the products. This characteristic can probably be attributed to the added thickener.

The traditional African product, Maas, is thus smoother and creamier than Kefir, with a less sour taste and less effervescence. The specific sensory properties of Kefir can, however, be slightly changed by: variation in the starter cultures used for Kefir production (Duitschaever *et al.*, 1988); the heat treatment of the milk (Mann, 1979; Marshall, 1993; Merin & Rosenthal, 1986); the starter concentration used (Garrote *et al.*, 1998); the fermentation temperature; and shortening or lengthening the fermentation time (Liu & Moon, 1983; Koroleva, 1988). The possibility of easily changing the main characteristics will be an important marketing factor if sensory studies indicate that changes in the taste of Kefir are needed to make it more acceptable for a certain selected target market.

Experimental Study 2 - The effect of different incubation temperatures on the sensory attributes of Kefir.

In any South African household the room temperature may vary considerably during a 24 h period. This is especially the case for the households of the low-income communities. This wide variation in temperature would also influence the storage of most perishable foodstuffs. This study was thus undertaken to determine if changes in the incubation temperature would result in detectable changes in the sensory properties of Kefir. A trained panel was used to rate the Kefir produced using the different temperature treatments.

The key differences between the sensory characteristics of the Kefir prepared using the three different treatments are illustrated as star charts and are shown in Fig. 4. The ANOVA for the data are given in Table 2. Of the nine variables evaluated, the ANOVA showed that the "creaminess" of the Kefir samples was significantly different at $p < 0.01$ (1% level). The "sourness" of the Kefir samples differed significantly at $p < 0.001$ (0.1% level) and the "sourness" and "creaminess" of

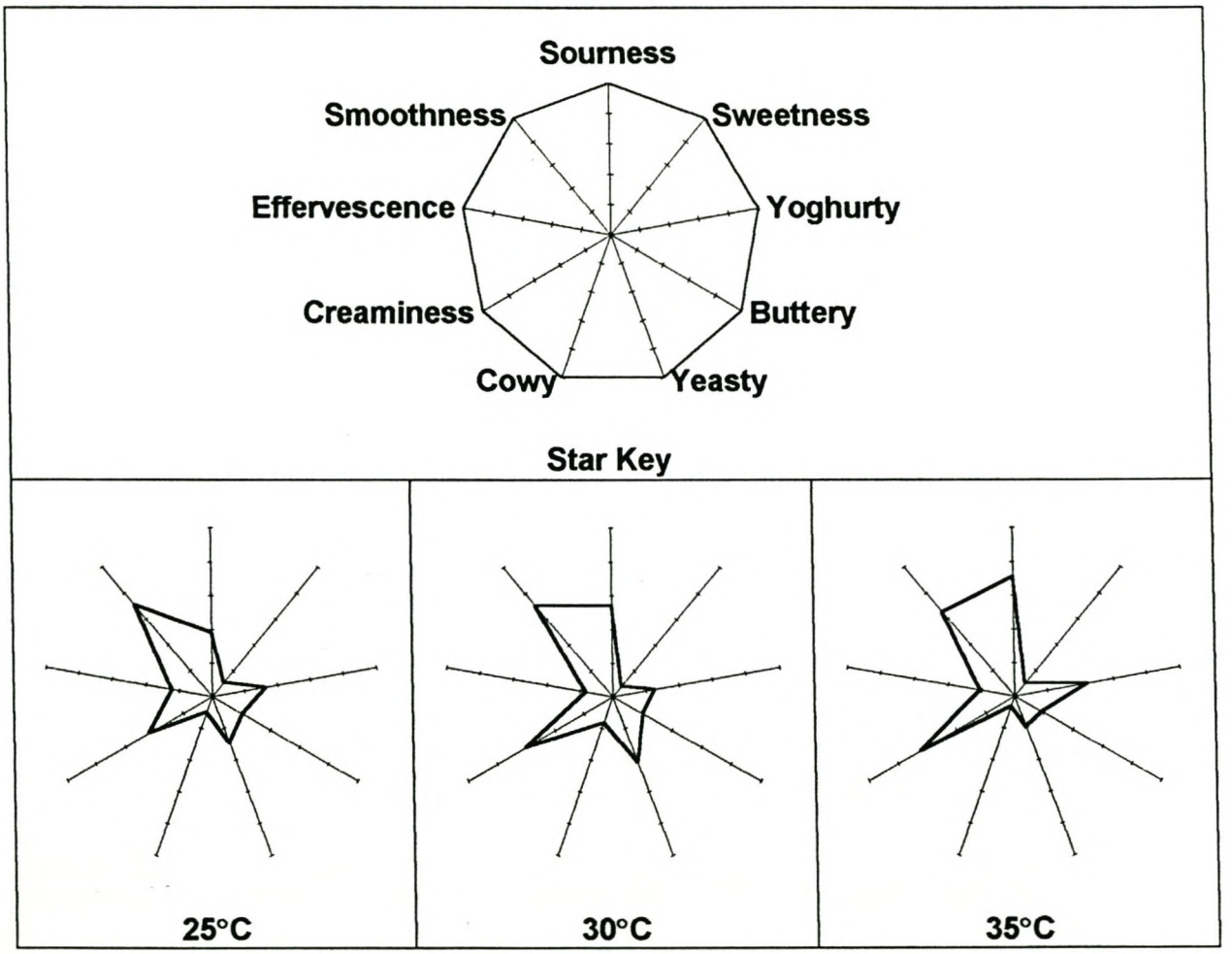


Figure. 4 Star charts of Kefir produced at different temperatures.

Table 2. Analyses of variance for sensory characteristics of Kefir produced at 25°, 30° and 35°C (8 panellists).

Source of variability	SS[a]	df[a]	MS[a]	F[a]	At 5% level		At 1% level		At 0.1% level	
					P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]
Sourness										
Panellists	8448.63	7	1206.95	8.82	0.00032	2.76	0.00032	4.28	0.00032*	7.08
Treatments	4072.02	2	2036.01	14.88	0.00034	3.74	0.00034	6.51	0.00034*	11.78
Error	1915.81	14	136.84							
Total	14436.46	23								
Sweetness										
Panellists	2409.67	7	344.24	5.33	0.0039	2.76	0.0039*	4.28	0.0039	7.08
Treatments	204.75	2	102.38	1.59	0.2938	3.74	0.2394	6.51	0.2394	11.78
Error	903.58	14	64.54							
Total	3518.00	23								
Yoghurt (green apple)										
Panellists	4827.33	7	689.62	5.99	0.0022	2.76	0.0022*	4.28	0.002249	7.08
Treatments	780.19	2	390.09	3.39	0.0630	3.74	0.0634	6.51	0.063048	11.78
Error	1611.48	14	115.11							
Total	7219.00	23								

*level of significant difference

[a]SS = sum of squares, df = degrees of freedom, MS = mean square, F = variance ratio, F_{crit} = the critical value of F, P-value = significance level

Table 2. Cont.

Source of variability	SS[a]	df[a]	MS[a]	F[a]	At 5% level		At 1% level		At 0.1% level	
					P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]
Buttery (caramel)										
Panellists	7687.29	7	1098.19	5.03	0.01	2.76	0.01*	4.28	0.01	7.08
Treatments	733.69	2	366.84	1.68	0.22	3.74	0.22	6.51	0.22	11.78
Error	3057.65	14	218.40							
Total	11478.63	23								
Yeasty (cheesy)										
Panellists	9593.16	7	1370.45	5.72	0.0028	2.76	0.0028*	4.28	0.0028	7.08
Treatments	298.08	2	149.042	0.62	0.5508	3.74	0.5508	6.51	0.5508	11.78
Error	3352.25	14	239.45							
Total	13243.49	23								
Cowy (barny)										
Panellists	4984.24	7	712.03	2.14	0.11	2.76	0.11	4.28	0.106525	7.08
Treatments	1631.31	2	815.66	2.45	0.12	3.74	0.12	6.51	0.121936	11.78
Error	4651.85	14	332.28							
Total	11267.41	23								

*level of significant difference

[a]SS = sum of squares, df = degrees of freedom, MS = mean square, F = variance ratio, F_{crit} = the critical value of F, P-value = significance level

Table 2. Cont.

