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A thesis submitted in partial fulfillment of the requirements for the degree of M
Science in Food Science and Technology of the University of Nairobi.

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2004

## DECLARATION

I declare that this is my original work and that it has not been presented for a degree in any other university.

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This thesis has been submitted with our approval as University supervisors.

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## DEDICATION

To my Heavenly Father, Almighty God, "in whom I live and move and have my being"

and

my earthly father, Professor Bill Lore, for his unfailing love and support.

*Everything comes from God alone.*
*Everything lives by his power,*
*and everything is for his glory.*

## ACKNOWLEDGEMENTS

I thank my supervisors, Prof SK Mbugua and Dr J Wangoh, for their guidance and invaluable advice in the course of my work and in the write-up of this thesis.

I also express my deep gratitude to the German Academic Exchange Service (DAAD) for granting me a scholarship and providing the funds necessary for the execution of the research work.

I extend my appreciation to the local authorities in Isiolo District, namely the District Officer I, Mr J Rotich, and the District Officer, Central Division, Mr M Mathioya, for their logistical assistance during the preliminary studies in Isiolo.

## TABLE OF CONTENTS


Page

DECLARATION ..... I

DEDICATION ..... II

ACKNOWLEDGEMENTS ..... III

LIST OF FIGURES ..... VIII

LIST OF ABBREVIATIONS ..... IX

ABSTRACT ..... X

CHAPTER 1: INTRODUCTION ..... 1

1.1. Background to the study ..... 1
1.2 Problem statement and study justification ..... 2
1.3 Expected beneficiaries ..... 4
1.4 Overall objective ..... 4
1.5 Specific objectives ..... 4

CHAPTER 2: LITERATURE REVIEW ..... 5

2.1 The camel ..... 5
2.2 Composition of camel milk ..... 7
2.3 Traditional fermented camel milk ..... 8
2.4 Microflora of other traditional fermented milk products ..... 9
*2.4.1 Lactic fermentations ..... 9*
2.4.1.1 Yoghurt ..... 9
2.4.1.2 Bulgarian milk ..... 10
2.4.1.3 *Leben, dahi*, and related products ..... 10
2.4.1.4 *Mursik* ..... 11
2.4.1.5 *Kule naoto* ..... 12
2.4.1.6 *Nono* ..... 12
2.4.1.7 *Ergo* ..... 12
*2.4.2 Yeast-lactic fermentations ..... 13*
2.4.2.1 *Kefir* ..... 13
2.4.2.2 *Koumiss* ..... 14
*2.4.3 Mold-lactic fermentations ..... 14*
2.4.3.1 *Viili* ..... 14
2.5 Starter cultures in fermented milk production ..... 15
*2.5.1 Functions of starter microorganisms in fermented dairy products ..... 16*
*2.5.2 Classification of LAB starter microorganisms in fermented milks ..... 17*
*2.5.3 Yeast starter microorganisms in fermented milks ..... 20*

CHAPTER 3: STUDY DESIGN AND METHODOLOGY ..... 21

3.1 Investigation on the traditional process of suusac fermentation ..... 21
3.2 Laboratory simulated method for suusac fermentation ..... 21
3.3 Analytical studies ..... 22
*3.3.1 Microbial analysis ..... 22*

3.3.1.1 Materials ..... 22
Camel milk ..... 22
Cow milk ..... 22
Laboratory chemicals and microbiological media ..... 22
API® microbiological identification kits ..... 22
3.3.1.2 Methods ..... 23
Enumeration of microorganisms ..... 23
Total viable count (TVC) ..... 23
Enumeration of lactic acid bacteria (LAB) ..... 23
Enumeration of yeasts and moulds ..... 23
Enumeration of coliforms ..... 24
*3.3.2 Microbial isolations ..... 24*
3.3.2.1 Isolation of lactobacilli ..... 24
3.3.2.2 Isolation of lactococci ..... 24
3.3.2.3 Isolation of yeasts ..... 25
*3.3.3 Primary classification - LAB ..... 25*
3.3.3.1 Production of carbon dioxide from glucose ..... 25
3.3.3.2 Production of ammonia from arginine ..... 26
3.3.3.3 Growth at 15°C and 45°C ..... 26
*3.3.4 Primary classification - yeasts ..... 27*
3.3.4.1 Colonial characteristics and morphology ..... 27
3.3.4.2 Production of ascospores ..... 27
*3.3.5 Microbial identification ..... 27*
3.3.5.1 Principle for the method ..... 27
3.3.5.2 Method for LAB identification ..... 28
3.3.5.3 Identification of lactic acid bacteria ..... 29
3.3.5.4 Method for yeasts identification ..... 30
3.3.5.5 Data processing for identification of yeasts ..... 32
*3.3.6 Biochemical and physiological characterisation ..... 32*
3.3.6.1 Production of lactic acid ..... 32
3.3.6.2 Production of diacetyl ..... 33
3.3.6.3 Hydrolysis of casein ..... 34
3.3.6.4 Activity in litmus milk ..... 34
*3.3.7 Fermentation trials using isolated single-strain cultures ..... 35*
*3.3.8 Acid and flavour development by mixed cultures ..... 35*
*3.3.9 Proximate composition ..... 36*
3.3.9.1 Determination of total solids ..... 36
3.3.9.2 Determination of butterfat content ..... 36
3.3.9.3 Determination of crude protein content ..... 36
3.3.9.4 Determination of ash ..... 37
3.3.9.5 Determination of lactose ..... 37
*3.3.10 Determination of pH ..... 37*
*3.3.11 Determination of titratable acidity ..... 37*
3.4 Statistical data analysis ..... 37

CHAPTER 4: RESULTS AND DISCUSSION ..... 39

4.1 Traditional production of suusac in Isiolo District ..... 39
4.2 Physico-chemical composition of suusac ..... 43
4.3 Microbial content profile in suusac ..... 45
4.4 Characteristics and identity of microbial isolates in suusac ..... 49
*4.4.1 Identification of lactococci ..... 51*
*4.4.2 Identification of lactobacilli ..... 54*
*4.4.3 Identification of yeasts ..... 56*
4.5 Functional properties of isolates in skim milk ..... 58
4.6 Production of acidity and flavour by single cultures in camel and cow milk ..60

4.7 Production of acidity and flavour by mixed cultures ..... 62
CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS ..... 66
CHAPTER 6: REFERENCES ..... 68
APPENDIX 1: INTERVIEW GUIDE FOR *SUUSAC* PRODUCTION ..... 75

## LIST OF TABLES

**Page**

*Table 2.1: Camel population in Kenya by district* ..... 6

*Table 2.2: Proximate composition of milk of various animal species* ..... 7

*Table 3.1: Composition of API 50 CHL Medium* ..... 29

*Table 3.2: Composition of API 20 C AUX C Medium* ..... 31

*Table 4.1: Average proximate composition (%), pH and total titratable acidity of traditional and laboratory-produced suusac* ..... 44

*Table 4.2: Average proximate composition (%), pH and total titratable acidity of raw cow and camel milk used in laboratory fermentation trials* ..... 44

*Table 4.3: Number of microorganisms in traditional and laboratory-produced suusac* ..... 46

*Table 4.4: Biochemical profiles of isolated lactococci on API 50 CHL test strips* ..... 53

*Table 4.5: Biochemical profiles of isolated lactobacilli on API 50 CHL test strips* ..... 55

*Table 4.6: Biochemical profiles of isolated yeasts on API 20 C AUX test strips* ..... 56

*Table 4.7: Production of acid and flavour by isolated lactic acid bacteria and yeasts* ..... 59

*Table 4.8: Hydrolysis of casein by isolated lactic acid bacteria and yeasts* ..... 60

## LIST OF FIGURES

**Page**

*Figure 3.1: Flow diagram of fermentation with starters isolated from camel milk* ..... 35

*Figure 4.1: Flow diagram of traditional suusac production* ..... 40

*Figure 4.2: Changes in microbial numbers and pH during spontaneous lactic acid fermentation of camel milk* ..... 49

*Figure 4.3: Proportions of lactic acid bacteria and yeast species isolated from traditional suusac* ..... 50

*Figure 4.4: Comparative pH of cow and camel milk after 24 hours' fermentation at 30°C using single strain isolates from camel milk* ..... 61

*Figure 4.5: Comparative diacetyl scores of cow and camel milk after 24 hours' fermentation at 30°C using single strain isolates from camel milk* ..... 62

*Figure 4.6: Comparative changes in pH of camel and cow milk during fermentation using mixed cultures (1:1) of Lactobacillus plantarum (LP), Lactobacillus curvatus (LC) and Candida krusei (CK)* ..... 63

*Figure 4.7: Comparative changes in pH of cow and camel milk during fermentation using mixed cultures (1:1 and 1:1:1) of Lactobacillus plantarum (LP), Lactobacillus curvatus (LC) and Candida krusei (CK)* ..... 63

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AOAC | Association of Official Analytical Chemists |
| BPCL | bromocresol purple chalk lactose |
| cfu | colony forming units |
| IDF | International Dairy Federation |
| LAB | lactic acid bacteria |
| MRS | deMan Rogosa Sharpe |
| PCA | plate count agar |
| PDA | potato dextrose agar |
| TVC | total viable count |
| VRBA | violet red bile agar |

## ABSTRACT

The purpose of the study was to determine the lactic acid bacteria (LAB) and yeasts associated with the traditional fermented camel milk product (*suusac*) of the Somali community in Kenya. The traditional method of *suusac* production was studied by use of questionnaire and documented. The microbial content profile and changes during fermentation were then determined.

From 15 samples of traditionally fermented *suusac*, 45 LAB and 30 yeast strains were isolated and identified using API 50 CHL and API 20C AUX identification systems, respectively. The total viable microorganisms, LAB, coliforms, and yeasts and molds were enumerated. The isolates were investigated for their functional roles in the fermentation process, namely, acidification, flavour/aroma production and proteolytic activity. Fermentation trials with single and mixed strain cultures were investigated to assess their acidification and flavour-producing properties.

The traditional production of *suusac* involves spontaneous fermentation of camel milk in smoked gourds at ambient temperature for 1–2 days. The milk is not subjected to heat treatment prior to fermentation. The isolated LAB species were identified as *Lactobacillus curvatus* (8% of total isolates), *Lactobacillus plantarum* (16%), *Lactobacillus salivarius* (8%), *Lactococcus raffinolactis* (4%) and *Leuconostoc mesenteroides* subsp. *mesenteroides* (24%). The isolated yeasts were *Candida krusei* (20%), *Geotrichum penicillatum* (12%) and *Rhodotorula mucilaginosa* (8%). In traditional *suusac*, LAB counts averaged 6.77 $\log_{10}$cfu/ml, while yeast counts were relatively lower (2.05 $\log_{10}$cfu/ml). Low coliform numbers were encountered (<1 $\log_{10}$cfu/ml).

The LAB produced considerable acidity and majority (60%) were homofermentative. The primary functional role of the LAB was fermentation of lactose to lactic acid, resulting in acidity levels ranging from 0.46–0.67% lactic acid equivalent. All the LAB isolates recorded high proteolytic activity, except for *L. raffinolactis*, which did not exhibit any proteolytic activity. The LAB showed varying degrees of diacetyl production. Of the LAB, *L. curvatus* recorded the highest diacetyl flavour score, corresponding to >30 mg diacetyl/100 ml of milk.

The yeast isolates showed limited carbohydrate-assimilating capabilities, but played a role in flavour development and proteolysis. *G. penicillatum* produced diacetyl (3.1–10 mg/100 ml), although it did not exhibit any proteolytic activity. *C. krusei* exhibited some proteolytic activity, although its diacetyl-producing capacity in camel milk was minimal (0.5–3 mg/100 ml).

*C. krusei* also played a role in mixed starter fermentation of camel milk by increasing the activity of the LAB cultures and improving product flavour. The use of *C. krusei* + *L. plantarum* (1:1) and *C. krusei* + *L. curvatus* (1:1) reduced the fermentation time by half as compared to the use of the cultures individually.

# CHAPTER 1: INTRODUCTION

## *1.1 Background to the study*

In Kenya, 80% of the land mass has been classified as arid and semi-arid lands (ASAL) and the low rainfall experienced in these areas renders them unfavourable for crop production (GoK, 2001). Thus, there is a high dependency on pastoralism here. The main pastoralist communities in Kenya are the Samburu, Somali, Turkana, Rendille, Boran and Gabbra and the livestock reared include cattle, sheep, goats, and camels. It is noteworthy that these arid and semi-arid areas support 25% of Kenya's human population and over 50% of Kenya's total livestock population. For this reason, development of ASAL is crucial for overall national development. In the 5th National Development Plan for the period 1984 - 1988 (GoK, 1988), the integration of the camel in Kenya's livestock development efforts was proposed. Kenya's camel population is about 900,000 and 95% of this number are found in the semi-arid and arid districts of Isiolo, Wajir, Marsabit and Garissa (Wangoh, 1997).

Food preservation in tropical and sub-tropical regions of the world has posed a major challenge from time immemorial. This problem is more pronounced in the arid and semi-arid regions, due to the elevated ambient temperatures (often 30°C and above), which promote the rapid growth of spoilage microorganisms in food. More often than not, these arid regions are less developed in terms of infrastructure; hence refrigeration facilities are virtually non-existent. As a result, food preservation methods are largely limited to low-cost appropriate technologies such as drying, salting and fermentation. Holzapfel (2002) has observed that traditional fermentation processes serve as a substitute where refrigeration and other means are unavailable for food preservation. Fermented foods also have added benefits of increased safety and enhanced sensory attributes.

Yagil (1982) noted that in the desert regions of the world, camel milk forms the mainstay of the human diet. The camel has been recognized as a superior provider of milk than the cow, because of its resistance to water stress that normally exists in arid areas. Indeed, the camel is the only animal that can exist for weeks without water and still produce milk (Mohamed, 1993).

Most camel milk is drunk fresh, although it may also be consumed when slightly sour or strongly soured (Yagil, 1982). Due to its high nutrient content, milk is a very good medium for microbial growth and is highly prone to rapid microbial spoilage by proteolysis and acidification. For this reason, raw camel milk does not keep for long under the warm tropical conditions. Since heat processing of camel milk is not widely practised as a method of preservation (Wangoh, 1993), fermentation provides a cheap, simple method of preserving the milk for a limited period of time (Yagil, 1982; Wangoh, 1997). Sour camel milk is usually prepared by allowing the milk to ferment spontaneously at ambient temperature. In addition, fermentation can be improved by back-slopping method, using portions of previously fermented product as starter. This soured milk has a storage life of about one week at ambient temperature (Mohamed, 1993).

### *1.2 Problem statement and study justification*

Spontaneous fermentation, which has long been used to preserve camel milk, is difficult to standardize and control. It is often not without concomitant variation in end product quality (Holzapfel, 2002). It has also been noted that spontaneous fermentation processes are widely prone to failure and may promote undesirable side effects (Tamime, 1990).

Developments in dairy microbiology have led to studies on the behaviour and metabolism of starter cultures. This in turn has made starter selection feasible, and has contributed to greater uniformity and predictability of the quality of fermented dairy products. However, this knowledge has been largely limited to starter cultures for fermented products based on cow milk. Characterisation of the microorganisms involved in fermentation of camel milk will form the scientific foundation of development of starter cultures for camel milk. Starter culture development would greatly improve the traditional camel milk fermentation technology through strain isolation, selection and identification. Currently, there is no commercial production of fermented camel milk in Kenya.

Few scientific studies have been carried out on the fermentation of camel milk by lactic acid cultures (Wangoh, 1997). Published information is also lacking on the characterisation of the microflora of fermented camel milk (Farah, 2001: personal communication). A study by Farah *et al.* (1990) found that traditionally fermented camel milk may be improved by the use of selected mesophilic lactic cultures. These investigators noted that lack of these starter cultures was a limiting factor for large-scale production of fermented camel milk. Accordingly, microbiological characterisation of the microflora of fermented camel milk is an important starting point in the development of starter cultures for commercial production of fermented camel milk. The use of camel milk starters would ensure the manufacture of a standardised product, whose quality is adequately controlled. Ultimately, commercialisation of camel milk production would result in economic development, both at household and national levels, through increased monetary earnings.

### *1.3 Expected beneficiaries*

The results of this study will initially be of benefit to the scientific community, regarding the microbiological profile involved in fermentation of camel milk, in terms of identity and functionality. The study results will thus add to the pool of scientific knowledge on the microbial diversity, thereby forming a basis for further research and exploration on any genes with unique functionalities on human health and well-being. The scientific knowledge unearthed by this study can also lead towards microbial strain selection and development, with the ultimate aim of developing camel milk starter cultures with desirable functionalities.

### *1.4 Overall objective*

The overall objective was to investigate the lactic acid bacteria and yeasts in *suusac*, a traditional fermented camel milk product of the Somali community in Kenya.

### *1.5 Specific objectives*

The specific objectives were to:

1. document the traditional art of *suusac* production as practised by the Somali community of Isiolo District, Eastern Province of Kenya;
2. isolate and identify the lactic acid bacteria and yeasts involved in the traditional fermentation of camel milk;
3. investigate the functional roles of the isolated microorganisms, namely acidification, flavour production and proteolytic activity; and
4. carry out laboratory-based trials of *suusac* production using pure cultures isolated from traditional *suusac*.

# CHAPTER 2: LITERATURE REVIEW

## *2.1 The camel*

The camel family, *Camelidae*, is classed into two genera, namely *Camelus* and *Lama*. The Old World genus of *Camelus* comprises two species: *C. dromedarius*, the one-humped dromedary and *C. bactrianus*, the two-humped Bactrian. The New World genus of *Lama* has four species; two of these (*L. guanacoe* and *L. vicugna*) are wild, while the other two (*L. glama* and *L. pacos*) are domesticated (Wilson, 1984). In some cases, *Lama vicugna* is classified as a separate genus, *Vicugna* (Yagil, 1982).

The dromedary camel is found in the desert and semi-desert regions of North Africa, Sudan, Ethiopia, the Near East and West-central Asia, while the Bactrian camel is found in Russia, Mongolia, East-central Asia and China (Wilson, 1984). The dromedary camel, found in Kenya, is the source of milk in the arid and semi-arid areas of Kenya.

The camel is physiologically and anatomically adapted to desert life (Wilson, 1984; Yagil, 1982). It can tolerate extreme heat and desiccation by sweating more efficiently than other mammals. Its large mass acts as a heat buffer (Wilson, 1984), allowing it to store its heat during the day and cool off by conduction and convection in the evening (Yagil, 1982). In most mammals, sub-cutaneous fat is spread over the body surface, reducing the rate of sweat evaporation. The camel's hump is used to store fat, leaving the sub-cutaneous tissues virtually fat-free. This enables evaporation of sweat more easily and efficiently (Wilson, 1984; Yagil, 1982). The camel also has a unique ability to concentrate its urine, thereby reducing the rate of water loss (Wilson, 1984). Camels can lose 25–30% of their body weight by loss of water, amounts that would have fatal consequences in other domestic animals. This loss can be made up in just 10 minutes

by drinking water (Wilson, 1984). Thus, the camel can use limited water resources more efficiently than other animals.

The camel has a smooth reflective coat that is neither too thick as to prevent evaporative heat loss, nor too thin as to allow too much heat to strike the skin (Wilson, 1984; Yagil, 1982). The camel's padded foot is well adapted to the loose sandy desert soils. However, it is less suited for walking on stony or muddy terrain. The foot forms a cushion, which spreads the camel's weight on sand (Wilson, 1984). The camel's gait enables it to cover longer walking distances with less effort than other animals (Wilson, 1984).

Kenya's camel population is about 900,000 and this number is concentrated mainly in Isiolo, Marsabit, Wajir and Garissa districts where camel husbandry is the major economic activity (Wangoh, 1997). Table 2.1 indicates the camel population densities in Kenya, as at 1994 census.

**Table 2.1: Camel population in Kenya by district**

| District | Population in 1000 units | | |
|---|---|---|---|
| | Camel | Human | Camel per kaput |
| Isiolo | 424 | 276 | 1.54 |
| Marsabit | 227 | 125 | 1.82 |
| Wajir | 153 | 125 | 1.22 |
| Garissa | 61 | 124 | 0.49 |
| Samburu | 14 | 114 | 0.12 |
| Mandera | 12 | 123 | 0.10 |
| Turkana | 10 | 179 | 0.06 |
| Baringo | 1 | 286 | 0.003 |
| West Pokot | 1 | 231 | 0.004 |
| Total | 903 | 1583 | 0.57 |

*Source: Wangoh, 1997*

## 2.2 *Composition of camel milk*

Camel milk is generally opaque white, and normally has a sweet, sharp taste though sometimes it tastes salty (Yagil, 1982). Camel milk is generally comparable in composition to milk of other domestic animals (Table 2.2), except for that of sheep and buffalo, which have higher fat contents (Wilson, 1984).

Camel milk is notably rich in vitamin C (Wilson, 1984; Yagil, 1982), but low in vitamin A (Wilson, 1984). Camel milk is thus an important contributor of vitamin C to the diets of inhabitants of arid regions, where fruits and vegetables are largely unavailable (Yagil, 1982). Levels of vitamin B complex in camel milk are comparable to those in milk of other domestic animals. Amounts of short chain fatty acids are generally lower than in cow's milk, and the fat globules of camel's milk are smaller than those of cow's milk (Wangoh, 1993).

**Table 2.2: Proximate composition of milk of various animal species**

| Species | Percentage composition | | | | | |
|---|---|---|---|---|---|---|
| | Moisture | SNF | Fat | Lactose | Protein | Ash |
| Camel | 86.3-87.6 | 7.0-10.7 | 2.9-5.4 | 3.3-5.8 | 3.0 –3.9 | 0.6-0.8 |
| Cow | 86.2-87.6 | 8.7-9.4 | 3.7-4.4 | 4.8-4.9 | 3.2-3.8 | 0.7 |
| Buffalo | 83.1 | 9.0-10.5 | 7.4 | 4.9 | 3.8 | 0.8 |
| Goat | 87.1-88.2 | 7.8-8.8 | 4.0-4.5 | 3.6-4.2 | 2.9-3.7 | 0.8 |
| Sheep | 79.5-82.0 | 11.6-12.0 | 6.9-8.5 | 4.3-4.7 | 5.6-6.7 | 0.9-1.0 |
| Horse | 90.1-90.2 | 8.6-8.9 | 1.0-1.2 | 6.3-6.9 | 2.0-2.7 | 0.3-0.4 |
| Pig | 82.8 | 12.1 | 5.1-6.7 | 3.7 | 7.1-7.3 | 1.0-1.1 |
| Human | 88.0-88.4 | 8.3-8.9 | 3.3-4.7 | 6.8-6.9 | 1.1-1.3 | 0.2-0.3 |

*Source: Wilson, 1984*

### *2.3 Traditional fermented camel milk*

Literature on traditional fermented camel milk is scant and is limited to the different methods used for its preparation. The few reviews available indicate that the fermentation process is largely spontaneous and is often carried out in special containers. The microorganisms involved have not been investigated conclusively and little is known of the exact nature of the microorganisms contributing to these fermentation processes (FAO, 1990).

Fermented camel milk has been given different names in different parts of the world, such as *kefir* in the Middle East; *lehben* in Egypt, Israel and Syria; *yoghurt* in Bulgaria; and *chal* or *shubat* in Russia (Mohamed, 1993; Yagil, 1982).

*Chal* is a white sparkling beverage with a sour flavour. It is traditionally prepared by souring fresh milk in a skin bag by adding previously soured milk. For 3–4 days, fresh milk is continually added to the mixture, such that the end product has 3–5 times the original volume of *chal* that was added initially (Yagil, 1982).

In Kenya, *suusac* is made by leaving camel milk for 1–2 days at ambient temperature to sour spontaneously. In most cases, the traditional fermented camel milk (*suusac*) was prepared by allowing fresh milk to ferment spontaneously without prior heat treatment (Wangoh, 1997).

In Sudan, Dirar (1994) reported a fermented camel milk product called *gariss*. It is prepared from whole fat milk, which is soured in a skin bag strapped on a camel's back. The fermentation thus occurs under continuous agitation as the camel goes about its grazing activity.

## *2.4 Microflora of other traditional fermented milk products*

The microflora usually involved in spontaneous lactic fermentation of traditional milk products are mixed, of undefined or only partially defined composition. In many investigations, the presence of various lactic acid bacteria (LAB), yeasts and milk molds with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was reported (Oberman, 1985).

Robinson and Tamime (1990) have classified fermented milk products into three groups, based on the method of fermentation. These are lactic, yeast-lactic and mold-lactic fermentations. This scheme of classification will be used to discuss the microflora of some traditional fermented milk products.

### 2.4.1 Lactic fermentations

#### 2.4.1.1 Yoghurt

In Bulgaria, traditional yoghurt is made from boiled cows' or goats' milk, which is inoculated at 40–45°C with a small quantity of previously soured milk. The pot containing the inoculated milk is wrapped in furs to maintain a constant incubation temperature. The milk is incubated for 8–10 hours in an oven until a smooth, viscous, firm curd is formed. Yoghurt is generally a highly acidic product (Oberman, 1985).

The yoghurt microflora have been divided into 3 groups (Oberman, 1985):
(i) Essential microflora, comprising *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These two microorganisms play the predominant role in yoghurt fermentation. At the start of fermentation, the metabolic activity of *S. salivarius* subsp. *thermophilus* brings about accumulation of moderate amounts of lactic, acetic and formic acids (Oberman, 1985). The presence of formic

acid, together with the reduction in redox potential as a result of $CO_2$ production by *S. salivarius* subsp. *thermophilus*, stimulates the growth of *L. delbrueckii* subsp. *bulgaricus* (Oberman, 1985; Robinson and Tamime, 1990).

(ii) Non-essential microflora represented by homofermentative LAB other than those in group (i) and by heterofermentative LAB e.g. *Lactobacillus acidophilus, Bifidobacterium bifidum, Propionibacterium shermanii,* and *Streptococcus lactis* subsp. *diacetylactis.*

(iii) Contaminants: yeasts, molds, coliforms and other undesirable microorganisms.

*L. delbrueckii* subsp. *bulgaricus* is primarily responsible for aroma production, through metabolism of the amino acid, threonine, to glycine and acetaldehyde. Although *S. salivarius* subsp. *thermophilus* also produces acetaldehyde as a product of its metabolism, the synthesis is less active at fermentation temperatures than synthesis by the former microorganism (Robinson and Tamime, 1990).

#### 2.4.1.2 Bulgarian milk

Bulgarian milk is an extremely sour product, prepared from boiled goats' or cows' milk inoculated with a portion of previously fermented milk. Fermentation occurs overnight at 40–45°C in or near an oven in a similar manner as yoghurt production detailed above (Oberman, 1985). *Str. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are the main microorganisms involved in the fermentation processes (Oberman, 1985; Robinson and Tamime, 1990).

#### 2.4.1.3 *Leben, dahi,* and related products

*Leben* is a concentrated yoghurt-like product from the Middle East. Concentration is achieved by hanging the fermented curd in a cloth bag that allows the whey to drain out. In Turkey, a goatskin bag is used, while in Egypt, a porous earthenware pot is

used in place of a bag (Oberman, 1985). The mixed microflora involved in *leben* production consist of *S. lactis* subsp. *lactis, S. salivarius* subsp. *thermophilus, L. delbrueckii* subsp. *bulgaricus* and lactose-fermenting yeasts (Marshall, 1987; Robinson and Tamime, 1990).

*Dahi* (or *dahdi*) is the Indian equivalent of yoghurt and is usually made from boiled cows', buffaloes' or mixed milk. After inoculation with a small quantity of previously soured milk, the milk is incubated overnight near an oven; the warm temperatures around the oven favour the growth of the thermophilic cultures involved in the fermentation process. The microflora involved in *dahi* fermentation have been identified as *S. lactis* subsp. *lactis, S. salivarius* subsp. *thermophilus, L. delbrueckii* subsp. *bulgaricus, Lactobacillus plantarum,* and lactose-fermenting yeasts (Robinson and Tamime, 1990).

#### 2.4.1.4 *Mursik*

*Mursik* is produced by the Nandi community of Kenya. It is prepared from cows' milk fermented in ash-treated gourds. Blood may be added to fresh milk before fermentation, or to already fermented milk. The milk (or blood-milk mixture) is heated to boiling point then cooled to ambient temperature, after which it is allowed to undergo spontaneous fermentation for 3–5 days (Mathara, 1999). Mathara (1999) found *L. plantarum* to be the most dominant of the LAB involved in *mursik* production. Other LAB isolated from *mursik* were *Leuconostoc mesenteroides* and *Enterococcus faecium. Saccharomyces* species of yeast and *Geotrichum candidum* mold species were also isolated.

#### 2.4.1.5 *Kule naoto*

*Kule naoto* is produced from cows' milk by the pastoralist Maasai community of Kenya. Raw milk mixed with fresh blood is placed in a pre-smoked gourd and spontaneously fermented at ambient temperature for upto 5 days (Mathara, 1999). The dominant LAB genus involved in this fermentation process was identified as *Lactococcus*. Other LAB isolated were from the *Leuconostoc*, *Pediococcus* and *Lactobacillus* genera. The isolated fungi were *Saccharomyces* sp., and *G. candidum*. The LAB play functional roles in acid production, proteolysis and anti-microbial activity. However, the roles of the isolated yeast and mold species still need elucidation (Mathara, 1999).

#### 2.4.1.6 *Nono*

*Nono* is a traditional fermented milk product made by the nomadic Fulani of Nigeria. It is produced by spontaneous fermentation of cow's milk. The procedure for *nono* preparation is documented by Bankole and Okagbue (1992). Milk is fermented in a calabash for 24 hours, and the top fat layer is removed with a wooden spoon before breaking the curd with the same spoon. The fermented product is then transferred to a gourd, which is corked and shaken vigorously for about 30 minutes. The cork is released periodically to release gas from the gourd. The product is then returned to the calabash and the floating curd pellets are removed. The remaining colloidal mixture is referred to as *nono*. Microbiological analysis of *nono* by Bankole and Okagbue (1992) revealed *Lactobacillus* species and *Saccharomyces cerevisiae* to be the predominant microorganisms.

#### 2.4.1.7 *Ergo*

Gonfa *et al.* (2001) have documented the method of preparation of *ergo*, an Ethiopian traditional, spontaneously fermented milk product. It is usually produced from cow's

milk but camel and goat milk may also be used. The raw milk is collected in pre-smoked vessels and left to ferment for 2–4 days at ambient temperatures of 16–18°C. Microbiological studies of *ergo* by Gonfa *et al.* (2001) revealed that its microflora is dominated by lactic cocci of the genera *Streptococcus*, *Lactococcus* and *Leuconostoc*. Lactobacilli, yeasts and molds were also present.

### 2.4.2 Yeast-lactic fermentations

#### 2.4.2.1 *Kefir*

*Kefir* is traditionally prepared by the inhabitants of the Caucasus Mountains in Russia. Boiled cows' milk is placed in leather bags and inoculated with *kefir* grains. *Kefir* grains are small, white-yellow grains that resemble cooked rice. The product is incubated at 23–25°C overnight. The main products of fermentation are lactic acid (0.8%), ethanol and $CO_2$ (1%) with traces of acetaldehyde, diacetyl and acetoin. *Kefir* has a mildly alcoholic flavour and foams when agitated (Oberman, 1985).

The mixed microflora of *kefir* grains comprise *Lactobacillus* sp., *Streptococcus* sp., *Leuconostoc* sp. and lactose-fermenting yeasts (Oberman, 1985; Robinson and Tamime, 1990). The key microorganisms involved in the fermentation are *Lactobacillus kefir*, *Saccharomyces cerevisiae* and *Candida kefyr* (Marshall, 1987). Traditional *kefir* contains 70% lactobacilli, 20% streptococci and 5% yeasts (Oberman, 1985).

Lin *et al.* (1999) studied the microbial composition of kefir grains in Taiwan. The LAB isolated from kefir grains were identified as *Lactobacillus helveticus* and *Leuconostoc mesenteroides*, and the yeasts were identified as *Kluyveromyces marxianus* and *Pichia fermentans*.