Source of variability	SS[a]	df[a]	MS[a]	F[a]	At 5% level		At 1% level		At 0.1% level	
					P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]	P-value[a]	Fcrit[a]
Creaminess										
Panellists	5656.07	7	808.01	5.06	0.0049	2.76	0.0049*	4.28	0.0049	7.08
Treatments	2283.58	2	1141.79	7.16	0.0072	3.74	0.0072*	6.51	0.0072	11.78
Error	2233.58	14	159.54							
Total	10173.24	23								
Effervescence (gassiness)										
Panellists	8559.00	7	1222.71	6.69	0.0013	2.76	0.0013*	4.28	0.0013	7.08
Treatments	209.08	2	104.54	0.57	0.5771	3.74	0.5771	6.51	0.5771	11.78
Error	2558.75	14	182.77							
Total	11326.83	23								
Smoothness										
Panellists	3616.63	7	516.66	5.01	0.0051	2.76	0.0051*	4.28	0.0051	7.08
Treatments	129.00	2	64.50	0.63	0.5495	3.74	0.5495	6.51	0.5495	11.78
Error	1444.50	14	103.18							
Total	5190.13	23								

*level of significant difference

[a]SS = sum of squares, df = degrees of freedom, MS = mean square, F = variance ratio, F_{crit} = the critical value of F, P-value = significance level

the Kefir samples increased with increase in incubation temperature from 25° to 30° and to 35°C (Fig. 4). There was no significant difference ($p > 0.05$) in the mean scores for the other sensory attributes ("sweetness", "yoghurt (green apple)", "buttery (caramel)", "yeasty (cheesy)", "cowy (barny)", "effervescence (gassiness)" and "smoothness") that were evaluated. At elevated temperatures (30° - 35°C) the growth of both the heterofermentative lactococci and the yeasts in Kefir, is inhibited. The growth of the other lactic acid bacteria is probably enhanced, which results in a Kefir with high acidity and a low ethanol concentration. The recommended incubation temperature for Kefir production is 25°C so as to achieve optimum flavour and consistency (Liu & Moon, 1983; Koroleva, 1988). In this study the panellists showed significant differences in their mean scores at $p < 0.05$ for all the sensory attributes. Differences were found at the 1% level for "sweetness", "yoghurt (green apple)", "buttery (caramel)", "yeasty (cheesy)", "creaminess", "effervescence (gassiness)" and "smoothness" and at the 0.1% level for "sourness" (Table 2).

Except for a variation ($p < 0.01$) in "sourness" and "creaminess", the different incubation temperatures did not affect the sensory properties of Kefir unfavourably. For example, no strong off-flavours such as a pronounced "yoghurt (green apple)", "buttery (caramel)", "yeasty (cheesy)" or "cowy (barny)" taste developed. A variation in room temperature when Kefir is made at home, should thus not result in huge taste variations with the exception of "sourness" and "creaminess".

Experimental Study 3 - Paired preference testing to determine if Kefir or commercial Maas is preferred by young African urbanites.

Maas is a traditional fermented milk beverage of the African population (Coetzee, 1982). Due to certain constraints (unavailability of unpasteurised milk in urban areas and high price of commercial Maas) urban, low-income African consumers are, however, distanced from this product. Kefir has the potential to fill this gap as it can be produced from pasteurised milk in contrast to traditional Maas. Kefir can also be produced at a cost lower than that of commercial Maas as Kefir grains are re-usable (Marshall, 1993; Steinkraus, 1996). In commercial Maas production an industrial starter culture has to be added to each batch leading to an increase in the cost. This study was done using 50 African school learners in an urban environment to determine if young Africans prefer Kefir or commercial Maas. If

Kefir were to be marketed to Africans, commercial Maas would be its main competitor.

The results for the paired preference testing are summarised in Table 3. Of the 50 young Africans 41 or 82% preferred commercial Maas to Kefir. Eighty-eight percent of females and 75% of males preferred Maas to Kefir. A question on their families' buying behaviour (Fig. 2) revealed that ninety-six percent of the subjects' families have purchased commercial Maas upon occasion. The young Africans were thus familiar with this product. This was surprising, as commercial Maas is expensive, although it can also be argued that there is no alternative product on the market to satisfy their need for a fermented milk product.

To investigate the significance of this data the Roessler Table for Paired Preference Tests (two tailed) (Stone & Sidel, 1993), was consulted. In this table the minimum number of agreeing judgements necessary to establish significance at various probability levels for the paired-preference test, are tabled. The data from this study showed that commercial Maas was significantly preferred to Kefir by young African males at $p = 0.05$ (18 agreeing judgements at $n = 24$) and by young African females at $p < 0.001$ (more than 21 agreeing judgements at $n = 26$). For the total number of young African tasters, the preference of commercial Maas to Kefir was significant at $p < 0.001$ (more than 37 agreeing judgements at $n = 50$).

As Kefir is a "new" product with which young Africans are totally unfamiliar, it can be assumed that they will initially regard this product with suspicion. In contrast commercial Maas is a well-known product to them, with the implication that they will immediately have recognised and selected it at the expense of the unflavoured "new" product. It is possible that they might have grown accustomed rather to the added flavourants, colourants and other food additives in commercial Maas than to the characteristic traditional Maas flavour. To be able to afford commercial Maas may also enhance their status in their community. One can only speculate if they would buy Kefir in the place of commercial Maas if both were commercially available. Kefir has numerous built-in benefits that enhance its commercial value and, as mentioned previously, it can be prepared at home from pasteurised or raw milk with grains that are re-usable. With subsequent use of the grains, the production price of Kefir will decrease, making it much cheaper than commercial Maas. Kefir also enhances the

Table 3. Paired preference testing of Kefir and commercial Maas by young Africans.

Product	Number of tasters			Percentage of tasters		
	Male	Female	Total	Male	Female	Total
Kefir	6	3	9	12	6	18
Maas	18	23	41	36	46	82
Total	24	26	50	48	52	100

user's health as it has numerous health benefits (Saloff-Coste, 1996; Buttriss, 1997) and possesses an inhibitory activity towards potential pathogens and spoilage organisms that may occur in milk (Gupta *et al.*, 1996; Garrote *et al.*, 2000).

Experimental Study 4 - Paired preference testing to determine if Kefir or Maas is preferred by adult Africans.

In rural areas, substantial amounts of traditional Maas are consumed by the African population of South Africa (M. Nomakaya, 1999, Department of Agricultural Economics, University of Fort Hare, personal communication). Maas, therefore, fills an important position in the African diet. The African population of South Africa is, however, urbanising at an accelerating rate (Myburgh, 1995) and the implication of this is an ever-growing low-income urban African consumer market. Urbanised Africans do not have easy access to unpasteurised (raw) milk to produce their own Maas and commercially manufactured Maas is too expensive for most members of low-income communities. This results in a situation where urban, low-income African consumers are distanced from a highly nutritional, traditional food product. Kefir, however, can easily be produced at home at a cost lower than that of commercial Maas as Kefir grains are re-usable (Marshall, 1993; Steinkraus, 1996). With subsequent use of the grains the production price of Kefir decreases, making it much cheaper than commercial Maas. In commercial Maas production an industrial starter culture has to be added to each batch, increasing the cost while packaging and transportation further increase the price of commercial Maas.

This study was done to determine how Kefir compares to laboratory Maas, which is comparable to traditional Maas, in preference testing by 89 adult Africans. The samples were tasted by seasonal workers who still keep homes in rural areas and, therefore, still have traditional taste preferences, such as that for traditional Maas. The results for the paired preference testing are summarised in Table 4. Of the 89 adult Africans who tasted the products, 50 (or 56.1%) preferred laboratory Maas to Kefir. It was also found that 55% of females and 56% of males preferred Maas to Kefir.