#### 2.4.2.2 *Koumiss*

This product is traditionally prepared in Central Asia. Although *koumiss* was originally made from mare's milk, a similar product made from cows' milk is nowadays produced (Oberman, 1985; Robinson and Tamime, 1990). Oberman (1985) has detailed the procedure for traditional preparation of *koumiss* as follows: Fresh mare's milk is mixed with a finished previous *koumiss* and placed in a special skin bag. Every 1 to 2 hours the milk is agitated and after 3–8 hours a strong foam and sour flavour are formed, indicating the end of the fermentation process. Incubation temperatures are usually 20–25°C, although further ripening may be done at lower temperatures.

The microflora in traditionally prepared *koumiss* are very variable, but are reported to comprise *L. delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* and lactose-fermenting yeasts, namely *Torula koumiss* and *Saccharomyces lactis* (Oberman, 1985). In addition, *Lactococcus sp.* and some spore-forming bacilli may be found (Marshall, 1987). The product is sour, alcoholic and effervescent but not curdled since the casein in mare's milk does not coagulate at the isoelectric point (Marshall, 1987; Oberman, 1985). Mare's milk contains about half the casein content of cow milk (Oberman, 1985). It is likely that this low casein content gives rise to the poor curd-forming properties of mare's milk.

### 2.4.3 Mold-lactic fermentations

#### 2.4.3.1 *Viili*

*Viili* is a spontaneously fermented cow's milk product from Finland. It has a characteristic stringy or ropy texture (Oberman, 1985) caused by capsule-producing strains of *Leuconostoc mesenteroides* subsp. *cremoris* (Marshall, 1987). Additional

fermentation microflora include *S. lactis* subsp. *diacetylactis,* lactose-fermenting yeasts, and the mold *G. candidum* (Marshall, 1987; Oberman, 1985; Robinson and Tamime, 1990). Because *viili* is made from unhomogenized milk, during incubation the cream rises to the top, which together with the surface growth of the mold gives the product its characteristic smooth, velvety appearance (Marshall, 1987; Robinson and Tamime, 1990).

### *2.5 Starter cultures in fermented milk production*

The processes involved in the production of the characteristic flavour and texture of fermented milks are the result of the presence of specific microorganisms and their enzymes in milk. These microorganisms may be bacteria, molds, yeasts or combinations of these (Marshall and Law, 1984; Tamime, 1990). Since these microorganisms initiate or "start" fermentation, they are often referred to as "starter cultures" or "starters" (Berg, 1988).

Sanders (1992) defines a starter culture as a microbial strain or mixture of strains, species or genera used to effect a fermentation and bring about functional changes in milk that lead to desirable characteristics in the fermented product. The most important dairy starter microorganisms are species of *Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus*, which form part of the lactic acid bacteria (LAB) (Berg, 1988; Marshall and Law, 1984; Tamime, 1990; Varnam, 1993). Recently, the inclusion of the genera *Pediococcus* has been proposed (Varnam, 1993). A further recent development, stimulated by interest in the therapeutic properties of fermented milks, is the use of the intestinal microorganism *Bifidobacterium* sp. in starter cultures (Tamime, 1990; Varnam, 1993). Some of the therapeutic benefits of *Bifidobacterium* sp. include enhancement of the immune system, restoration of the balance of intestinal

microflora and anti-carcinogenic activity (Shah, 2001). Yeasts are also included since they are used together with LAB in the production of *kefir* and *koumiss* through lactic-alcoholic fermentation (Oberman, 1985; Tamime, 1990; Varnam, 1993).

### 2.5.1 Functions of starter microorganisms in fermented dairy products

The main role of starters is the production of lactic acid by fermentation of lactose. Lactic acid is responsible for the distinctive acidic flavour of fermented milks. The acidic conditions produced in fermented milks (pH 4.8 and below) suppress the growth of pathogenic and some spoilage microorganisms (Sanders, 1992; Tamime, 1990; Varnam, 1993).

In addition, starters also produce volatile compounds such as diacetyl from citrate and acetaldehyde from threonine or sugars (Varnam, 1993). These volatile compounds contribute to the flavour/aroma of the fermented products (Sanders, 1992; Tamime, 1990; Varnam, 1993). Citrate in milk is converted by citrate-utilizing LAB such as *L. lactis* and *L. mesenteroides* to pyruvate, which is further converted to α-acetolactate and then diacetyl. In most LAB, such as *L. lactis*, *L. mesenteroides* subsp. *cremoris* and *L. mesenteroides* subsp. *dextranicum*, α-acetolactate is enzymatically decarboxylated to acetoin. However, some strains do not possess the enzyme α-acetolactate decarboxylase, resulting in an accumulation of α-acetolactate, which is subsequently oxidized to diacetyl (Hugenholtz *et al.*, 2002).

Acetaldehyde is the main flavour compound in yoghurt and is produced by the yoghurt bacteria, *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Though the exact mechanisms of acetaldehyde production are not well established, Hugenholtz *et al.* (2002) have reported two major possibilities: (i) from pyruvate via the

pyruvate-formate lyase or the pyruvate dehydrogenase reaction, and (ii) from threonine via the threonine aldolase reaction resulting in glycine and acetaldehyde production.

The synthesis of proteolytic and lipolytic enzymes by starters is desirable in ripening of certain types of cheese (Tamime, 1990; Varnam, 1993). Milk does not contain sufficient free amino acids and peptides to allow the growth of LAB. Thus, proteolytic enzymes are required to degrade milk casein to oligopeptides, which are degraded by peptidases to peptides and amino acids (Shah, 2001).

### 2.5.2 Classification of LAB starter microorganisms in fermented milks

Since the publication of the ninth edition of Bergey's manual of systematic bacteriology (Sneath *et al.*, 1986), several changes have occurred in the taxonomy of bacteria (Jay, 1992). Many of the new taxonomic groups were created as a result of the use of developed modern methods, either alone or in combination with traditional methods. Modern taxonomic methods include rRNA sequencing, DNA base composition, DNA homology, cell wall analysis, serological profiles and enzyme profiles (Garvie, 1984; Jay, 1992).

According to the latest classification, the LAB group is comprised of at least 8 genera. Four new genera, namely *Carnobacterium, Enterococcus, Lactococcus* and *Vagococcus*, have been included together with the 4 traditional genera of *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus*. The carnobacteria were previously classified as lactobacilli, while *Enterococcus, Lactococcus* and *Vagococcus* were formerly grouped with the streptococci (Jay, 1992).

The LAB group is loosely defined with no precise boundaries. However, all its members share the property of producing lactic acid from hexoses (Jay, 1992). Based

on the products of glucose metabolism, LAB may be divided into either homofermentative or heterofermentative LAB. Homofermentative LAB produce over 50% of total acid as lactic acid from glucose fermentation. Heterofermentative LAB produce equal molar amounts of lactic acid, ethanol and $CO_2$ from hexoses (Garvie, 1984; Jay, 1992).

All members of the genera *Pediococcus, Streptococcus, Lactococcus* and *Vagococcus*, together with some lactobacilli are homofermentative. All leuconostocs and some lactobacilli are heterofermentative (Jay, 1992; Tamime, 1990). Homofermentative LAB possess the enzymes aldolase and hexose isomerase, but lack phosphoketolase. They use the Embden-Meyerhof-Parnas (EMP) pathway to produce mainly lactic acid from glucose. Heterofermentative LAB possess mainly phosphoketolase enzyme and hence ferment glucose mainly by Hexose Monophosphate (HMP) pathway (Garvie, 1984; Jay, 1992). The LAB are either mesophilic (optimum growth at 30°C) or thermophilic (optimum growth at 40–45°C). *Lactococcus* and *Leuconostoc* are mesophilic, while *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are thermophilic (Tamime, 1990).

In 1919, the genus *Lactobacillus* was classified by Orla-Jensen into 3 groups *viz.* Thermobacterium, Streptobacterium and Betabacterium, based on whether glucose fermentation was homofermentative or heterofermentative and optimum growth temperature. In the ninth edition of Bergey's manual of systematic bacteriology (Sneath *et al.*, 1986) a newer classification scheme for this genus divides it into 3 groups (I, II, and III) resembling Orla-Jensen's three genera, but without designating them as formal subgeneric taxa. Although most of the strains in each of the new groups fit the original descriptions of thermobacteria, streptobacteria and betabacteria,

many of the newly-described species do not. Therefore, the new classification method does not include growth temperature.

Group I organisms comprise obligate homofermentative lactobacilli, which ferment hexoses almost exclusively by the EMP pathway. Pentoses and gluconate are not fermented. This group consists of all Orla-Jensen's thermobacteria plus many newly-described species. They have been grouped into two complexes of related species or subspecies namely: (i) *L. delbrueckii* subsp. *bulgaricus*, subsp. *lactis* and subsp. *leichmanii* and (ii) *L. acidophilus, L. helveticus, L. gasseri* and *L. crispatus.*

Group II lactobacilli are facultatively heterofermentative, fermenting hexoses almost exclusively by the EMP pathway. Some species ferment hexoses to lactic acid, ethanol and formic acid, under low glucose conditions. Pentoses are fermented to lactic and acetic acids via an inducible phosphoketolase. Included in this group are all of Orla-Jensen's streptobacteria and other newly described strains. They have been grouped into three complexes of related species or subspecies namely: (i) *L. plantarum* strains, (ii) *L. casei* strains and (iii) *L. sake, L. curvatus* and *L. bavaricus.*

Group III lactobacilli are obligately heterofermentative and ferment hexoses to lactic acid, ethanol and $CO_2$. Pentoses are fermented to lactic and acetic acids. Both pathways involve phosphoketolase. The group comprises all obligately heterofermentative gas-forming lactobacilli of Orla-Jensen's betabacteria and some new species. Examples are *L. kefir, L. divergens, L. buchneri* and *L. bifermentans.* However, Tamime (1990) notes that apart from *L. kefir,* the betabacteria are not significant as dairy starters.

Important starter organisms in the genus *Lactococcus* include *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar diacetylactis. The first two are important as acid producers, while the third is primarily a flavour producer (Tamime, 1990). *Leuconostoc mesenteroides* subsp. *dextranicum* and subsp. *cremoris* produce diacetyl from citrate, thus are important in flavour production in fermented milks (Tamime, 1990). Of the genus *Pediococcus*, only two species may occur in milk *viz. P. pentosaceus* and *P. acidilactici*. However, these species are less important than the other LAB (Sneath *et al.*, 1986).

### 2.5.3 Yeast starter microorganisms in fermented milks

The presence of yeasts in milk, besides the LAB, results in lactic-alcoholic fermentation used in the production of *kefir* and *koumiss* (Tamime, 1990). *Kluyveromyces marxianus* var. *marxianus* and *K. marxianus* var. *lactis* are used as starters in *koumiss*, producing ethanol and $CO_2$. Other microorganisms involved in *koumiss* fermentation are the LAB, namely: *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Varnam, 1993). *Kefir* grains contain *Candida kefyr, K. marxianus* var. *marxianus, Saccharomyces cerevisiae, Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, L. acidophilus, L. kefir, L. kefiranofaciens* and *L. casei* (Varnam, 1993).

# CHAPTER 3: STUDY DESIGN AND METHODOLOGY

## *3.1 Investigation on the traditional process of suusac fermentation*

Information on the traditional art of *suusac* production was obtained by conducting key-informant interviews, with the aid of a semi-structured interview guide (Appendix 1). Four key informants were interviewed. Convenient sampling was used to identify camel-owners of Somali origin, resident in Isiolo Town with camel herds near the town, and who were knowledgeable on the traditional method of *suusac* production.

## *3.2 Laboratory simulated method for suusac fermentation*

Figure 4.1 in the section on results and discussion illustrates the process adapted for the laboratory-based production of *suusac*. The skimming of the top layer of cream from the fermented milk was done to remove contaminating microorganisms, particularly molds, which would preferentially grow at the surface of the product where oxygen is more readily available.

Based on the traditional method of *suusac* production as above, a laboratory simulation of the same was carried out for the purposes of enumerating certain groups of microorganisms in *suusac*. Pre-smoked gourds used for the laboratory-based fermentation were sourced from Isiolo. Smoking of the interior of the gourds was done using an acacia twig. One end of the twig was heated in an open flame till red-hot and then immediately introduced into the gourd, which was then covered in order to trap the smoke. The gourd was occasionally shaken to allow pieces of charcoal to break off from the twig. This process of gourd smoking was repeated upto five times, after which the gourd was left to cool and the pieces of broken charcoal brushed out using root fibres. The smoked gourd was then ready for use. Spontaneous fermentation of the camel milk was carried out in an incubator at 30°C for 24 hours.

## *3.3 Analytical studies*

### 3.3.1 Microbial analysis

#### 3.3.1.1 Materials

*Camel milk*

Samples of fermented camel milk from Isiolo were collected in sterile sample bottles, cooled to 4–6°C and transported to the laboratory in an insulated cool box containing ice packs. The samples were analysed within 12 hours of collection, and were maintained at 4–6°C during the period between collection and analysis. Raw camel milk used in the laboratory experiments was also obtained from Isiolo and maintained at 4–6°C between collection and analysis. The raw milk was analysed within 24 hours of collection.

*Cow milk*

Raw cow milk was obtained from the University of Nairobi's Kanyariri farm. The milk was analysed within 4 hours of collection. Between collection and analysis, the milk was maintained at 4–6°C.

*Laboratory chemicals and microbiological media*

Analytical grade laboratory chemicals and microbiological media were bought from Marty Enterprises Limited, Nairobi.

*API® microbiological identification kits*

API® (Analytical Profile Index) microbiological identification kits (bioMérieux, France) for lactic acid bacteria and yeasts (API 50 CHL and API 20C AUX, respectively) were bought from Hass Scientific and Medical Supplies Limited, Nairobi.

### 3.3.1.2 Methods

*Enumeration of microorganisms*

For each of the following microbiological analyses, serial dilutions of the *suusac* samples, ranging from $10^{-1}$ to $10^{-7}$, were prepared in sterile 0.85% NaCl physiological saline solution in distilled water. The pour plate method of Harrigan and McCance (1986) was used for all the analyses except for yeasts and molds count, which was done according to the spread plate method. Duplicate plates were prepared per dilution. All counts were reported as $\log_{10}$ colony-forming units per ml ($\log_{10}$cfu/ml) of *suusac*.

*Total viable count (TVC)*

One ml of the $10^{-4}$ to $10^{-7}$ dilutions was used to inoculate pour plates using plate count agar (PCA). The plates were incubated at 30°C for 48 hours. The colonies on plates with between 30 and 300 colonies were then counted.

*Enumeration of lactic acid bacteria (LAB)*

One ml of the $10^{-4}$ to $10^{-7}$ dilutions was used to inoculate pour plates of MRS (deMan Rogosa Sharpe) agar. The plates were incubated anaerobically in GasPak® jars at 30°C for 3 days after which the colonies on plates with between 30 and 300 colonies were counted.

*Enumeration of yeasts and moulds*

0.1 ml of the $10^{-1}$ to $10^{-3}$ dilutions was used to inoculate spread plates of potato dextrose agar (PDA), which was acidified to pH 3.5 with sterile 10% tartaric acid. The plates were then incubated at 22–25°C for 5 days, followed by colony counting of plates with between 20 and 200 colonies.

*Enumeration of coliforms*

One ml of the $10^{-2}$ to $10^{-5}$ dilutions was used to inoculate pour plates of violet red bile agar (VRBA). The plates were then incubated at 37°C for 48 hours after which the colonies on plates with between 30 and 300 colonies were counted. On VRBA, typical coliform colonies appeared dark red surrounded by a violet-red precipitate of bile salts.