To investigate the significance of this data the Roessler Table for Paired Preference Tests (two tailed) (Stone & Sidel, 1993), was consulted. In this table the minimum number of agreeing judgements necessary to establish significance at

Table 4. Paired preference testing of Kefir and laboratory Maas by older Africans.

Product	Number of tasters			Percentage of tasters		
	Male	Female	Total	Male	Female	Total
Kefir	34	5	39	43.6	45.5	43.8
Maas	44	6	50	56.4	54.5	56.2
Total	78	11	89	87.6	12.4	100

various probability levels for the paired-preference test, are tabled. The data showed that Kefir and laboratory Maas were preferred equally by males at $p > 0.05$ (less than 49 agreeing judgements at $n = 78$, according to the Roessler Table), by females at $p > 0.05$ (less than 10 agreeing judgements at $n = 11$, according to the Roessler Table) and by the total number of subjects at $p > 0.05$ (less than 55 agreeing judgements at $n = 89$, according to the Roessler Table). It was concluded that no significant preference exists and thus that adult Africans prefer Kefir and laboratory Maas equally.

One can assume, therefore, that Kefir and traditional Maas are comparable in taste. Both these products contained no added flavourants, colourants and other food additives, in contrast to the commercial product. If Kefir is to be marketed commercially, adult Africans, who still value their traditional eating culture, would be a logical target market for this product. Kefir would not be competing with traditional Maas as urban Africans do not have easy access to this product, for reasons previously mentioned.

Experimental Study 5 - Paired preference testing to determine if Kefir or laboratory Maas is preferred by different population groups.

This study was done to determine if there is a difference in the preference for Kefir or traditional Maas between the different population groups in South Africa. The value of this study is principally academic as Maas is not a traditional foodstuff of White or Coloured South Africans. Although the panellists had to make a 'forced' choice in preference for Kefir or Maas, the White and Coloured panellists do not necessarily 'like' the products, as their 'liking' for Kefir or Maas was not measured in this study.

The results for the paired preference testing are summarised in Table 5. Of the 371 people (40% White, 48% Coloured and 12% African) who tasted the products, 207 (or 56%) preferred Kefir to laboratory Maas. It was found that 54% of the White panellists, 58% of the Coloured panellists and 56% of the African panellists preferred Kefir to laboratory Maas.

To investigate the significance of this data the Roessler Table for Paired Preference Tests (two tailed) (Stone & Sidel, 1993), was consulted. In this table the

Table 5. Paired preference testing of Kefir and laboratory Maas by different population groups.

Product	Number of tasters				Percentage of tasters			
	White	Coloured	African	Total	White	Coloured	African	Total
Kefir	80	103	24	207	53.7	57.5	55.8	55.8
Maas	69	76	19	164	46.3	42.5	44.2	44.2
Total	149	179	43	371	40.2	48.2	11.6	100

minimum number of agreeing judgements necessary to establish significance at various probability levels for the paired-preference test, are tabled. According to this table there is no significant difference ($p > 0.05$) between the preference of Kefir and laboratory Maas between the different population groups. The panellists thus preferred Kefir and laboratory Maas equally.

A general discussion with the panellists indicated that none of the tasters were familiar with Kefir. Primarily the African panellists were familiar with Maas. The African panellists (between the ages of 18 and 25) preferred Kefir and laboratory Maas equally. This study, once again, proved that, although Kefir is a "new" product almost totally unknown to South Africans, it is comparable to Maas in preference.

Conclusions

In this study the main sensorial differences between Kefir, Maas and commercial Maas were identified by trained panellists. Kefir was found to be more sour than laboratory and commercial Maas while the "yeasty" and "cowy" tastes of commercial Maas were more pronounced than that of Kefir and laboratory Maas. Kefir is the most effervescent of the three products. Maas is generally smoother than Kefir. The differences found between Kefir and Maas can mainly be ascribed to the unique yeast-lactic acid fermentation that occurs during Kefir production, in contrast to the lactic acid fermentation that occurs during Maas production. Commercial Maas is the creamiest of the products. The specific properties of commercial Maas can probably be ascribed to the added flavourants, colourants and other food additives.

The effect of different incubation temperatures on the sensory attributes of Kefir was also studied. It was found that the "sourness" and "creaminess" of Kefir increase with increase in incubation temperature from 25° to 35°C. This is probably due to the growth promotion of different groups of Kefir microorganisms at the different temperatures (Liu & Moon, 1983; Koroleva, 1988). The different incubation temperatures did not affect the sensory properties of Kefir unfavourably as no strong off-flavours developed. A variation in room temperature when Kefir is made at home would thus not result in huge taste variations with the exception of "sourness" and "creaminess". The preferred "sourness" and "creaminess" for Kefir might be

achieved by varying the fermentation time when the incubation temperature is either too high or too low. It would be of interest in future to study the effect on the taste of Kefir when the incubation temperature fluctuates during the Kefir making process as it will be difficult to maintain a constant temperature when Kefir is made at home, especially in the informal housing of low-income African communities.

The data clearly showed that commercial Maas is preferred to Kefir by young African urbanites. This could be attributed to the fact that this is the only Maas product they are familiar with and accustomed to and that this was the first contact they had made with Kefir. Older Africans, who have not yet adopted the modern "township" culture, however, showed no significant preference for laboratory (representing traditional) Maas over Kefir. This suggests that adult Africans, whose traditions are still preserved, might be the appropriate starting target market for Kefir. It is interesting to note that there was no significant difference in the preference for Kefir or Maas among the different population groups. Although this study did not indicate if White and Coloured panellists actually/specifically "liked" Kefir or Maas, the possibility for the marketing of Kefir as a "natural" product with numerous health benefits, does exist. This presents an opportunity for further research in the sensory preferences of White and Coloured persons. In such a study it would be appropriate to compare the sensory profiles and preferences of Kefir and a product such as natural yoghurt.

Although Kefir was not significantly preferred to Maas in any of the studies, the commercial advantages Kefir has over Maas, could give it a marketing edge. Kefir can be made from pasteurised or raw milk, in contrast to traditional Maas, which can only be produced from raw milk. Due to legislation (Anon., 1997) raw milk is not freely available to the public. Pasteurised milk, however, can be purchased at any food store. Kefir can be made at a cost slightly higher than that of milk from grains that are re-usable. With subsequent use of the grains the cost of Kefir will decrease, making it much cheaper than commercial Maas. Kefir is a "natural" product with no additives, it enhances the user's health as it has numerous health benefits (Saloff-Coste, 1996; Buttriss, 1997) and it possesses an inhibitory activity towards potential pathogens and spoilage organisms that may occur in milk (Gupta *et al.*, 1996; Garrote *et al.*, 2000). Most importantly, the taste of Kefir is comparable to that of

traditional Maas, making it an appropriate substitute for a product that is in demand but currently unavailable in urban areas.

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CHAPTER 5

ARGUMENTS SUPPORTING KEFIR MARKETING TO THE LOW-INCOME URBAN AFRICAN POPULATION IN SOUTH AFRICA

Abstract

Low-income urban African communities in South Africa are demanding low-cost products, mainly because of their extremely low income. Although they are urbanised, these communities' traditional rural food consumption behaviour is still present. Traditionally Africans consume large volumes of sour milk or Maas. The low-income urban African is, however, deprived of this product due to numerous factors, resulting in nutritional shortages in the diet. Therefore, the demand exists in low income urban African communities for a low cost fermented milk product with high nutritional value, which is safe to consume and which is comparable in taste to traditional Maas. Kefir, a fermented milk of Caucasian origin, has the ability to satisfy these needs.