### 3.3.2 Microbial isolations

#### 3.3.2.1 Isolation of lactobacilli

From the MRS agar pour plates of the highest dilutions, discrete colonies were selected, Gram-stained and examined microscopically. Gram-positive rods were then subcultured into litmus milk and incubated at 30°C for 3 days, after which the cultures were re-examined for morphology, Gram stain and catalase reaction. Gram-positive, catalase-negative rods were tentatively considered as lactobacilli, subject to further genus and species identification. Pure cultures for further identification were then obtained by streaking onto MRS agar. The streak plates were incubated anaerobically in GasPak® jars at 30°C for 3 days. The isolated lactobacilli from the streaked plates were transferred to screw-capped bottles of yeast extract glucose chalk litmus milk, incubated at 30°C for 24 hours and then stored at 4–6°C.

#### 3.3.2.2 Isolation of lactococci

Pour plates of the $10^{-6}$ and $10^{-7}$ dilutions were prepared using bromocresol purple chalk lactose (BPCL) agar. After 3 days' incubation at 30°C, discrete colonies were selected for microscopic examination. Gram-positive cocci were then subcultured into litmus milk and incubated at 30°C for 3 days, after which the cultures were re-examined for morphology, Gram stain and catalase reaction. Gram-positive, catalase-

negative cocci were tentatively considered as presumptive lactococci, subject to further genus and species identification. Pure cultures for further identification were then obtained by streaking onto BPCL agar. The streaked plates were incubated at 30°C for 3 days. The isolated cocci from the streaked plates were transferred to screw-capped bottles of yeast extract glucose chalk litmus milk, incubated at 30°C for 24 hours then stored at 4–6°C.

#### 3.3.2.3 Isolation of yeasts

Discrete yeast colonies from the PDA spread plates were isolated by streaking onto PDA plates to ascertain the purity of the cultures. The pure cultures were then subcultured onto PDA slants, incubated at 22–25°C for 3 days then stored at 4–6°C.

### 3.3.3 Primary classification – lactic acid bacteria (LAB)

Primary classification of LAB was based on the results of Gram staining, microscopic cell morphology and catalase reaction. Gram-positive, catalase-negative rods and cocci were tested for their ability to produce $CO_2$ from glucose and ammonia from arginine. Ability to grow at 15°C and 45°C was also examined. The methods described by Harrigan and McCance (1986) were used.

#### 3.3.3.1 Production of carbon dioxide from glucose

The medium used to test for $CO_2$ production was Gibson's semi-solid tomato juice medium. This medium consisted of 4 parts reconstituted skim milk + 1 part nutrient agar with the addition of 0.25% yeast extract, 5% glucose and 10% tomato juice. Tomato juice provided manganese ions necessary for the growth of LAB. The medium was distributed into test tubes to a depth of 5–6 cm, steam-sterilized and then cooled to 45°C in a water bath. 0.5 ml of 24-hr-old LAB culture in yeast extract glucose chalk

litmus milk was aseptically inoculated into the tubes and mixed. The tubes were then cooled in tap water and molten nutrient agar at 50°C was then poured on top of the medium to give a layer 2–3 cm deep above the medium surface. The inoculated tubes were incubated at 30°C for 3 days. Any carbon dioxide gas produced was trapped by the semi-solid medium and agar plug. This was seen by upward disruption of the agar plug and presence of gas bubbles.

#### 3.3.3.2 Production of ammonia from arginine

The medium used was MRS broth containing 0.3% arginine monohydrochloride and 2% glucose. Five milliliters of the sterile medium was dispensed into tubes and a loopful of young culture in yeast extract glucose chalk litmus milk was aseptically inoculated into the tubes and incubated at 30°C for 2–7 days. To detect ammonia production, a loopful of culture was added to a loopful of Nessler's reagent on a slide. The development of an orange-brown colour indicated the presence of ammonia. A pale yellow colour or no colour reaction indicated the absence of ammonia.

#### 3.3.3.3Growth at 15°C and 45°C

MRS broth was used as the basal medium for the above test. The medium was dispensed into tubes in 5 ml amounts and sterilized by autoclaving at 121°C for 15 minutes. A loopful of young culture from yeast extract glucose chalk litmus milk was aseptically inoculated into each of 2 tubes of sterile medium. One of the tubes was incubated at 15°C and the other at 45°C for 2–7 days. Growth was indicated by turbidity of the medium.

### 3.3.4 Primary classification – yeasts

#### 3.3.4.1 Colonial characteristics and morphology

Colonial characteristics (colour and shape) on PDA were observed. Cellular morphology and mode of vegetative reproduction were observed by microscopic examination of Gram-stained heat-fixed smears.

#### 3.3.4.2 Production of ascospores

Production of ascospores was induced by subculturing a young culture onto Gorodkowa agar, a sporulation medium containing 1% peptone, 0.1% D-glucose, 0.5% sodium chloride and 2% agar. The cultures were incubated for 3 days at 25°C and examined microscopically for presence of ascospores. Ascospore formation was confirmed by spore staining of heat-fixed smears.

### 3.3.5 Microbial identification

#### 3.3.5.1 Principle for the method

The API 50 CH strip allows for the identification of lactic acid bacteria by observation of carbohydrate metabolism. The strip consists of 50 micro-tubes each containing an anaerobic zone (tube portion) and an aerobic zone (cupule portion) for the study of fermentation and assimilation, respectively. The first micro-tube contains no carbohydrate substrate and serves as a negative control. The other micro-tubes contain a defined amount of specific dehydrated carbohydrate substrates. Fermentation of the substrate is indicated by a colour change in the tube portion due to the anaerobic production of acid, detected by a pH indicator in the API 50 CHL medium. A standardised suspension of the test organism (equivalent to 600 x $10^6$ cells/ml) is made in the medium and each tube of the strip is then inoculated. During

incubation, carbohydrates are fermented to acids, which results in a decrease in pH observed by a colour change of the bromocresol purple indicator from purple to yellow. The series of positive and negative results makes up the biochemical profile of the test organism and is used for its identification using identification software.

The API 20 C AUX yeast identification system comprises 20 cupules containing dehydrated substrates, which enable the performance of 19 assimilation tests. One of the cupules is a negative control, containing no substrate. The cupules are inoculated with a semi-solid minimal medium and the yeasts will only grow if they are capable of utilizing the substrate as the sole carbon source. The reactions are read by comparing them to the negative growth control and identification is achieved using identification software.

#### 3.3.5.2 Method for LAB identification

The API 50 CH strips were used to identify the LAB. The purity of the isolated LAB was ascertained by streak plating onto MRS agar and confirming that only single type colonies occurred on the agar plates. A heavy bacterial suspension was prepared by transferring several bacteria colonies from the agar plate to an ampoule containing 2 ml of sterile distilled water, using a sterile swab. Using aseptic techniques, a certain number of drops of the suspension was transferred to an ampoule of 5 ml sterile distilled water in order to obtain a suspension with a turbidity equivalent to McFarland Standard no. 2.

The McFarland Standard is a series of standards of Barium sulphate suspensions of known different opacities, allowing the estimation of the density of bacterial suspensions. The density of the bacterial suspension is compared to that of a

suspension of known opacity contained in an ampoule of the same diameter. The McFarland standard no. 2 is composed of 9.60 x $10^{-5}$ mol/l of Barium sulphate. It has a theoretical optical density of 0.50 at 550 nm, which is equivalent to a bacterial concentration of 600 x $10^6$ cells/ml. The number of drops added to the 5-ml ampoule was noted and twice this number of drops was used to inoculate a 10 ml ampoule of API 50 CHL medium. The medium composition is shown in Table 3.1.

**Table 3.1: Composition of API 50 CHL Medium**

| Component | Quantity |
|---|---|
| Polypeptone | 10 g |
| Yeast extract | 5 g |
| Tween 80 | 1 ml |
| Dipotassium phosphate | 2 g |
| Sodium acetate.$3H_20$ | 5 g |
| Diammonium citrate | 2 g |
| Magnesium sulphate.$7H_20$ | 0.2 g |
| Magnesium sulphate.$4H_20$ | 0.05 g |
| Bromocresol purple | 0.17 g |
| Distilled water | to make 1 litre |

*Source: BioMérieux (2001b)*

The medium was homogenized gently with a pipette then inoculated into the tubes, which were overlaid with mineral oil to ensure anaerobic conditions. The strips were incubated at 30°C for 48 hours, after which the results were read. A positive test was recorded by a colour change in the pH indicator from purple to yellow. For the esculin test, a positive test was recorded by a colour change from purple to black.

#### 3.3.5.3 Identification of lactic acid bacteria

The biochemical profiles of the microorganisms were recorded and entered into a computer for identification using the APILAB Plus® software. The APILAB Plus®

software database enables the identification of various groups or taxa of microorganisms.

The identification of the observed profile is based on calculation of (i) how closely the profile corresponds to the taxon, relative to all other taxa in the database (percentage of identification) and (ii) how closely the profile corresponds to the most typical set of reactions for each taxon (T-index). The T-index is a value ranging between 0 and 1 and is inversely proportional to the number of atypical tests.

The taxa are sorted by decreasing values of the percentage of identification (% ID). For the first four taxa, the ratio of their % ID is calculated to that of the following taxon. The taxon with the highest ratio is selected for identification, as well as the taxa situated before it in the classification, if any. The T-indices are calculated using modal frequencies.

If only one taxon is chosen and its % ID is ≥ 80%, it is proposed for identification. If several taxa are chosen and if the sum of the % ID is ≥ 80%, they are proposed for identification, with a comment based on the value of the sum of the % ID and the average of the T-indices.

#### 3.3.5.4 Method for yeasts identification

The API 20 C AUX strip was used to identify the isolated yeast strains. The purity of the isolate was first ascertained by streaking onto potato dextrose agar and checking for growth of single-type colonies only. The inoculum was then prepared by aseptically transferring portions of the colony into 2 ml of sterile distilled water in order to obtain a suspension with a turbidity equal to McFarland Standard no. 2. One drop of the

yeast suspension was dispensed onto Rice Agar Tween morphology medium to enable observation of hyphae/pseudohyphae. Three drops of the suspension were transferred into a 7-ml ampoule of sterile API 20 C AUX C medium and gently homogenized with a pipette. The composition of the medium is indicated in Table 3.2.

The cupules on the strip were inoculated with the suspension obtained in the ampoule of C Medium. The strip was then incubated at 30°C for 48 hours, after which the results were read. Growth in each cupule was compared to the negative control cupule. A cupule more turbid than the control was taken as a positive reaction. The presence or absence of hyphae/pseudohyphae was also recorded.

**Table 3.2: Composition of API 20 C AUX C Medium**

| Component | Quantity |
|---|---|
| Ammonium sulphate | 5 g |
| Monopotassium phosphate | 0.31 g |
| Dipotassium phosphate | 0.45 g |
| Sodium chloride | 0.1 g |
| Calcium chloride | 0.05 g |
| Magnesium sulphate | 0.2 g |
| Histidine | 0.005 g |
| Tryptophan | 0.02 g |
| Methionine | 0.02 g |
| Agar | 0.5 g |
| Vitamin solution | 1 ml |
| Trace elements | 10 ml |
| Distilled water | to make 1000 ml |

*Source: BioMérieux (2001a)*

#### 3.3.5.5 Data processing for identification of yeasts

The pattern of positive and negative reactions was recorded, coded into a 7-digit numerical profile, and entered into a computer for identification using the APILAB Plus® software. The 7-digit profile was generated by converting the binary results observed (+ or -) into a numerical profile. This was done by dividing the tests on the strip into groups of three and giving each positive reaction a value of 1, 2 or 4 depending on its position in the group. The sum of these three values (0 for negative reactions) gave the corresponding digit with a value between 0 and 7. For example, (+ - +) was given the digit 5, i.e. (sum of 1, 0 and 4) and (+++) the digit 7, i.e. (sum of 1, 2 and 4). Details on the identification of the observed profile using the software are as indicated in section 3.3.5.3.

### 3.3.6 Biochemical and physiological characterisation

Functional characterisation tests described below were carried out according to the methods of Harrigan and McCance (1986).

#### 3.3.6.1 Production of lactic acid

The basal medium used to test for production of lactic acid consisted of 10% reconstituted skim milk powder and 0.5% D-glucose. The isolated starter culture was inoculated into the basal medium at the rate of 2% then incubated at 30°C for 2 days. The amount of lactic acid produced was determined by titration of 25 ml milk with 0.1 N NaOH (phenolphthalein indicator) to first persistent pink colour. The volume of titre was used to calculate the titratable acidity as % lactic acid equivalent, based on the following equation:

$$\% \text{ lactic acid equivalent} = \frac{T \times 0.1 \times 9}{\text{sample volume}} = \frac{0.9T}{25}$$

where T = titre in ml of 0.1N NaOH

### 3.3.6.2 Production of diacetyl

This was determined by the Voges-Proskauer test for the production of acetoin from glucose. The principle of the method is that in the presence of an alkali, any acetoin present is oxidized to diacetyl. Diacetyl combines with creatine to give a pink colouration. The intensity of the pink colour is a direct indicator of the amount of diacetyl produced.

The basal medium used to test for production of diacetyl consisted of 10% reconstituted skim milk powder and 0.5% D-glucose. The isolated starter culture was inoculated into the basal medium at the rate of 2% then incubated at 30°C for 2 days. A knife-point of creatine was added to the inoculated medium after incubation, followed by 5 ml of 40% NaOH. The intensity of the colour developed within 30 minutes was noted and assigned a score objectively using the scale below. The corresponding amount of diacetyl, in mg/100 ml of milk, is indicated in parentheses.

| Score | Colour intensity |
|---|---|
| 0 | no pink colour (< 0.5) |
| 1 | slightly pale pink (0.5 - 3) |
| 2 | pale pink (3.1 - 10) |
| 3 | red (10.1 - 30) |
| 4 | dark red (> 30) |

### 3.3.6.3 Hydrolysis of casein

A poured and dried plate of milk agar was inoculated with the isolated starter culture by streaking once across the agar surface. The inoculated plate was then incubated at 30°C for 2 days. Production of a clear zone after incubation was recorded as a presumptive positive result. To confirm that the clearing was as a result of casein hydrolysis (and not due to acid production from lactose), the plate was flooded with 1% hydrochloric acid, a protein precipitant. A true positive clear zone did not disappear on addition of the acid. The width of the clear zone was recorded in mm.

### 3.3.6.4 Activity in litmus milk

Bottles of steam-sterilized litmus milk were inoculated with a loopful of 24-hour-old isolated starter culture. The bottles were incubated at 30°C for 2 days and examined for the following changes in the medium:

- Acid production shown by a change in the colour of the litmus from light purple to pink and clotting of the milk (acid clot).
- Reduction of the litmus and loss of colour.
- Coagulation of the milk as a result of proteolytic enzyme activity affecting the casein, the litmus colour remaining light purple (sweet clot).
- Hydrolysis of casein as a result of proteolytic enzyme activity causing clearing and loss of opacity in the milk (peptonisation).
- Utilisation of citrate in the milk medium resulting in the production of an alkaline medium shown by colour change to a deep purple colour.

### 3.3.7 Fermentation trials using isolated single-strain cultures

Fermentation trials using the isolated LAB and yeasts from traditional *suusac* were carried out to assess the product's characteristics with respect to acidity development and flavour production. For comparison purposes, the fermentation was carried out in camel and cow milk using single-strain cultures. The process flow diagram for the fermentation trials is shown in Figure 3.1. Determinations of pH and diacetyl production were done after 24 hours' fermentation.

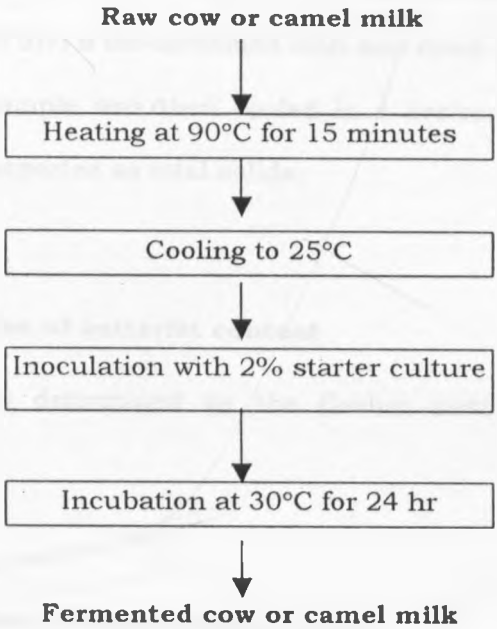


**Figure 3.1: Flow diagram of fermentation with starters isolated from camel milk**

### 3.3.8 Acid and flavour development by mixed cultures

Based on the acid- and flavour-producing capabilities of the isolates, three species were selected to assess the performance of mixed cultures in camel and cow milk. The selection criteria used were diacetyl and acidity levels produced in camel milk by the

single-strain cultures (for LAB) and degree of proteolytic activity (for yeast isolates). Section 4.7 on results indicates the specific isolates that were used in the experiment.