Introduction

The ultimate objective of economic activity is the satisfaction of human needs, therefore, the nature of such needs will direct economic activity. Food consumption behaviour should, for instance, provide important guidelines for food production, marketing activities and government intervention.

Accepting that a consumer market segment is determined by *inter alia* income and food tradition and subsequent consumer tastes and preferences, it is clear that different income and cultural groups fall into different market segments. The low-income urban African consumer market is unique in its characteristics and needs and consequently, the dairy consumption behaviour of this market warrants special attention.

Commercial dairy products in South Africa have traditionally been developed and produced for sophisticated and affluent consumers. Both the price and the technology (including processing, packaging, storage and distribution) make these products unsuited to the majority of South Africa's population with their extremely low purchasing power and their specific living conditions. For this market, low cost products have to be produced with the help of low cost technologies. According to Bachmann (1987), the characteristics of low cost products include the following: manufacturing with relatively simple equipment; good shelf-life under natural climatic conditions; no need for expensive packaging; the provision of essential nutritional elements; and complementation to the traditional local diet.

Several factors deprive the low-income urban consumer market of their traditional fermented milk drink, Maas. Kefir, a fermented milk drink of Caucasian origin, has certain properties that make it suitable for this market, as well as conforming to the definition (Bachmann, 1987) for a low cost product. In this chapter the case for Kefir marketing to the low-income urban African population in South Africa, is argued.

Dairy product consumption behaviour

On the rural scene

Milk is a favourite foodstuff in all traditional southern African cultures (Coetzee, 1982). Although milk is a very nutritious product, it spoils quickly and it is generally recognised that after 5 – 6 h raw milk will sour or start fermenting (Marshall, 1987). The lack of refrigeration and hygienic facilities has forced the rural African population to keep milk in its least perishable form, namely as curd. It is, therefore, not surprising that many communities acquired a taste for "sour milk" and that, with time, techniques were developed to ensure that the process of souring (fermentation) followed a particular traditional pattern (Tamime & Robinson, 1988).

Spontaneously fermented milk is the most common dairy product in Africa. In rural African communities it is an old tradition that herd boys milk the cows during the course of a day and when the wooden milking pails are full (which could take several hours), the milk is poured into calabashes or leather milk sacks to curdle.

Traditional Maas (called *Amasi* by the Zulu and Xhosa and *Mafi* by the Sotho) was and still is made in clay pots and calabashes. The calabashes have wooden stoppers and whey is drawn off through a hole in the bottom of the calabash (Coetzee, 1982). The basic method is still a batch add-and-withdraw technique and milk is periodically added to the containers. The bacteria on the surface of the containers serve as the starter culture for the traditionally produced Maas (Keller & Jordaan, 1990).

Africans use Maas as a whole meal or as part of a meal for breakfast, lunch or dinner (Joubert & De Lange, 1992). The creamy fraction of Maas separates into lumps of a cheesy mass called "*Ingqaka*" and when Maas is ready for consumption, it is either drunk as it is or mixed with maize meal crumbs ("*Umphokoqo*"). When mixed together with "*Umphokoqo*" the dish is called "*Umvubo*" or "African salad". A recent food consumption study conducted in the rural areas of Eastern Cape province revealed an average consumption of 1.4 litres of Maas per day per adult equivalent (M. Nomakaya, 1999, Department of Agricultural Economics, University of Fort Hare, 1999, personal communication).

An important consequence of the traditional usage of "sour" milk by Africans through the ages was the evolutional development of the phenomenon of lactose intolerance under Africans. Lactose intolerance is the inability of individuals to digest the lactose in milk which is due to a lack of the enzyme ß-D-galactosidase in the gut (Buttriss, 1997). It is estimated that 70% of adult Africans in Africa have this deficiency. In South Africa, an estimated 87% of Zulu's, 65% of Sothos, 82% of Xhosas and 86% of Shangaans have a low concentration of ß-D-galactosidase (Joubert & De Lange, 1992). Such lactose intolerant individuals experience gastrointestinal symptoms when consuming fresh milk and then as a result tend to avoid milk and other dairy products. This has important implications for the dairy industry as well as for human nutrition. Milk is an excellent source of calcium and other nutrients such as vitamin B_{12}, riboflavin and phosphorus, as well as some of the essential amino acids. A high proportion of lactose-intolerant individuals is, therefore, malnourished, especially with regard to calcium and a relationship between lactase deficiency and osteoporosis has been reported (Shah, 1993). People who are lactose-intolerant are, however, able to drink fermented milks due to the

presence of microbial β-galactosidase and subsequent lower lactose levels (Shanani & Chandan, 1979; Shah, 1993; Buttriss, 1997).

On the urban scene

The abolishment of the designated area laws of the former South African apartheid legislation caused an abnormally high rate of urbanisation, especially under the African population. This high rate of urbanisation was further assisted by South Africa's high population growth. The mass urbanisation over the past twelve years, in particular of the low-income households from the rural areas of the former self-governing areas and the TBVC-states, has caused enormous instant urban residential areas, mainly in the form of squatter areas and informal settlements in and around almost every town and city in South African (Myburgh, 1999). An estimated 1 million people are urbanised per year in South Africa (Britz, 1999) and today approximately half of South African Africans live in cities (more than 15 million people according to the 1996 census). In an era of insufficient economic growth, this rapid urbanisation has led to high urban unemployment and subsequently, the establishment of large communities of urban poor.

Rural traditions and culture regarding food consumption are still present among these urban low-income African communities. The high population density and geographic and economic reservedness of these communities preserve this culture and traditions (Myburgh, 1995). However, due to several constraints, urban low-income communities are often unable to follow their traditional African diet.

One of the direct outcomes of the urbanisation process is that unpasteurised milk is not as freely available as in rural areas for use in the traditional production of Maas (Dr. A.S. Myburgh, 1999, Department of Agricultural Economics, University of Stellenbosch, personal communication). Legislation now stipulates that raw (unpasteurised) milk or raw cream may not be sold unless it is to be used for further processing (Anon., 1997). The production of Maas is not considered as "further processing" (Viall, 1999). Local authorities may apply to be listed to allow the sale of raw milk in their areas if they can control the safety of the raw milk but, in many cases, this is highly unlikely. According to legislation, the herds of cattle farmers who wish to sell unpasteurised milk must annually be certified by a veterinarian to be free of tuberculosis and brucellosis and the farmers must register with their local

authorities. Farmers who sell unpasteurised milk are legally obliged to have their milk regularly tested for the presence of antibiotics or other antimicrobial substances, pathogenic organisms, coliform bacteria, *Escherichia coli*, somatic cells as well as the viable bacterial count (Anon., 1997).

In informal settlements there are individuals with their own cows who, regardless of legislation, still sell unpasteurised milk or Maas, without any proper certification, to their local communities. These small farmers usually have small herds (3 - 19 cows per farmer) that they often keep in their backyards in residential areas or give free wander in the informal settlements, which in itself creates an environmental health problem. The milking is done by hand twice a day, the product sieved through a 'clean' cloth and poured into 25 litre plastic or stainless steel containers. Cooling facilities are a problem and the temperature of the milk may vary between 10° and 35°C. The bulk of the milk is sold 'as is' for household use and the rest is 'soured' to produce traditional Maas. The customers supply their own containers and the product is scooped from the 25 litre holding tanks (H. Schrader, 2000, Cape Metropolitan Council, personal communication). Concern is generally expressed over health, hygiene and environmental hazards resulting from this practice, and not without reason.

A study, conducted by the Cape Metropolitan Council into the quality of Maas produced by 35 small farmers within informal settlements in the metropolis, revealed a total viable cell count of more than 5×10^4 cfu. ml^{-1} in 25 (30%) of the 84 samples tested. Seventy-eight (93%) of the samples contained more than 20 coliforms per ml and 32% of the samples tested positive for presence of *E. coli* (H. Schrader, 2000, Cape Metropolitan Council, personal communication). These results clearly indicate there is reason for concern regarding the health risks in selling this type of Maas to low-income urban communities. According to legislation "raw milk that has become sour" may not be sold when it gives a standard plate count of more than 5×10^4 cfu.ml^{-1} of the product, if it contains more than 20 coliform bacteria per ml or if it is found to contain any *E. coli* in 1 ml of fluid (Anon., 1997).

Africans that belong to the lower income group and who live in informal settlements and rural areas are prevented from buying commercial Maas and fresh milk for the following reasons: the absence of refrigeration in the dwellings and *spazas*; extremely low disposable income; early departure time of workers from their

homes to the workplace and late arrival from their workplace; shortage in transport facilities; and a lack of proper distribution of fresh milk in African townships (Myburgh, 1995). Commercial Maas is also a poor equivalent of the traditional variety as it contains colourants, thickeners and preservatives (Berry, 1999). These factors lead to a situation where urban, low-income African consumers are distanced from a highly nutritional traditional product.

With urbanisation, the consumption of dairy products by Africans has decreased substantially and has had certain impacts on the nutritional status of low-income urban Africans. The BRISK study, conducted in the Cape Peninsula in 1994 to evaluate the dietary intake pattern in the urban African population (Bourne *et al.*, 1994), revealed a very low milk intake of less than 200 ml per adult per day. The recommended milk intake per adult per day is 400 ml, which is required in order to meet calcium needs. This quantity provides 476 mg calcium or just over half the recommended dietary allowance (RDA) of 800 mg for an adult per day (the balance coming from the rest of the diet). The intake of other dairy products was negligible. As much as 42% of the subjects consulted during the study reported consuming no dairy products during a 24 h recall period. The inadequate milk consumption by urban Africans was reflected by a too low intake of micronutrients such as calcium, zinc and riboflavin, and low levels of riboflavin in the blood, which can again lead to nutrition-related diseases (Langenhoven *et al.*, 1995).

A problem and opportunity identified

Low-income urban African consumers are prevented from making their own traditional Maas such as they did whilst still living in rural areas. The quality of the traditional Maas they can buy in urban areas is questionable and may pose a serious health risk. They are unable or do not want to buy commercial Maas and abstain from consuming non-fermented milk products due to a high level of lactose intolerance. Subsequently, a too low intake of dairy products results and nutritional deficiency diseases follow. Such a situation would certainly threaten food security in low-income urban settlements.

There is definitely a huge demand in low-income urban African communities for a low cost fermented milk product with high nutritional value, which is safe to consume and which is comparable in taste to traditional Maas. Such a product that satisfies these needs, is Kefir.

What is Kefir?

Kefir is a traditional fermented milk that originated in the Caucasian Mountains in Russia (Duitschaever, 1989) and is commonly manufactured by fermenting milk with Kefir grains (Kwak *et al.*, 1996). These grains have a structure similar to tiny florets of cauliflower, which may vary in size from 0.3 to 3.5 cm diameter and contains several organisms that co-exist in a symbiotic association. These organisms are responsible for a lactic acid-alcoholic fermentation which gives Kefir its typical flavour that can be described as mildly alcoholic, yeasty-sour, with a tangy effervescence (Liu & Moon, 1983; Duitschaever, 1989; Pintado *et al.*, 1996; Garrote *et al.*, 1998). The grains are formed during the process of making Kefir and as far as is known, only from existing grains (Steinkraus, 1996). These grains are generally known to the public in South Africa as a "joghurtplantjie" (yoghurt plant) (Keller & Jordaan, 1990).

Kefir is still manufactured in Russia and Europe under a variety of names, such as Kephir, Kiaphur, Kefer, Knapon, Kepi, and Kippi (Kwak *et al.*, 1996). It is also popular in Eastern European countries and is produced in small quantities in Czechoslovakia, Poland, Sweden, Finland as well as in Germany, Greece, Austria, Brazil and Israel (Koroleva, 1988; Libudzisz & Piatkiewicz, 1990). It is currently available in the United States and its popularity is growing in Japan (Saloff-Coste, 1996). Numerous overseas companies sell Kefir grains over the Internet (Anon., 2000). Neither Kefir, nor Kefir grains are as yet marketed in South Africa, creating an excellent opportunity to launch these products locally.

Characteristics that make Kefir suitable for the low-income urban African market

Ease of preparation

Kefir is sufficiently easy to produce at home. It requires no more facilities than what is normally found in a low-income family's kitchen. Approximately 18 g of Kefir grains are placed in 1 litre of milk in a clean container (Schoevers, 2000). This mixture is then incubated at room temperature for approximately 24 h or until the desired consistency is reached. The Kefir is strained through a clean sieve or cloth into a bowl to retrieve the Kefir grains, which can immediately be used to ferment the next batch of milk or be stored in a cool place (Schoevers, 2000).

Kefir can be made using milk with 3.2, 2.5, 1.0% (m/v) or no fat (Koroleva, 1988). The milk can be obtained from ewes, goats, mares or cows (Kneifel & Mayer, 1991) and either raw or pasteurised milk can be used for Kefir manufacture (Marshall, 1993). This is a particular important point in favour of Kefir, as high-quality traditional Maas cannot be produced from pasteurised milk. If pasteurised milk, however, is used to produce Maas, putrefaction sets in before fermentation (due to the loss of natural lactic acid bacteria), resulting in a product with a putrid taste and aroma. If one considers the fact that health authorities for obvious health reasons discourage the sale of unpasteurised milk making it almost impossible for urban Africans to obtain, Kefir manufacture has a differential advantage over Maas in this regard. Kefir can even be made using UHT-treated milk or powdered milk (Merin & Rosenthal, 1986).

In any South African household the room temperature may vary considerably during a 24 h period. This is especially the case for the households of the low-income communities. Sensory studies, as shown in Chapter 4 of this thesis, indicated that a variation in room temperature when Kefir is made at home would not result in huge taste variations or the development of any strong off-flavours. It will, however, have a slight effect on the "sourness" and "creaminess". It was found in the previous study that the "sourness" and "creaminess" of Kefir increases with increase in incubation temperature from 25° - 35°C, due to the growth promotion of different groups of Kefir microorganisms at the different temperatures.

The specific sensory properties of Kefir can be slightly changed by: variation in the starter cultures used for Kefir production (Duitschaever *et al.*, 1988); the heat treatment of the milk (Mann, 1979; Marshall, 1993; Merin & Rosenthal, 1986); the starter concentration used (Garrote *et al.*, 1998; Schoevers, 2000); the fermentation temperature; and shortening or lengthening of the fermentation time (Liu & Moon, 1983; Koroleva, 1988). The possibility of easily changing the main characteristics, as was shown in Chapter 4 of this thesis, will be an important marketing factor if sensory studies indicate that changes in the taste of Kefir are needed to make it more acceptable for a certain selected target market.

Acceptability by lactose-intolerant individuals

A number of reports have shown that lactose malabsorbers can consume, without harmful effects, certain fermented dairy products, of which Kefir (like Maas) is one (Roginski, 1988; Shah, 1993). The most likely explanation for an improved tolerance of lactose when it is consumed as part of Kefir is the presence of microbial ß-galactosidase derived from the bacterial starter cultures used in fermented milk production, which like intestinal lactase, can break down lactose to its component sugars (Buttriss, 1997). Another theory proposed by Gurr (1987) states that cultured products, because of their acidity and the consequent finer dispersion of protein in the stomach, retard the emptying of the stomach's contents into the small intestine. Any capacity to break down lactose, whether it be of microbial or indigenous origin, would then have a longer period to take effect and consequently lactose digestion would theoretically be more efficient, even when the specific activity of the enzyme is low (Gurr, 1987). The lactose concentration of Kefir (ca. 4%) is also lower than that of milk (ca. 4.7%). This is due to the metabolic activity of the lactic acid bacteria that occurs naturally as part of Kefir grains (Shah, 1993).