### 3.3.9 Proximate composition

All the samples were analysed for proximate composition in triplicate. In all cases crude fibre was assumed to be nil.

#### 3.3.9.1 Determination of total solids

The total solids were determined according to the IDF Standard 21B: 1987. Two grams of the sample were weighed into a flat-bottomed dish and dried in an air oven at 100°C for 3 hours. The dried sample was then cooled in a desiccator and weighed. The percentage of residue was reported as total solids.

#### 3.3.9.2 Determination of butterfat content

The butterfat content was determined by the Gerber method according to IDF Standard 105:1981.

#### 3.3.9.3 Determination of crude protein content

Nitrogen content was determined by the Kjeldahl method and used to calculate the crude protein content, according to the IDF Standard 20A: 1986. Crude protein (grams per 100g product) was calculated as 6.38 x N.

### 3.3.9.4 Determination of ash

Ash was determined according to the official methods of analysis of the AOAC (1984). Two grams of the sample were weighed into a previously tared crucible and ignited in a muffle furnace at 550°C for 2 hours until the ash was Carbon-free. The sample was then cooled in a desiccator and weighed. Ash was calculated as % of the weight of the sample.

### 3.3.9.5 Determination of lactose

Lactose content was calculated by difference, according to the official methods of analysis of the AOAC (1984).

### 3.3.10 Determination of pH

The pH was measured using a Pye Unicam pH meter model 290 Mk 2.

### 3.3.11 Determination of titratable acidity

Titratable acidity was determined according to the methods of AOAC (1984). Details are explained in Section 3.3.6.1.

## *3.4 Statistical data analysis*

Descriptive statistics (means and standard deviations) were used to summarize the data on microbial numbers, proximate composition, pH, titratable acidity and diacetyl flavour scores. Data on microbial counts were first transformed by a logarithmic ($\log_{10}$) transformation before computing the mean $\log_{10}$ counts and standard deviations.

The independent samples *t*-test was used to determine whether or not a significant difference existed between camel and cow milk raw materials, and between traditional

and laboratory-produced *suusac*, with respect to proximate composition, pH and titratable acidity. The *t*-test was also used to test for significant differences in pH and flavour between fermented milk produced using camel and cow milk. In both cases, the level of significance used was 0.05.

One-way analysis of variance (ANOVA) was used to test for significant difference in titratable acidity, pH and proteolytic activity between the isolated LAB and yeasts. The level of significance used was 0.05.

Microsoft® Excel 2000 package was used to carry out all the above statistical analyses.

# CHAPTER 4: RESULTS AND DISCUSSION

## *4.1 Traditional production of suusac in Isiolo District*

The traditional art of *suusac* production was documented, based on the information obtained during field interviews. The flow diagram of traditional processing of *suusac* is illustrated in Figure 4.1. The camel milk is milked directly into a gourd (called *toobke* or *bire* in the Somali language), whose interior has been cleaned out, smoothed and treated with smoke. The milking gourd, usually with a capacity of 2–4 litres, is obtained from the *garas* tree (*Dobera glabra*) and is long and narrow. Smoking of the gourd is done using the smouldering twig of the acacia tree (*Acacia seyal)* after rinsing it with cold water. The hot smoking chips are introduced into the gourd, which is then covered with its lid for a few minutes. The gourd is then left to cool and the charcoal pieces brushed out with root fibres. Gourd smoking is said to aid in extending the storage life of fresh milk and also gives *suusac* its characteristic taste and aroma.

After milking, the milk is bulked in a larger gourd (called *haan* or *dhiil*). This larger gourd is rounder in shape with a short neck and is secured in a woven basket. The milk is not subjected to any heat treatment. The gourd containing the fresh milk (*dhaay*) is closed and kept under ambient conditions for 2–3 days to allow for the milk to ferment spontaneously. When fermentation is complete, the top fat layer is skimmed off the *suusac,* which is stirred and is then ready for consumption. The product is stored in a cool place at ambient temperature and has a storage life of upto one week. The range of ambient daytime temperatures noted in Isiolo town during the study period was 26–29°C.

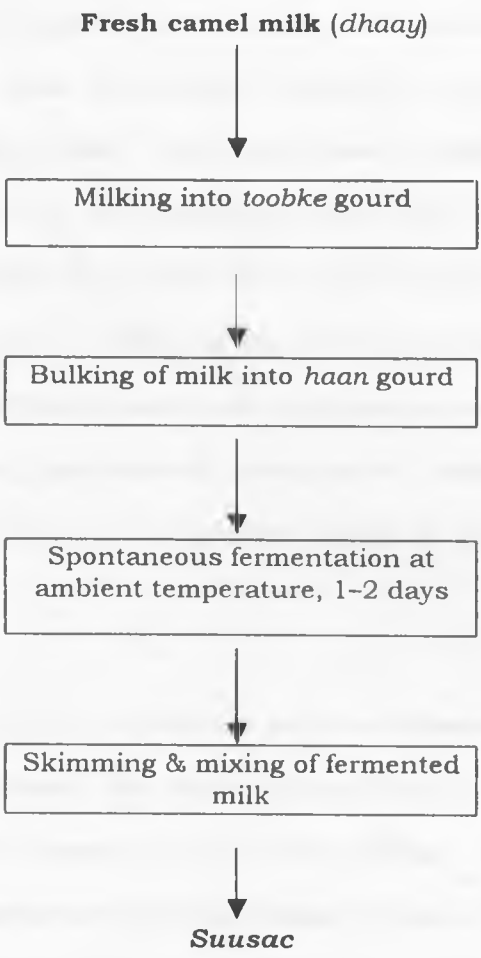


**Figure 4.1: Flow diagram of traditional *suusac* production**

*Suusac* is a white, low-viscosity, fermented camel milk product and has a distinct smoky flavour and astringent taste. When kept in a container for several days *suusac* undergoes syneresis, separating into whey and precipitated protein. However, homogeneity of the product is easily restored by shaking the container in which the milk is held.

The preparation of *suusac* by natural fermentation results in a product whose quality characteristics exhibit wide variations. This is inevitable due to the unregulated nature of spontaneous fermentation processes. In many cases, little is known of the exact nature and role of the microorganisms contributing to the fermentation (FAO,

1990). Holzapfel (2002) observed that in many traditional fermentation processes, the repeated use of the same fermentation container, e.g. calabash, as a source of inoculum is generally practised. This is the case for traditional *suusac* preparation. Other authors (Gonfa *et al.*, 2001; Mathara, 1999; FAO, 1990; Bankole and Okagbue, 1992) have also documented the use of the same gourd or calabash as a source of culture inoculum for various traditional African fermented milk products. FAO (1990) notes that clay pots and gourds with wide openings are commonly used as traditional milking vessels among agro-pastoral communities around Lake Victoria. Some pastoralists, including Boran in Ethiopia and Maasai in Kenya, use vessels woven out of animal skins.

According to Sanders (1991), in products such as fermented milks where processing steps and aids are minimal, the starter culture flora is the most important factor dictating the type and character of the final product. This view is supported by Tamime (1990), who notes that the fermentation process of any cultured dairy product relies entirely on the purity and activity of the starter culture, provided that the milk is free from inhibitory agents. However, because of lack of appropriate starter culture for fermented products in developing countries, wide variation in the quality attributes of the products is to be expected (Holzapfel, 2002).

The practice of smoking of traditional milk vessels is a common feature among pastoral and agro-pastoral communities in Eastern and Southern Africa (FAO, 1990). Grass, shrubs and hardwoods used by communities in Kenya, Ethiopia and Tanzania have been reviewed by FAO (1990) and include: *Olea africana, Balanities aegyptica, Diplorhynchus condylaccarpon* and *Combretum* spp. In Ethiopia, the milk vessels are smoked by burning wooden chips of specific trees and shrubs by introducing hot smoking chips into the container and whirling them inside for a few minutes with the

container lids in place (Gonfa *et al.*, 2001). The vessels may also be inverted over the smoking chips until the smoke dies out (Gonfa *et al.*, 2001). Among the Nandi and Maasai of Kenya, gourds are smoked with red-hot smoking chips and the residual charcoal pieces brushed out with special twigs (Mathara, 1999). The Nandi and Maasai use twigs from the *Cassia marylandica* and *Olea africana* trees, respectively, to smoke the gourds (Mathara, 1999).

Traditionally, milk is either consumed raw or after spontaneous fermentation but is rarely boiled unless used for tea making. In fact in some East African communities, such as the Maasai, it is a taboo to heat milk (FAO, 1990). The spontaneous fermentation of unheated milk takes advantage of the action of naturally occurring mixed microflora inherent in the milk (Sanders, 1991). In most camel-herding societies, camel milk is allowed to ferment naturally without prior heat treatment (Wangoh, 1997). This is also practised in the preparation of *kule naoto* (Mathara, 1999) and *nono* (Bankole and Okagbue, 1992), fermented cow milks of the Maasai of Kenya and Fulani of Nigeria, respectively. However, in the production of *mursik*, a traditional milk of the Nandi of Kenya, the milk is boiled first then cooled before spontaneous fermentation is allowed to occur (Mathara, 1999).

Raw milk is a highly nutritious and perishable commodity and is a vehicle for several pathogenic agents that cause food-borne diseases as brucellosis, typhoid fever and tuberculosis, among others. According to Sanders (1991), mixed-culture fermentation is an important method of preserving the safety of fermented dairy products. The main contributing factor to this is the rapid production of lactic acid by LAB during fermentation, which effectively inhibits the growth of most pathogenic bacteria (Holzapfel, 2002; FAO, 1990). Other inhibitory compounds produced by LAB starters include natural antibiotics and bacteriocins such as organic acids, nisin, reuterin and

hydrogen peroxide. These metabolic products exhibit antimicrobial properties against putrefactive and gram-negative bacteria, some fungi and endospore-forming gram-positive bacteria (Holzapfel, 2002).

### *4.2 Physico-chemical composition of suusac*

The physico-chemical properties of traditional and laboratory-produced *suusac* are shown in Table 4.1. Butterfat and lactose contents of the two products were significantly different ($p<0.05$), possibly due to compositional differences in the raw milk from which the products were made. The laboratory-produced *suusac* was made from raw camel milk, whose proximate composition is indicated in Table 4.2.

Comparison of the proximate analysis of fresh and fermented camel milk revealed that the contents of total solids, butterfat and lactose were significantly lower in fermented camel milk. However, protein and ash contents were not significantly different in the two products. The lower butterfat content in *suusac* may be attributed to the practice of skimming the top layer of cream at the end of fermentation. The lower content of lactose in the fermented product is as a result of the biotransformation of lactose to lactic acid through the fermentative action of the LAB involved in *suusac* production. This biotransformation of lactose substrate was also observed in a significant increase in the titratable acidity from 0.19% in the fresh milk to 0.90% in traditional *suusac*.

Table 4.2 shows the proximate composition and acidity of raw cow and camel milk used in the laboratory fermentation trials. Protein, lactose and butterfat contents were different in the two milks ($p<0.05$). This may be attributed to the inherent differences in the two animal species.

**Table 4.1: Average proximate composition (%), pH and total titratable acidity of traditional and laboratory-produced *suusac***

| Component | Traditional | Laboratory-produced |
|---|---|---|
| Total solids | 8.8 (2.7) | 8.5 (2.4) |
| Solids-not-fat (SNF) | 6.5 (0.8) | 5.7 (2.0) |
| Butterfat | 2.3* (0.5) | 2.8* (0.4) |
| Crude protein (N x 6.38) | 3.4 (0.8) | 3.3 (0.8) |
| Total ash | 1.1 (0.7) | 1.0 (0.2) |
| Lactose (by difference) | 2.0* (0.8) | 1.4* (0.3) |
| pH | 4.1 (0.1) | 3.8 (0.0) |
| Total titratable acidity (% lactic acid) | 0.90 (0.10) | 0.88 (0.05) |

Standard deviation in parentheses
Number of replicates = 3
* Significant difference between traditional and laboratory-produced *suusac* ($p<0.05$)

**Table 4.2: Average proximate composition (%), pH and total titratable acidity of raw cow and camel milk used in laboratory fermentation trials**

| Component | Cow milk | Camel milk |
|---|---|---|
| Total solids | 13.6 (0.5) | 11.5 (2.4) |
| Solids-not-fat (SNF) | 9.4 (0.1) | 8.4 (0.4) |
| Butterfat | 4.2* (0.1) | 3.1* (0.4) |
| Crude protein (N x 6.38) | 3.8* (0.2) | 3.2* (0.8) |
| Total ash | 0.7 (0.1) | 1.0 (0.3) |
| Lactose (by difference) | 4.9* (0.5) | 4.1* (0.1) |
| pH | 6.2 (0.1) | 5.8 (0.3) |
| Total titratable acidity (% lactic acid) | 0.15 (0.00) | 0.19 (0.06) |

Standard deviation in parentheses
Number of replicates = 3
* Significant difference between camel and cow milk ($p<0.05$)

A high titratable acidity (0.88%) was achieved in the laboratory-produced *suusac* from an initial acidity of 0.19% in raw camel milk. This is an important contributory factor to the keeping quality of *suusac*, since pathogens and other spoilage organisms are

inhibited by the high acidic conditions (Tamime, 1990; Johnson, 1991). According to Attia *et al.* (2001), the maximum buffering capacity of camel milk is obtained at lower pH values, which seems to present a higher physical stability towards increase in acidity. However, the same authors also note that camel milk does not form an acid curd during lactic fermentation but rather a fragile heterogeneous coagulum of dispersed casein flakes. Camel milk does not form a firm curd due to its low content of κ-casein, the casein fraction that directly influences the clotting ability of milk (Ramet, 2001).

### *4.3 Microbial content profile in suusac*

Table 4.3 shows the counts of total viable microorganisms, LAB, yeasts and molds, and coliforms in traditional and laboratory-produced *suusac*. High total viable counts were observed, with the LAB predominating. Relatively lower numbers of fungal flora and coliforms were encountered. The predominance of LAB infers that the expected effect of the microbial profile of *suusac* on the proximate composition of the product (Table 4.1) is a reduction in lactose content as a result of fermentation by the LAB.

There was no significant difference ($p<0.05$) in the numbers of total aerobic mesophilic organisms and coliforms between traditional and laboratory-produced *suusac*. However, the laboratory-produced *suusac* had significantly higher numbers of LAB and yeast and molds than traditional *suusac*. A probable reason for this difference is that the time of sampling of the two products was not synchronous with respect to the growth curve age of the microorganisms, hence the observed slight differences in counts of 1–2 log. Sampling of the laboratory-based product was done after 24 hours, based on the observed duration of traditional camel milk fermentation in Isiolo. It is likely that the LAB and fungal flora in traditional *suusac* were counted when just into

the death phase, whereas the organisms in the laboratory-produced product were counted towards the end of the stationary phase.