Nutritional value

Fermented milk products are just as nutritious as raw milk and in some ways even more so and have longer shelf-life stability than most other liquid milk products. The nutrient composition of Kefir is similar to that of milk with Kefir containing more vitamin B_1, B_2 and folic acid (Roginski, 1988; Libudzisz & Piatkiewicz, 1990). Propionibacteria can even be added to Kefir grains to increase the vitamin B_{12}

concentration (Cerna & Grabova, 1997; J. van Wyk, 2000, Department of Food Science, University of Stellenbosch, personal communication). The concentrations of lactic acid, galactose, free amino acids and fatty acids are also increased as a result of the Kefir fermentation process (Gurr, 1987). The fermentation process has little effect on the mineral content of milk (Buttriss, 1997).

Packaging, distribution and storage

Kefir grains can be successfully preserved by a variety of techniques, such as air-drying, freeze-drying, cold storage and freezing (A. Cilliers, 2000, Department of Food Science, University of Stellenbosch, personal communication). The freeze-dried Kefir grains can also be successfully packaged in a variety of plastic films (A. Cilliers, 2000, Department of Food Science, University of Stellenbosch, personal communication) and the distribution of Kefir grains to low-income urban consumers, therefore, will pose no problem.

The packaging and distribution of Kefir itself may prove more complicated. The carbon dioxide that forms in Kefir as a result of the yeast-lactic acid fermentation may cause "bulging" of containers. Appropriate containers, which can withstand the escaping gas pressure or allow for the CO_2 to escape, should be used. Kefir can be kept for 8 – 19 days at refrigeration temperatures (Roginski, 1988).

Food retailing in informal settlements and squatter areas is unique to South Africa. It takes place exclusively through a large number of geographically dispersed informal traders dealing from informal structures, known as "shops" or "spazas" who, in turn, do their purchases from wholesalers and "cash and carry" outlets such as Metro and Makro that are situated nearby on the outskirts of the townships (Myburgh, 1996). These informal traders and communities have cooling and storage constraints and freeze-dried Kefir grains will thus be perfect to distribute through these channels. Kefir also has keeping-ability at room temperature and does not need urgent cooling as pasteurised milk would.

Price

Since the manufacturing of Kefir is simple and Kefir grains reusable, the cost of making Kefir would only be the price of the milk purchased and the initial acquisition of the Kefir grains. In contrast, commercially manufactured Maas is fairly

expensive. The retail cost of a 500 ml carton or plastic bottle is approximately R 3.65 (November 2000 price, Shoprite). Fifty percent of the people of the informal communities are unemployed and have an extremely low income and food purchasing makes out the largest part of the household budget (Myburgh, 1995). They can, therefore, not afford commercial Maas, eliminating commercial Maas as a product competitive to Kefir.

Inhibitory activity against potential spoilage and pathogenic organisms

Studies have indicated that Kefir possesses an antimicrobial activity against a wide variety of Gram-positive and negative bacteria, as well as some fungi (Saloff-Coste, 1996; Garrote *et al.*, 2000). In Chapter 3 of this thesis, the inhibitory activity of Kefir towards certain spoilage microorganisms and potential pathogens that may occur in milk was studied. The test organisms used in this study included strains of: *Escherichia coli*; *Staphylococcus aureus*; *Bacillus cereus*; *Listeria monocytogenes* and *Clostridium tyrobutyricum.* These test organisms (10^3 - 10^4 cfu.ml^{-1}) were inoculated into pasteurised milk together with Kefir grains (18 g.l^{-1}). No Kefir grains but only test organisms, were added to the control milk samples. In Fig. 1, for example, the survival of *E. coli* in Kefir and in milk, are shown. *Escherichia coli* was selected because of the general use of this bacterium in the food industry as an indicator of food microbial quality and safety and as indicative of faecal contamination. The *E. coli* in the Kefir samples showed total growth inhibition after 16 h of incubation, when compared to the growth of *E. coli* in control milk samples. The same pattern was observed for all the test organisms. It can be concluded from this that Kefir does have a strong inhibitory effect on the growth of certain microorganisms that may cause spoilage of milk or, more importantly, human diseases. According to Khedkar *et al.* (1991), studies on factors affecting the viability of potential spoilage and pathogenic microorganisms in fermented milks, have indicated that at the beginning of fermentation the decrease in growth of these organisms is probably due to antimicrobial compounds, peroxide and decrease in redox-potential. Later on, the low pH, the presence of organic acids and perhaps diacetyl contribute to the inhibition of potential spoilage and pathogenic microorganisms in fermented milks.

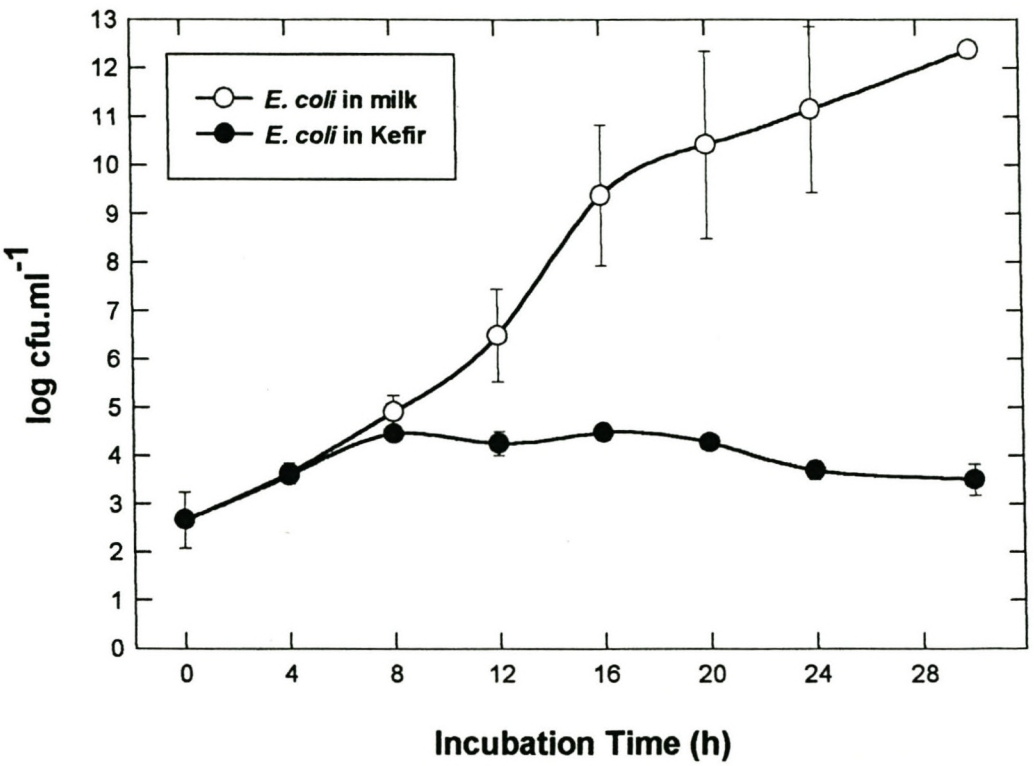

Figure 1. The viable counts of *Escherichia coli* in Kefir and in milk.

As Kefir grains possess an inhibitory activity towards certain pathogens that may occur in either raw or inferior pasteurised milk, the grains have the ability to make milk safer to consume. Considering the inhibitory effect of Kefir towards *E. coli*, a Gram-negative bacterium usually isolated from faeces, Kefir could be potentially considered as a probiotic. If gut colonisation with Kefir grain organisms could be achieved, resulting acid production could inhibit colonisation with pathogenic microorganisms, protecting the host against food-borne diseases. Furthermore, as a home-made product, Kefir presents a low risk of putrefaction due to its ability to inhibit spoilage microorganisms. Kefir is thus an excellent way of preserving milk, especially when refrigeration facilities are not available.