**Table 4.3: Number of microorganisms in traditional and laboratory-produced *suusac***

| | Log count ($log_{10}$cfu/ml) | |
|---|---|---|
| | Traditional | Laboratory-produced |
| Total viable count | 9.03 (0.07) | 9.15 (0.11) |
| Lactic acid bacteria (LAB) | 6.77* (0.25) | 8.93* (0.30) |
| Yeasts and Molds | 2.05* (0.17) | 3.76* (0.40) |
| Coliforms | 1.00 (0.02) | 1.00 (0.00) |

Standard deviation in parentheses
Number of replicates = 3
* Significant difference between traditional and laboratory-produced *suusac* ($p<0.05$)

The results obtained for total viable and LAB counts in *suusac*, and the predominance of LAB are similar to those reported by authors (Hamama and Bayi, 1991; Samolada *et al.*, 1998; Mathara, 1999; Abdelgadir *et al.*, 2001) who have studied the microbial composition of traditional fermented milks from different animals. Hamama and Bayi (1991) reported mean log counts of 8.54 and 6.41 log cfu/ml for total aerobic flora and lactobacilli, respectively in *raib*, a Moroccan traditional fermented cow milk product. Samolada *et al.* (1998) studied the changes in microbial flora in ewe's milk fermented for 5 days and reported mean total and LAB log counts as 9.0 and 8.46 log cfu/ml, respectively. In a study of the dominant microorganisms in *rob*, a Sudanese traditional fermented milk, Abdelgadir *et al.* (2001) found the range of log counts of lactococci and lactobacilli to be 7.0–8.0 log cfu/ml. Mathara (1999) recorded the ranges of total viable counts and LAB counts in Kenyan milks *mursik* and *kule naoto* as 7.86–8.85 log cfu/ml and 8.04–8.85 log cfu/ml, respectively.

With respect to coliform counts, the same investigators (Hamama and Bayi, 1991; Samolada *et al.*, 1998) reported higher counts than those obtained in *suusac*, ranging from 5.23 to 7.0 log cfu/ml. However, similarly low numbers of coliforms have been reported in two traditional fermented cow milk products in Kenya (Mathara, 1999). The counts of yeasts and molds in *suusac* were lower than those in other traditional fermented products, which are reported as <2 to 8.08 log cfu/ml in Zimbabwean *amasi* (Gadaga *et al.*, 2000), 6 to 7 log cfu/ml in Sudanese *rob* (Abdelgadir *et al.*, 2001), 4.64 log cfu/ml in Moroccan *raib* (Hamama and Bayi, 1991) and 6.32 and 7.32 log cfu/ml in Kenyan *mursik* and *kule naoto*, respectively (Mathara, 1999). These differences in the numbers of coliforms and fungal flora indicate the unique microbial diversity of the different spontaneously fermented milk products, as influenced by the acidification profiles and specific processing conditions.

The changes in microbial numbers and pH during spontaneous fermentation of camel milk are shown in Figure 4.2. The pH showed a steady decline from an initial value of 6.1 to 4.3 after 24 hours. Initial counts of coliforms and fungal flora were low relative to the LAB, which dominated throughout the fermentation. Within the first 12 hours, there was a steady increase in total, LAB and coliform counts by about 2 log cycles.

Between 16 and 20 hours of fermentation, coliform numbers reduced sharply from 5.2 to 1 log cfu/ml, against a slight increase in LAB numbers from 7.5 to 7.7 log cfu/ml. During that time, the pH decreased from 5.0 to 4.6. This low pH as a result of the production of lactic acid by the LAB is likely to have caused the suppression of coliform numbers in *suusac*. Other authors (Garotte *et al.*, 2000; Gran *et al.*, 2003) have reported the inhibition of *E. coli* and other coliforms by low pH caused by the production of lactic acid in fermented milk products.

The yeast and mold counts remained fairly constant throughout the fermentation period, with only a slight increase of about 1 log cycle from 2.5 to 3.8 log cfu/ml. This limited increase of yeasts and molds in fermenting camel milk is similar to results obtained by Samolada *et al.* (1998) in a study on the microbial flora during manufacture of fermented ewe's milk. Here, low numbers of yeasts were counted during fermentation and the pH decreased from 6.58 to 4.71 in the final product.

However, the levels of yeasts and molds achieved after 24 hours (3.8 log cfu/ml) were lower than those reported in other traditional fermented milks, namely 6–7 log cfu/ml in Sudanese *rob* (Abdelgadir *et al.*, 2001), 4.64 log cfu/ml in Moroccan *raib* (Hamama and Bayi, 1991) and 6.32 and 7.32 log cfu/ml in Kenyan *mursik* and *kule naoto*, respectively (Mathara, 1999). Despite the relatively low levels of yeasts and molds after 24 hours, these organisms are likely to be significant in flavour development in *suusac*. The proteolytic activity of yeasts has been reported to contribute to the flavour of fermented products (Jay, 1992). However, the possibility of inhibition of yeast growth by fungistatic agents produced by the LAB needs to be investigated.

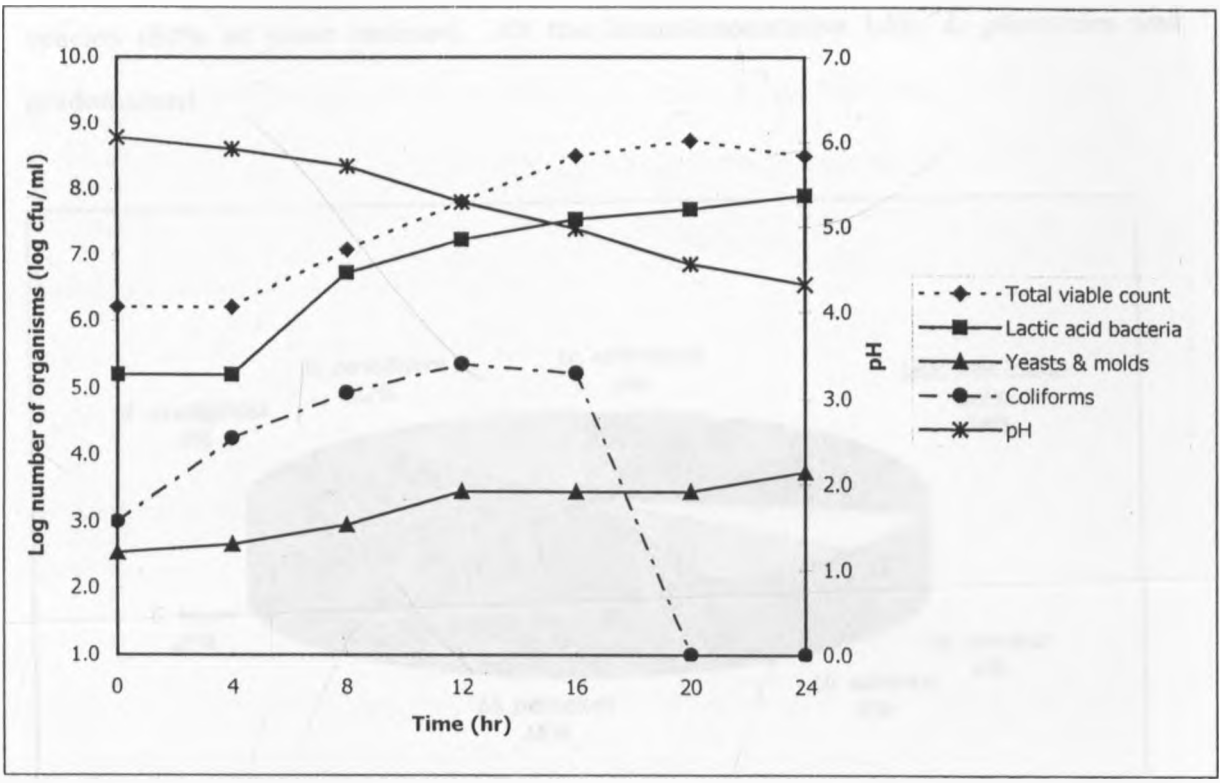


**Figure 4.2: Changes in microbial numbers and pH during spontaneous lactic acid fermentation of camel milk**

### *4.4 Characteristics and identity of microbial isolates in suusac*

From the 15 samples of traditional *suusac* analyzed, 45 LAB and 30 yeast isolates were identified. The 45 LAB isolates were composed of five LAB species, namely, *Lactococcus raffinolactis*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Lactobacillus curvatus*, *Lactobacillus salivarius* and *Lactobacillus plantarum*. The 30 yeast isolates were composed of three species: *Candida krusei*, *Geotrichum penicillatum* and *Rhodotorula mucilaginosa*. The proportions of the isolated LAB and yeast species as percentages of total number of isolates are illustrated in Figure 4.3. *L. mesenteroides* subsp. *mesenteroides*, a heterofermenter, was the most predominant of all the LAB species (40% of LAB isolates), while *C. krusei* was the most commonly isolated yeast

species (50% of yeast isolates). Of the homofermentative LAB, *L. plantarum* was predominant.

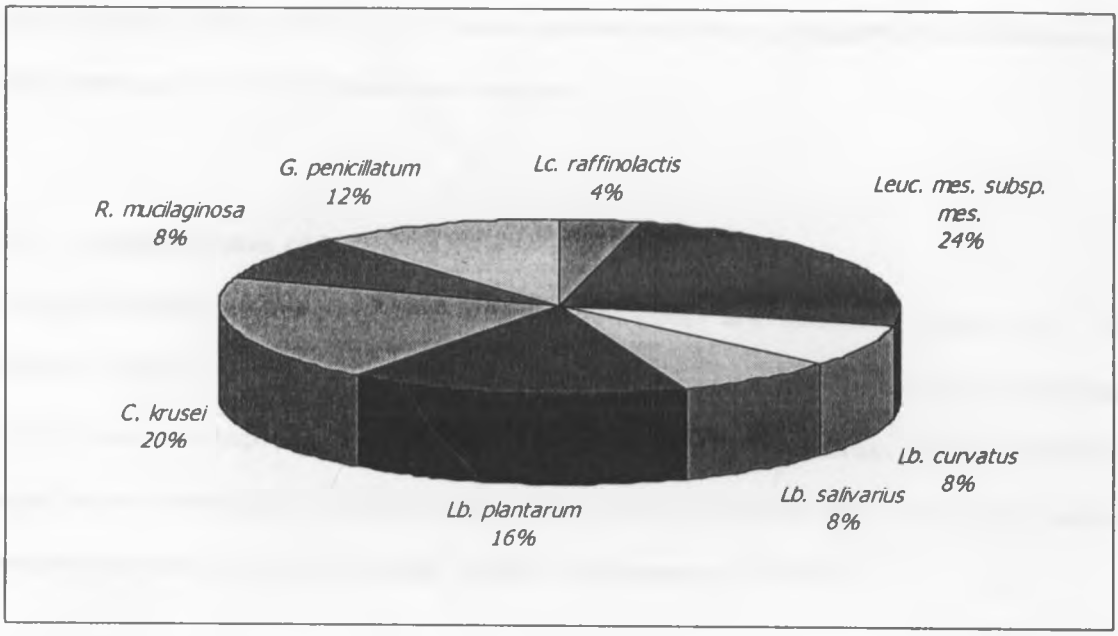


**Figure 4.3: Proportions of lactic acid bacteria and yeast species isolated from traditional *suusac***

The predominance of LAB in various traditionally fermented milks has been reported by several authors, the main genera being leuconostocs, lactobacilli and lactococci (Abdelgadir *et al.*, 2001; Beukes *et al.*, 2001; Gadaga *et al.*, 2001; Lin *et al.*, 1999; Mathara, 1999; Samolada *et al.*, 1998; Hamama and Bayi, 1991; Oberman, 1985). Some investigators (Beukes *et al.*, 2001; Mathara, 1999; Samolada *et al.*, 1998) have also encountered the presence of enterococci and pyogenic streptococci. A wide range of LAB species is to be found in different traditional fermented milks. These products differ very much from each other due to the wide spectrum of metabolic activity and specificity of the bacterial strains, even though they belong to the same species (Oberman, 1985).

The occurrence of yeast species in traditional fermented milk products has been variously reported (Abdelgadir *et al.*, 2001; Lin *et al.*, 1999; Mathara, 1999; Gadaga *et al.*, 2000; Oberman, 1985). *Saccharomyces cerevisiae* and *Candida kefyr* commonly occur though other species of the same genera and other genera such as *Geotrichum*, *Kluyveromyces* and *Pichia* have been isolated.

### 4.4.1 Identification of lactococci

The identification profiles of the isolated lactococci are shown in Table 4.4. The species of lactic-acid cocci were identified as *Lactococcus raffinolactis* and *Leuconostoc mesenteroides* subsp. *mesenteroides*. Both these species were Gram-positive, catalase-negative and mesophilic, capable of growth at 15°C but not at 45°C. They were able to ferment fructose, glucose, sucrose, lactose, galactose and maltose.

*Lactococcus raffinolactis* was homofermentative and produced ammonia from hydrolysis of arginine. It was capable of acidifying, coagulating and reducing litmus milk. Lactose was fermented to lactic acid, and this is a key functional characteristic of the organism in the fermentation of camel milk. Being homofermentative, *L. raffinolactis* does not produce $CO_2$ and lactic acid is the predominant end product of fermentation. The lactococci produce only the L(+) isomer of lactic acid and to a limited extent, impart flavour to fermented dairy products due to the production of certain organic acids (Jay, 1992).

*Leuc. mesenteroides* subsp. *mesenteroides* was heterofermentative, capable of producing $CO_2$ from glucose. It acidified and coagulated litmus milk weakly, and did not produce ammonia from arginine. Fermentation of lactose was positive. Additional products of heterofermentative utilisation of lactose via the 6-

phosphogluconate/phosphoketolase pathway include ethanol, acetic acid and $CO_2$ (Jay, 1992). The genus *Leuconostoc* is capable of converting citrate to aroma compounds such as diacetyl and acetoin (Frazier and Westhoff, 2001), a characteristic important for aroma production in *suusac*.

**Table 4.4: Biochemical profiles of isolated lactococci on API 50 CHL test strips**

| | Isolate code | | |
|---|---|---|---|
| | C201 | C301, C401 | C501, C01, C02, C03 |
| **Characteristic** | | | |
| $CO_2$ from glucose | - | + | + |
| $NH_3$ from arginine | + | - | - |
| Growth at 15°C | + | + | + |
| Growth at 45°C | - | - | - |
| Action on litmus milk: | | | |
| Acidification | + | +/- | +/- |
| Coagulation | + | +/- | +/- |
| Reduction | + | - | - |
| **Substrate** | | | |
| Glycerol | - | - | - |
| Erythritol | - | - | - |
| D-Arabinose | - | - | - |
| L-Arabinose | +/- | + | - |
| Ribose | + | + | +/- |
| D-Xylose | + | + | + |
| L-Xylose | - | - | - |
| Adonitol | - | - | - |
| β-Methyl-D-xyloside | - | - | - |
| Galactose | + | + | + |
| Glucose | + | + | + |
| Fructose | + | + | + |
| Mannose | + | + | + |
| Sorbose | - | - | - |
| Rhamnose | - | - | - |
| Dulcitol | - | - | - |
| Inositol | - | - | - |
| Mannitol | + | - | +/- |
| Sorbitol | + | - | - |
| α-Methyl-D-mannoside | - | - | - |
| α-Methyl-D-glucoside | + | + | + |
| N-Acetyl-Glucosamine | + | + | + |
| Amygdalin | +/- | - | - |
| Arbutin | + | - | - |
| Esculin | + | + | - |
| Salicin | + | - | - |
| Cellobiose | + | + | + |
| Maltose | + | + | + |
| Lactose | + | + | + |
| Melibiose | + | + | - |
| Sucrose | + | + | + |
| Trehalose | + | + | + |
| Inulin | - | - | - |
| Melezitose | - | - | - |
| D-Raffinose | + | + | - |
| Starch | +/- | - | - |
| Glycogen | - | - | - |
| Xylitol | - | - | - |
| Gentiobiose | +/- | - | - |
| D-Turanose | + | + | + |
| D-Lyxose | - | - | - |
| D-Tagatose | - | - | - |
| D-Fucose | - | - | - |
| L-Fucose | - | - | - |
| D-Arabitol | - | - | - |
| L-Arabitol | - | - | - |
| Gluconate | +/- | - | +/- |
| 2-Keto-Gluconate | +/- | - | +/- |
| 5-Keto-Gluconate | - | - | - |
| Identity | *Lactococcus raffinolactis* | *Leuconostoc mes* ssp. *mesenteroides* | *Leuconostoc mes* ssp. *mesenteroides* |