Taste

Trained panel evaluation - Kefir, commercial Maas and Maas prepared in the laboratory with a commercial culture ("laboratory Maas") were sensory evaluated by a trained panel using descriptive analysis with scaling (Chapter 4 of this thesis). This was done to characterise the main differences between these products. The key differences between the products are illustrated by the star charts shown in Fig. 2. Kefir was found to be more sour than laboratory and commercial Maas while the "yeasty" and "cowy" tastes of commercial Maas were more pronounced than that of Kefir and laboratory Maas. Kefir was the most effervescent of the three products and Maas was generally smoother than Kefir. Commercial Maas was the creamiest of the products. The differences found between Kefir and Maas can mainly be ascribed to the unique yeast-lactic acid fermentation that occurs during Kefir production, in contrast to the lactic acid fermentation that occurs during Maas production. The specific properties of commercial Maas can probably be ascribed to the added flavourants, colourants and other food additives. The specific sensory properties of Kefir can, however, be slightly changed if required by variation in: the starter culture used for Kefir production (Duitschaever *et al.*, 1988); the heat treatment of the milk (Mann, 1979; Marshall, 1993; Merin & Rosenthal, 1986); the starter concentration used (Garrote *et al.*, 1998; Schoevers, 2000); the fermentation temperature (Chapter 4 of this thesis); and the fermentation time (Liu & Moon, 1983; Koroleva, 1988).

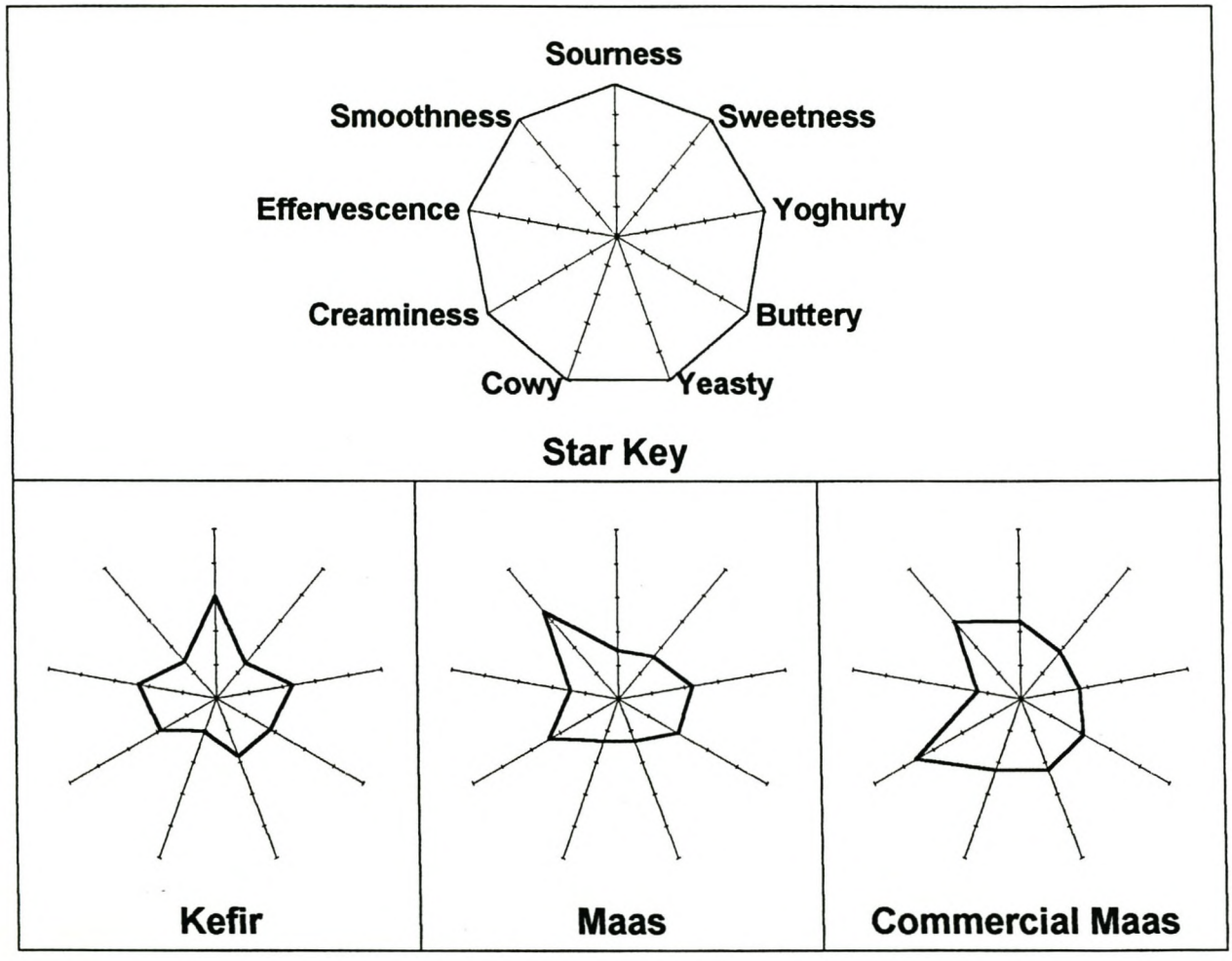

Figure 2. The key differences between Kefir, Maas and Commercial Maas.

Consumer panel evaluation – Paired preference studies were done with Kefir (an unknown product to South African consumers), commercial Maas and laboratory Maas by consumer panels consisting of panellists of different ages and population groups to indicate if one of these products is significantly preferred by the specific panels (Chapter 4 of this thesis). A summary of the different experimental studies and results obtained is given in Table 1. To investigate the significance of this data the Roessler Table for Paired Preference Tests (two tailed) (Stone & Sidel, 1993) was consulted. In this table the minimum number of agreeing judgements necessary to establish significance at various probability levels for the paired-preference test, are tabled.

The first study was done using 50 African school learners in an urban environment to determine if young African urbanites prefer Kefir or commercial Maas. If Kefir were to be marketed to Africans, commercial Maas would be the main competitor. Of the 50 young Africans 41 or 82% preferred commercial Maas to Kefir. Eighty-eight percent of females and 75% of males preferred Maas to Kefir. The data from this study showed that commercial Maas was significantly preferred to Kefir by young African males at $p = 0.05$ (18 agreeing judgements at $n = 24$) and by young African females at $p < 0.001$ (more than 21 agreeing judgements at $n = 26$). For the total number of young African tasters, the preference of commercial Maas to Kefir was significant at $p < 0.001$ (more than 37 agreeing judgements at $n = 50$). A question on their families' buying behaviour revealed that ninety-six percent of the subjects' families have purchased commercial Maas upon occasion. The young Africans were thus familiar with this product. This was surprising, as commercial Maas is expensive, although it can also be argued that there is no alternative product on the market to satisfy their need for a fermented milk product. It can be concluded that commercial Maas is preferred to Kefir by young Africans living in "townships." This could be attributed to the fact that this is the only Maas product they are familiar with and accustomed to and that this was the first contact they had made with Kefir.

The second study was done using 89 adult Africans to determine if they prefer laboratory Maas, which is comparable to either traditional Maas or Kefir. The panel consisted of seasonal workers who still keep homes in rural areas and, therefore, still have traditional taste preferences such as that for traditional Maas. Of the 89 adult Africans who tasted the products, 50 (or 56.1%) preferred laboratory Maas to Kefir. It

Table 1. Paired preference testing of Kefir, commercial Maas and laboratory Maas by different panels.