**Key:** + **positive reaction**
+/- **weak reaction**
- **negative reaction**

### 4.4.2 Identification of lactobacilli

The identification profiles of the isolated lactobacilli are shown in Table 4.5. They were identified as *Lactobacillus curvatus, Lactobacillus salivarius* and *Lactobacillus plantarum.* All of these species were Gram-positive, catalase-negative, homofermentative and mesophilic, capable of growth at 15°C but not at 45°C. *L. curvatus* and *L. salivarius* exhibited a weak reaction in producing ammonia from arginine, whereas *L. plantarum* did not hydrolyse arginine. Litmus milk was acidified and coagulated by all the three species, while litmus milk reduction was positive only in *L. curvatus* and *L. plantarum.*

The fermentation of carbohydrate substrates by the isolated lactobacilli indicated a diverse result, with *L. plantarum* fermenting more substrates than the other two organisms. However, all of the isolated *Lactobacillus* species fermented lactose, a key functional characteristic in the fermentation of camel milk. Since the isolated lactobacilli are homofermenters, lactic acid is the predominant product of lactose fermentation via the glycolytic pathway (Jay, 1992). Other key sugars fermented by all three species of lactobacilli were glucose, fructose, galactose and maltose. Ability to ferment sucrose was exhibited only by *L. plantarum.*

**Table 4.5: Biochemical profiles of isolated lactobacilli on API 50 CHL test strips**

| | Isolate code | | |
|---|---|---|---|
| | R401, R403 | R02, R06 | R01, R03, R04, R05 |
| **Characteristic** | | | |
| $CO_2$ from glucose | - | - | - |
| $NH_3$ from arginine | +/- | +/- | - |
| Growth at 15°C | + | + | + |
| Growth at 45°C | - | - | - |
| Action on litmus milk: | | | |
| Acidification | + | + | + |
| Coagulation | +/- | + | + |
| Reduction | + | - | + |
| **Substrate** | | | |
| Glycerol | - | - | - |
| Erythritol | - | - | - |
| D-Arabinose | - | - | - |
| L-Arabinose | - | + | + |
| Ribose | + | + | + |
| D-Xylose | + | + | - |
| L-Xylose | - | - | - |
| Adonitol | - | - | - |
| β-Methyl-D-xyloside | - | - | - |
| Galactose | + | + | + |
| Glucose | + | + | + |
| Fructose | + | + | + |
| Mannose | + | + | + |
| Sorbose | +/- | + | - |
| Rhamnose | - | + | - |
| Dulcitol | - | - | - |
| Inositol | - | - | - |
| Mannitol | - | - | + |
| Sorbitol | - | + | + |
| α-Methyl-D-mannoside | - | - | + |
| α-Methyl-D-glucoside | + | - | - |
| N-Acetyl-Glucosamine | +/- | + | + |
| Amygdalin | - | - | + |
| Arbutin | +/- | - | + |
| Esculin | + | - | + |
| Salicin | +/- | - | + |
| Cellobiose | - | - | + |
| Maltose | + | + | + |
| Lactose | + | + | + |
| Melibiose | +/- | - | + |
| Sucrose | - | - | + |
| Trehalose | - | + | + |
| Inulin | - | - | - |
| Melezitose | - | - | + |
| D-Raffinose | - | + | + |
| Starch | - | - | - |
| Glycogen | - | - | - |
| Xylitol | - | - | - |
| Gentiobiose | - | - | + |
| D-Turanose | - | - | + |
| D-Lyxose | - | - | - |
| D-Tagatose | - | - | - |
| D-Fucose | - | - | - |
| L-Fucose | + | + | - |
| D-Arabitol | - | - | - |
| L-Arabitol | - | - | - |
| Gluconate | - | + | + |
| 2-Keto-Gluconate | - | - | - |
| 5-Keto-Gluconate | +/- | - | - |
| Identity | *Lactobacillus curvatus* | *Lactobacillus salivarius* | *Lactobacillus plantarum* |

**Key:** + **positive reaction**
+/- **weak reaction**
- **negative reaction**

### 4.4.3 Identification of yeasts

Table 4.6 indicates the identification profiles of the isolated yeast species, based on colony characteristics and assimilation patterns of carbohydrate substrates. The yeasts were identified as *Rhodotorula mucilaginosa*, *Geotrichum penicillatum* and *Candida krusei*.

**Table 4.6: Biochemical profiles of isolated yeasts on API 20 C AUX test strips**

| | Isolate code | | |
|---|---|---|---|
| | Y01,Y02 | Y101, Y201, Y202 | Y102, Y301, Y302, Y303, Y304 |
| **Characteristic** | | | |
| Colony colour | pink | white | white |
| Colony shape | circular, smooth | circular, butyrous | circular, butyrous |
| Ascospores | absent | absent | absent |
| Budding cells | present | absent | present |
| Hyphae/pseudohyphae | absent | absent | present |
| **Substrate** | | | |
| Glucose | + | + | + |
| Glycerol | + | + | + |
| 2-Keto-D-gluconate | - | - | - |
| L-Arabinose | + | - | - |
| D-Xylose | + | + | - |
| Adonitol | + | - | - |
| Xylitol | - | - | - |
| Galactose | + | + | - |
| Inositol | - | - | - |
| Sorbitol | + | + | - |
| α-Methyl-D-glucoside | - | - | - |
| N-Acetyl-Glucosamine | - | - | + |
| Cellobiose | - | - | - |
| Lactose | - | - | + |
| Maltose | + | - | - |
| Sucrose | + | - | - |
| Trehalose | + | - | - |
| Melezitose | + | - | - |
| Raffinose | + | - | - |
| Identity | *Rhodotorula mucilaginosa* | *Geotrichum penicillatum* | *Candida krusei* |

**Key:** **+ positive reaction**
**- negative reaction**

Colonies of *R. mucilaginosa* were smooth and pink. The organism exhibited multilateral budding, did not form ascospores or pseudohyphae and assimilated glucose, galactose, maltose and sucrose. It did not assimilate lactose. Members of the genus *Rhodotorula* are non-fermenters of carbohydrates (Jay, 1992). *R. mucilaginosa* and *R. glutinis* are the two most common species in foods. They produce pink or red pigments and cause discolouration of foods such as fresh poultry, fish, beef and sauerkraut (Jay, 1992; Frazier and Westhoff, 2001). They have also been reported to cause spoilage in dairy products such as yoghurt, butter, cream and cheese (Pitt and Hocking, 1999). It may therefore be inferred that the presence of *Rhodotorula* in *suusac* is undesirable because its presence in dairy products leads to spoilage.

*Geotrichum penicillatum* formed white, butyrous colonies and did not form hyphae or pseudohyphae. It assimilated glucose, galactose and sorbitol. The genus *Geotrichum* may be classified as yeast-like fungi or molds. *G. candidum*, also known as *Oospora lactis*, is the most important species in food. It is referred to as the dairy mold and contributes to flavour and aroma development in cheese and is also responsible for the characteristic taste and aroma of *gari*, a fermented cassava product. (Jay, 1992). It may therefore be expected that *G. penicillatum* plays a functional role in the development of the taste and flavour of *suusac*.

*Candida krusei* formed white, butyrous colonies, reproduced by budding and formed pseudohyphae. It was able to assimilate glucose, lactose and glycerol. Frazier and Westhoff (2001) report that *C. krusei* has been used together with lactic starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria although the exact mechanisms of this association are not confirmed. This could imply a symbiotic association between *C. krusei* and the lactic acid bacteria involved in *suusac* production. *C. krusei* is involved in fermentation of cacao beans, playing an

essential role in development of the desirable chocolate flavour of roasted beans. The flavour development is due to the proteolytic activity of the yeasts (Jay, 1992). *C. krusei* may possibly play a functional role in flavour development in *suusac*.

### *4.5 Functional properties of isolates in skim milk*

The functional characteristics of the isolates, with respect to acidification and diacetyl production are indicated in Table 4.7. The fermentation medium used was 10% reconstituted skim milk + 0.5% D-glucose. *Lactococcus raffinolactis* produced significantly more lactic acid ($p<0.05$), resulting in an average pH of 3.5 as compared to 4.3–4.5 produced by the rest of the LAB isolates.

The low pH achieved in *suusac* may be attributed to most (60%) of the LAB being homofermentative; *Leuconostoc mesenteroides* was the only heterofermentative LAB species isolated. Homofermentative LAB ferment lactose or glucose almost exclusively to lactic acid while forming only small quantities of formic acid, acetic acid and ethanol (Oberman, 1985).

*L. raffinolactis* and *C. krusei* were the only two species that did not produce detectable levels of diacetyl. The recorded inability of the isolated *Candida* strain to utilize citrate is similar to the results reported by Gadaga *et al.* (2000), who isolated from Zimbabwean traditional fermented milk strains of *C. kefyr* that could assimilate lactose and DL- lactate but not citrate.

*L. curvatus* produced more diacetyl than the rest of the isolated species. Members of the genus *Leuconostoc* are able to ferment citrate to produce flavour compounds such as diacetyl, acetoin and 2,3-butanediol (Frazier and Westhoff, 2001), although the levels of diacetyl produced by *L. mesenteroides* in *suusac* were slightly lower than

those of *L. plantarum* and *L. curvatus*. In addition to diacetyl, other flavour compounds, such as acetaldehyde, ethanol and organic acids, are important contributors to the overall flavour and aroma profile of fermented milks (Oberman, 1985).

**Table 4.7: Production of acid and flavour by isolated lactic acid bacteria and yeasts**

| | pH | Titratable acidity | Diacetyl score |
|---|---|---|---|
| *Rhodotorula mucilaginosa* | 5.0[a] (0.2) | 0.28[a] (0.03) | 2 |
| *Lactobacillus salivarius* | 4.3[b] (0.1) | 0.46[b] (0.08) | 1 |
| *Lactobacillus curvatus* | 4.3[b] (0.0) | 0.47[b] (0.11) | 4 |
| *Leuconostoc mes.* subsp. *mes.** | 4.4[b] (0.1) | 0.52[b] (0.07) | 2 |
| *Lactobacillus plantarum* | 4.5[b] (0.0) | 0.57[b] (0.13) | 3 |
| *Geotrichum penicillatum* | 3.5[c] (0.1) | 0.64[c] (0.14) | 2 |
| *Lactococcus raffinolactis* | 3.5[c] (0.1) | 0.67[c] (0.14) | 0 |
| *Candida krusei* | 3.3[c] (0.1) | 0.83[c] (0.05) | 0 |

* *Leuconostoc mesenteroides* subsp. *mesenteroides*
Fermentation medium 10% reconstituted skim milk + 0.5% D-glucose. Determinations after 30°C incubation, 24 hr.
For each attribute, means with the same superscript are not significantly different ($p \leq 0.05$)
Mean (s.d.)
Number of replicates = 3

Results of the proteolytic activity (casein hydrolysis) of the LAB and yeast isolates are indicated in Table 4.8. *L. salivarius* recorded the highest proteolytic activity. *C. krusei* was the only yeast isolate that showed proteolytic activity, although this was lower than that exhibited by the LAB isolates. Essential amino acids and low molecular weight peptides are present in milk in low concentrations, hence proteolytic activity of LAB is important to facilitate their growth. The free amino acid content in fermented milks is several times higher than in whole milk. Significant amounts of peptides are liberated during fermentation, but the concentration varies depending on the bacterial strain (Oberman, 1985).

**Table 4.8: Hydrolysis of casein by isolated lactic acid bacteria and yeasts**

| | Width of agar clearing (mm) | Rating of proteolysis |
|---|---|---|
| *Lactobacillus salivarius* | 8.3[a] (1.8) | High |
| *Leuconostoc mes.* subsp. *mes.* | 6.9[ab] (1.5) | High |
| *Lactobacillus curvatus* | 5.7[b] (1.7) | High |
| *Candida krusei* | 2.3[c] (0.4) | Low |
| *Lactobacillus plantarum* | 0 | Negative |
| *Lactococcus raffinolactis* | 0 | Negative |
| *Geotrichum penicillatum* | 0 | Negative |
| *Rhodotorula mucilaginosa* | 0 | Negative |

Mean (s.d.)
Number of replicates = 3
Means with the same superscript are not significantly different ($p<0.05$).

### *4.6 Production of acidity and flavour by single cultures in camel and cow milk*

Figures 4.4 and 4.5 illustrate the pH and diacetyl scores, respectively, of single-strain cultures in cow and camel milk. There was no significant difference ($p<0.05$) between the pH of cow and camel milk for all the isolates, except for *L. plantarum* and *R. mucilaginosa*, which produced more acid in cow milk.

*R. mucilaginosa* produces rhodotorulic acid when grown in iron-limited conditions (Andersen *et al.*, 2003). Cow milk contains significantly less iron than camel milk (Wangoh, 1997). Thus, it is likely that *R. mucilaginosa* produced rhodotorulic acid when cultured in cow milk, hence the observed lower pH in cow milk as compared to camel milk. The reason why *L. plantarum* produced more acid in cow milk is not readily clear. Since the lactose contents of camel milk and cow milk are similar (Wangoh, 1997), it would be expected that *L. plantarum* would produce fairly similar

levels of lactic acid in the two milk products. The property exhibited by *L. plantarum* of higher lactic acid production in cow milk warrants further investigation.

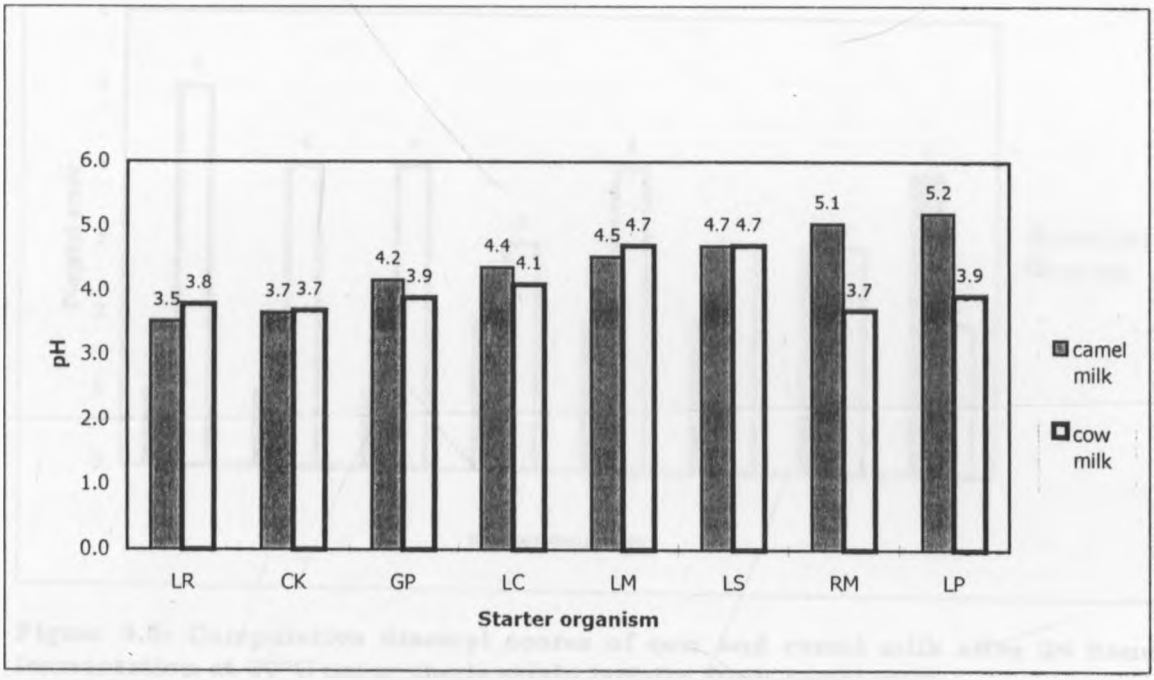


**Figure 4.4: Comparative pH of cow and camel milk after 24 hours' fermentation at 30°C using single strain isolates from camel milk**

Key: LR = *Lactococcus raffinolactis*, CK = *Candida krusei*, GP = *Geotrichum penicillatum*, LC = *Lactobacillus curvatus*, LM = *Leuconostoc mesenteroides* subsp. *mesenteroides*, LS = *Lactobacillus salivarius*, RM = *Rhodotorula mucilaginosa*, LP = *Lactobacillus plantarum*.