Experimental Study	Description of panel	Total number of tasters	Samples tested	Number of tasters preferring sample	Percentage of tasters preferring sample
1	Young Africans	50	Kefir	9	18
			Commercial Maas	41	82
2	Adult Africans	89	Kefir	39	43.8
			Laboratory Maas	50	56.2
3	Different population groups	371	Kefir	207	55.8
			Laboratory Maas	164	44.2

was also found that 55% of females and 56% of males preferred Maas to Kefir. The data from this study showed that Kefir and laboratory Maas were preferred equally by males at $p > 0.05$ (less than 49 agreeing judgements at $n = 78$, according to the Roessler Table), by females at $p > 0.05$ (less than 10 agreeing judgements at $n = 11$, according to the Roessler Table) and by the total number of subjects at $p > 0.05$ (less than 55 agreeing judgements at $n = 89$, according to the Roessler Table). It was concluded that no significant preference exists and thus that adult Africans prefer Kefir and laboratory Maas equally.

One can assume, therefore, that Kefir and traditional Maas are comparable in taste. Both these products contained no added flavourants, colourants and other food additives, in contrast to the commercial product. If Kefir is to be marketed commercially, adult Africans, who still value their traditional eating culture, would be a logical target market for this product. Kefir would not be competing with traditional Maas as urban Africans do not have easy access to this product for reasons mentioned earlier in this chapter.

In a third study Kefir and laboratory Maas were evaluated to determine if there is a difference in the preference for Kefir or traditional Maas between the different population groups in South Africa. Of the 371 people (40% White, 48% Coloured and 12% African) who tasted the products, 207 (or 56%) preferred Kefir to laboratory Maas. It was found that 54% of the White panellists, 58% of the Coloured panellists and 56% of the African panellists preferred Kefir to laboratory Maas. According to the Roessler Table there is no significant difference ($p > 0.05$) between the preference of Kefir and laboratory Maas between the different population groups. The panellists thus preferred Kefir and laboratory Maas equally.

A general discussion with the panellists indicated that the tasters were not familiar with Kefir, but the African panellists in this study were familiar with Maas. This study, once again, proved that, although Kefir is a "new" product almost totally unknown to South Africans, it is comparable to Maas in preference. The taste of Kefir is comparable to that of traditional Maas, making it an appropriate substitute for a product that is in demand but currently unavailable in urban areas.

Conclusions

Kefir has various differential advantages to commercial and traditional Maas. It can easily be made from pasteurised or raw milk, in contrast to traditional Maas, which can only be produced from raw milk. As mentioned earlier in this Chapter good-quality raw milk is not freely available to the public. Pasteurised milk, however, is readily available in the market at consumer level. Kefir can be made at a cost slightly higher than that of milk from Kefir grains that are re-usable. With subsequent use of the grains, the cost of Kefir will decrease, making it much cheaper than commercial Maas. The distribution of Kefir grains on large scale does not present any problems.

Kefir is a "natural" product with no additives, and it also enhances the user's health as it has numerous health benefits (Saloff-Coste, 1996; Buttriss, 1997) and it can be tolerated by lactose-intolerant individuals. As Kefir grains possess an inhibitory activity towards certain spoilage organisms and health-threatening pathogens that may occur in either raw or inferior pasteurised milk, it has the ability to make milk safer to consume and lengthen the shelf-life. Sensory studies indicated that the taste of Kefir is comparable to that of traditional Maas, making it an appropriate substitute for a product that is in demand but currently unavailable in urban areas.

In view of al these factors, Kefir and Kefir grains are suitable low-cost products for marketing to the low-income urban African consumer market.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Background

The rapid urbanisation of rural Africans has led to the establishment of large communities of urban poor. Due to their high population density the Africans' traditional customs and eating behaviour are still present (Myburgh, 1995). Maas, a traditional fermented milk made from unpasteurised milk, plays an important part in their daily diet as it provides all the nutrients found in fresh milk. Several factors such as legislation and subsequent unavailability of good-quality unpasteurised milk, are, however, preventing low-income urban Africans from making Maas. Their low income also often prevents them from purchasing commercial Maas. This situation may result in incidences of malnutrition in these communities (Bourne *et al.*, 1994). It can thus be concluded that there is definitely a large demand by low-income urban African communities for a low cost fermented milk product with high nutritional value, which is safe to consume and which is comparable in taste to traditional Maas. Such a product, with the ability to satisfy these needs, is Kefir.

Inhibitory activity of Kefir

The quality of unpasteurised/pasteurised milk that is available in low-income urban communities is often inferior, resulting in serious threats to the health of consumers. Due to a lack of proper refrigeration facilities, shop owners and consumers in these communities are unable to keep dairy products from spoiling. The safety and spoilage rate of dairy products available in low-income communities, therefore, presents problems.

The antimicrobial activity of Kefir organisms *in vitro* against a wide variety of Gram-positive and negative bacteria, as well as some fungi have been reported (Saloff-Coste, 1996; Garrote *et al.*, 2000). However, information on the antagonistic activity of Kefir organisms was limited and warranted further study. The inhibitory

activity of Kefir on the survival of some common spoilage organisms and foodborne pathogens was, therefore, studied. The organisms that were evaluated included strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Clostridium tyrobutyricum*. Growth of all the test organisms was strongly inhibited (≥99.9%) in Kefir over a 24 h incubation period. As this is the time usually required to prepare Kefir, this inhibitory quality of Kefir is regarded as a key element in favour of Kefir marketing. Pathogens and spoilage organisms that may occur in milk will, therefore, not multiply in Kefir and might even be eliminated, making Kefir much safer to consume than non-fermented dairy products and also increasing its shelf-life. This has obvious benefits for the low-income urban consumer.

Sensory testing

A question that inevitably arises when the commercialisation of a "new" product is undertaken is whether or not the product is acceptable to the proposed target market. Kefir can be regarded as a "new" product as it is virtually unknown in South Africa and studies to determine if consumers will accept it are fundamental. In the market Kefir will have to compete with Maas warranting comparative sensory testing of Kefir and Maas.

In the first study a trained panel using descriptive analysis determined the specific sensory properties of Kefir, Maas prepared in the laboratory and, therefore, not containing any flavourants, colourants and thickeners, and commercial Maas. The main differences in Kefir and Maas were thus determined. In a second study the ease of changing the sensory properties of Kefir was studied by variation of the incubation temperature. This was also done to determine the effect of changes in room temperature during home Kefir production on the taste of Kefir. It was found that the "sourness" and "creaminess" of Kefir increases with increase in incubation temperature from 25° to 35°C, but no strong off-flavours developed.

Consumer preference testing was done to determine if Kefir, commercial Maas or laboratory Maas was significantly preferred to one another by panels consisting of panellists of different ages and population groups. The data obtained clearly showed that commercial Maas was preferred to Kefir by young African urbanites. Adult

Africans who have not yet adopted the modern "township" culture, however, preferred Kefir and laboratory Maas (representing traditional Maas) equally, identifying this segment of the African population as the appropriate starting target market for Kefir.

'The champagne of fermented milks'

Kefir's unique refreshing taste sets it apart from other fermented milk products. The current challenge to the dairy industry is to find a way to benefit from this and all the other amazing qualities that Kefir offers. These include Kefir's incredible health benefits (Saloff-Coste, 1996; Buttriss, 1997), its simplistic production technology, its good keeping quality and its similarity to Maas.

The main factors responsible for the microbial inhibitory activity that Kefir exhibits will have to be further investigated to determine the precise nature. This might highlight other uses for Kefir and even lead to the discovery of other health benefits.

As Kefir is so advantageous to the user's health (Buttriss, 1997) the promotion of Kefir not only to the lower-income community but also as an upmarket health product with probiotic qualities presents ample opportunity for entrepreneurs. Sensory testing with Kefir flavoured with fruit, for example, presents much opportunity for further research.

The marketing of Kefir or Kefir grains to low-income urban African communities presents, without doubt, a wonderful opportunity. However, the parties opting to commercialise Kefir grains will have to do test marketing to determine if low-income African consumers will have the capability of producing their own Kefir. The distribution of Kefir grains to low-income communities as a part of a community upliftment project can prove highly successful as Kefir not only has the ability to enhance the nutritional status of low-income Africans, but also to increase food safety in these communities. This alone is enough reason to start popping the champagne corks!

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