Figure 4.5 indicates that *L. curvatus* produced more diacetyl in camel milk than cow milk, while the diacetyl scores for *L. plantarum* were equal in both cow and camel milk. The rest of the isolates produced more diacetyl in cow milk. The likely reason for higher diacetyl production in cow milk is a higher citrate content in cow milk as compared to camel milk. Citrate is the substrate for diacetyl production by citrate-utilizing LAB species (Hugenholtz *et al.*, 2002). The citrate content in cow milk is 2% (IDF, 1990) while that of camel milk is slightly lower, ranging from 1.4–1.7% (Wangoh, 1997). The higher diacetyl production in cow milk is likely to impart to the product a

different flavour profile from camel milk, since diacetyl is responsible for the distinct buttermilk flavour of fermented dairy products (Frazier and Westhoff, 2001).

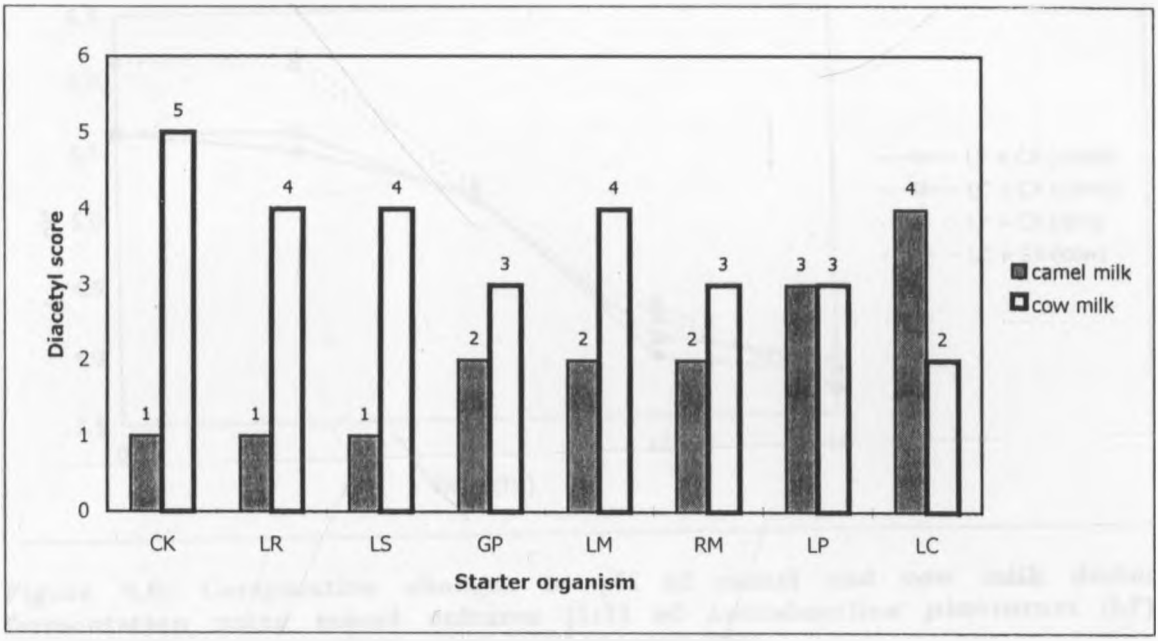


**Figure 4.5: Comparative diacetyl scores of cow and camel milk after 24 hours' fermentation at 30°C using single strain isolates from camel milk**

Key: CK = *Candida krusei*, LR = *Lactococcus raffinolactis*, LS = *Lactobacillus salivarius*, GP = *Geotrichum penicillatum*, LM = *Leuconostoc mesenteroides* subsp. *mesenteroides*, RM = *Rhodotorula mucilaginosa*, LP = *Lactobacillus plantarum*, LC = *Lactobacillus curvatus*.

### *4.7 Production of acidity and flavour by mixed cultures*

Three isolates were selected for the mixed-culture fermentation trials to assess acid and flavour producing capabilities. Two LAB isolates (*L. curvatus* and *L. plantarum*) were selected on the basis of having the two highest diacetyl flavour scores (Table 4.7), while *C. krusei* was selected because it was the only yeast isolate to exhibit some degree of proteolytic activity (Table 4.8). Figures 4.6 and 4.7 show the comparative changes in pH of cow and camel milk during fermentation using four combinations of the selected cultures. The fermentation was done according to the flow diagram in Figure 3.1, with mixed cultures in the ratio of 1:1 or 1:1:1 as appropriate. Diacetyl scores were determined after 16 hours of fermentation.

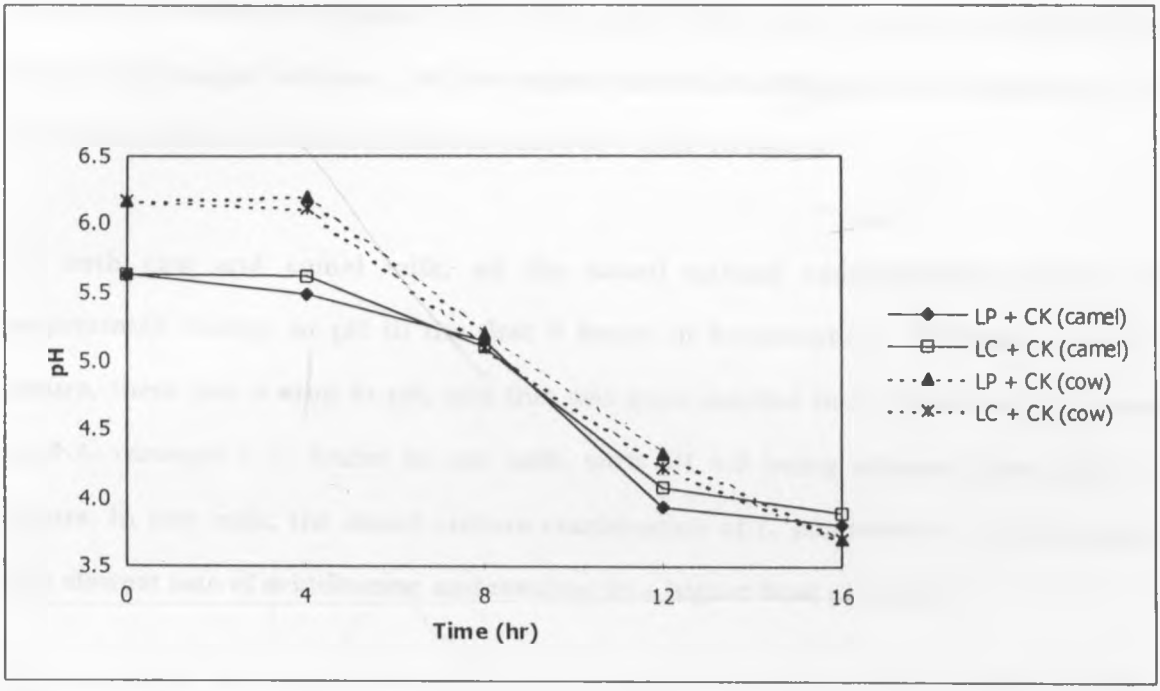


**Figure 4.6: Comparative changes in pH of camel and cow milk during fermentation using mixed cultures (1:1) of *Lactobacillus plantarum* (LP), *Lactobacillus curvatus* (LC) and *Candida krusei* (CK)**

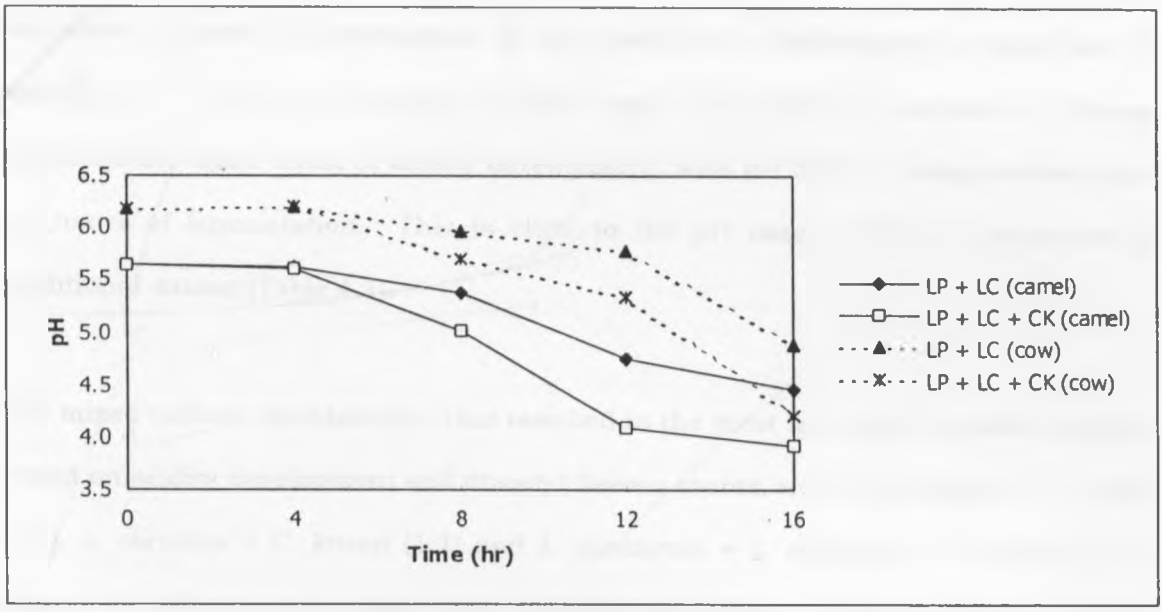


**Figure 4.7: Comparative changes in pH of cow and camel milk during fermentation using mixed cultures (1:1 and 1:1:1) of *Lactobacillus plantarum* (LP), *Lactobacillus curvatus* (LC) and *Candida krusei* (CK)**

Regarding flavour development, the performance of the mixed cultures was superior to that of the single cultures. All the mixed cultures combinations in both camel and cow milk achieved a diacetyl flavour score of 4 after 16 hours.

In both cow and camel milk, all the mixed culture combinations recorded no appreciable change in pH in the first 4 hours of fermentation. Between 4 and 12 hours, there was a drop in pH, and this was most marked in *L. plantarum* + *C. krusei* and *L. curvatus* + *C. krusei* in cow milk, with pH 4.5 being attained after about 10 hours. In cow milk, the mixed culture combination of *L. plantarum* + *L. curvatus* had the slowest rate of acidification and resulted in a higher final pH of 5.0.

In camel milk, the combination of *L. plantarum* + *L. curvatus* also showed the slowest rate of acidification, attaining a final pH of 4.5 after 16 hours as compared to the other three mixed culture combinations, which achieved the isoelectric point of casein (pH 4.6) after 10 hours of fermentation. All the other three combinations in camel milk (*L. plantarum* + *C. krusei*, *L. curvatus* + *C. krusei* and *L. plantarum* + *L. curvatus* + *C. krusei*) exhibited the same trend of acidity development, with pH 3.9–4.1 being attained after 12 hours of fermentation. This is close to the pH range of 4.0–4.2 measured in traditional *suusac* (Table 4.1).

The mixed culture combinations that resulted in the most favourable *suusac* products, based on acidity development and diacetyl flavour scores, were *L. plantarum* + *C. krusei* (1:1), *L. curvatus* + *C. krusei* (1:1) and *L. plantarum* + *L. curvatus* + *C. krusei* (1:1:1). Figure 4.4 indicates that single-strain fermentation using *L. plantarum* and *L. curvatus* resulted in pH 5.2 and 4.4, respectively, after 24 hours. However, the use of *C. krusei* together with these LAB in the ratio 1:1 resulted in attainment of pH 3.9 (*L. plantarum*) and 4.1 (*L. curvatus*) after 12 hours' fermentation. The above results imply that *C.*

*krusei* plays a role in enhancing the activity of the LAB starters, *L. plantarum* and *L. curvatus*, by increasing the rate of acidification of camel milk during fermentation. Holzapfel (2002) also reports a stimulating effect of *C. krusei* on *Lactobacillus fermentum* and *Lactobacillus brevis* during mixed starter culture fermentation of *mawé*, a fermented maize product. This symbiotic relationship has also been recorded by Frazier and Westhoff (2001), whereby *C. krusei* is used together with dairy starters to increase the activity and longevity of lactic acid bacteria.

# CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The traditional method of *suusac* production as practised by the Somali community of Isiolo, Kenya involves spontaneous fermentation of camel milk at ambient temperature for 1–2 days. Pre-smoked gourds are used as the fermenting vessels and the milk is not subjected to heat treatment prior to fermentation. The fermentation process involves the interaction of mixed microflora of lactic acid bacteria and yeasts.

The predominant LAB and yeast species isolated from *suusac* were *Leuconostoc mesenteroides* subsp. *mesenteroides* (24% of total isolates) and *Candida krusei* (20%), respectively. However, homofermentative LAB were more commonly isolated; *L. mesenteroides subsp. mesenteroides* was the only heterofermentative LAB isolated. The other LAB species isolated from traditional *suusac* were *Lactococcus raffinolactis* (4%), *Lactobacillus curvatus* (8%), *Lactobacillus salivarius* (8%) and *Lactobacillus plantarum* (16%). *Candida krusei* was the only lactose-assimilating yeast isolated. The other isolated yeast species were *Geotrichum penicillatum* (12%) and *Rhodotorula mucilaginosa* (8%).

The primary functional role of the LAB was fermentation of lactose to lactic acid. All the LAB isolates recorded high proteolytic activity except for *Lactococcus raffinolactis*, which did not exhibit any proteolytic activity. The LAB isolates showed varying degrees of diacetyl production. Because of their limited carbohydrate assimilating capabilities, the main functional role of the yeast cultures in *suusac* appears to be flavour development and proteolysis.

*Candida krusei* plays an important role in mixed starter fermentation of camel milk by increasing the activity of the LAB cultures and improving the flavour of *suusac*. The

use of *C. krusei* with *L. plantarum* and *L. curvatus* reduced the fermentation time by half as compared to the use of the cultures individually. The diacetyl flavour scores of the products made using the above mixed cultures were superior compared to those made using the single cultures.

The present study has isolated and identified 5 lactic acid bacteria and 3 yeast species from traditional *suusac*. However, the study findings have established that the use of mixed cultures of *C. krusei:L. plantarum* and *C. krusei:L. curvatus* in the ratio 1:1 produces an acceptable *suusac* product from the standpoint of acidification and flavour production. It is therefore recommended that these mixed cultures be used for large-scale production trials of *suusac*. However, the sensory quality (taste and consistency) of the starter-fermented milk should also be investigated.

Another aspect of traditional fermentation of camel milk that warrants further investigation is the effect of gourd smoking on the microbiological and biochemical properties of *suusac*. The plants used for gourd smoking need to be characterised to aid in understanding the role of the smoke in milk.

Following the observation of a drastic decline in coliform numbers after 16 hours during spontaneous fermentation of camel milk, further investigation is needed on the antimicrobial activity of LAB in *suusac* against coliforms. The LAB should be screened for bacterial antagonistic potential and their spectrum of antimicrobial activity established. This phenomenon could be of significance in contributing to the safety and keeping quality of *suusac*.

## CHAPTER 6: REFERENCES


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## APPENDIX 1: Interview guide for *suusac* production

**Milking procedure**

1. Is the milk collected in special containers? How are these containers handled?
2. How is the milk handled after milking and before fermentation?
3. What are the local names of the containers/gourds used to collect and ferment milk? What are the local names of the trees from which these gourds are made?
4. Describe the process of preparing these gourds for use. How are the containers stored?
5. Describe the process of smoking the containers. What are the local names of the trees that are used for smoking? What are some of the noted effects of smoking containers (e.g. effects on *suusac* flavour, storage life)?

**Fermentation**

1. Is the milk left to ferment spontaneously or is some previously fermented milk added as a starter?
2. Is the milk heated prior to fermentation?
3. Are there any additives to the milk?
4. How long does the fermentation take? (Measure range of fermentation temperature)
5. How is the product treated after fermentation is complete?
6. Describe the appearance of the fermented product (colour; smell; taste; appearance – even, curdled, smooth, etc.)
7. How is the product stored? How long is the product's storage life, under these conditions?