

# **Lactic acid bacteria in South African indigenous fermented milks and the evaluation of selected strains for application in the manufacturing of cultured milk**

by

**Elisabeth Maria Beukes**

**Submitted in partial fulfillment of the  
requirements for the degree**

**MSc (Agric) in Food Science**

**Department of Food Science  
Faculty of Biological and Agricultural Sciences  
University of Pretoria**

**Study Leader: Prof. B. H. Bester  
Co-Study Leader: Dr. J. F. Mostert**

**8 November 1999**

## DECLARATION

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

*EMBekkers*

8 November 1999

EMB  
Bekkers

## ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following persons and institutions for their contributions to the successful completion of this study.

- Dr. J. F. Mostert (ARC-Animal Nutrition and Animal Products Institute), as co-study leader and head of the Division Quality, Safety and Environmental Protection, for his guidance and constant encouragement throughout this study.
- Prof. B. H. Bester (Department of Food Science, University of Pretoria), as study-leader, for his vital contribution and support.
- My colleagues B. Lategan, T. Loretan, J. J. McDonald, N. A. Prinsloo and A-M Vögel (ARC-Animal Nutrition and Animal Products Institute), for their teamwork on this project and loyal backing.
- Dr. H. C. Schönfeldt and Ms. M. Scheepers (ARC-Animal Nutrition and Animal Products Institute) for their guidance and practical assistance in the sensory evaluation study.
- Dr. D. van Zyl and Ms. K. Kruger (Department of Statistics and Data Analysis, University of Pretoria) for analysing the results.
- All other personnel of the Agricultural Research Council who gave their assistance and support.
- The Agricultural Research Council, for providing the funds necessary to conduct the research work.
- My family and friends for their interest, patience and loving support.

- To the Almighty Father, without Whom I cannot do anything.

## ABSTRACT

### Lactic acid bacteria in South African indigenous fermented milks and the evaluation of selected strains for application in the manufacturing of cultured milk

by

E. M. Beukes

Leader: Prof. B. H. Bester

Co-leader: Dr. J. F. Mostert

Department: Food Science

Degree: MSc (Agric) Food Science and Technology

Fifteen samples of traditional fermented milk were obtained from individual households in South Africa and Namibia. The microflora of these samples were dominated by lactic acid bacteria, especially by the genera *Leuconostoc* (35%), *Lactococcus* (28%) and *Lactobacillus* (23%). Eighty-three percent of the leuconostocs were identified as *Leuconostoc mesenteroides* subsp. *dextranicum*, while *Leuconostoc citreum* and *Leuconostoc lactis* occurred in much lower numbers. Other species identified included *Lactococcus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus plantarum*. The species *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris* frequently used in commercial mesophilic starter cultures were, however, not encountered in the fermented milk samples.

Two-hundred-and-thirty-six selected lactic acid bacterial strains were subsequently screened for technological important properties. Tests for acid development showed that 55 of the 103 strains of *Lactococcus lactis* subsp. *lactis* coagulated reconstituted non-fat milk within 16 h of incubation at 22 °C. Of the nine representative strains which were screened for the production of flavour compounds only one, namely *Leuconostoc lactis*, produced diacetyl. This strain, which also grew well in milk, may be successfully used in starter cultures. *Leuconostoc citreum* and *Leuconostoc dextranicum* were unable to ferment lactose and to grow in milk. Two strains of *Lactobacillus plantarum* coagulated non-fat milk within 48 h. A fermented

milk product made with specific strains of *Lactococcus lactis* subsp. *lactis* and *Leuconostoc lactis* combined with a strain of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* received the most favourable comments during sensory evaluation.

Less than half of the fermented milk samples from households complied with the local Health regulations regarding the presence of coliform bacteria for this specific product. Furthermore, the presence of *Escherichia coli* and *Staphylococcus aureus* in some of the products emphasized the importance of production hygiene during manufacturing of dairy products in small-scale operations. This study also brought to light the intricate task involved in starter culture research, especially in the field of mesophilic cultures for fermented milk.

**Key words:** Traditional fermented milk, lactic acid bacteria, technologically important properties, food safety

## UITTREKSEL

### Melksuurbakterieë in Suid-Afrikaanse inheemse gefermenteerde melk en die evaluering van geselecteerde stamme vir gebruik in die vervaardiging van aangesuurde melk.

deur

E. M. Beukes

Studieleier: Prof. B. H. Bester

Mede-studieleier: Dr. J. F. Mostert

Departement: Voedselwetenskap

Graad: MSc (Agric) Voedselwetenskap en -tegnologie

Vyftien tradisioneel-gefermenteerde melkmonsters is versamel vanaf individuele huishoudings in Suid-Afrika en Namibië. Melksuurbakterieë het die mikrobepopulasie oorheers met *Leuconostoc* (35%), *Lactococcus* (28%) en *Lactobacillus* (23%) die belangrikste genera. Drie-en-tig persent van die leukonostoks is geïdentifiseer as *Leuconostoc mesenteroides* subsp. *dextranicum*, terwyl *Leuconostoc citreum* en *Leuconostoc lactis* in aansienlik laer getalle voorgekom het. *Lactococcus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *lactis* en *Lactobacillus plantarum* is ook aangetref. Die spesies *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* en *Leuconostoc mesenteroides* subsp. *cremoris* wat algemeen in kommersiële mesofiele suurselkulture gebruik word, was afwesig in die tradisioneel-gefermenteerde melk.

Twee-honderd-ses-en-dertig geselecteerde melksuurbakteriestamme is voorts geëvalueer ten opsigte van tegnologies-belangrike eienskappe. Toetse vir die ontwikkeling van melksuur het getoon dat 55 van die 103 stamme van *Lactococcus lactis* subsp. *lactis* aangemaakte afgeroomde melk binne 16 h by 22 °C gekoaguleer het. Beide *Leuconostoc citreum* en *Leuconostoc dextranicum* benut nie laktose nie en het gevolglik nie in die melk gegroeи nie. Twee stamme van *Lactobacillus plantarum* kon aangemaakte afgeroomde melk binne 48 h koaguleer. Van die nege verteenwoordigende stamme wat ondersoek is vir die produksie van geurkomponente kon slegs een stam, *Leuconostoc lactis*, diasetiel produseer. Hierdie stam het

ook goed gegroei in melk en toon potensiaal vir gebruik in suurselkulture vir gefermenteerde melk. Sensoriese evaluering van gefermenteerde melkprodukte vervaardig met spesifieke stamme van *Lactococcus lactis* subsp. *lactis* en *Leuconostoc lactis*, in kombinasie met 'n stam van *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, het getoon dat hierdie stamme ook in suurselkulture vir gefermenteerde melk gebruik kan word.

Minder as die helfte van die tradisioneel-gefermenteerde melkmonsters het voldoen aan die plaaslike gesondheidsregulasies rakende die teenwoordigheid van kolivormige bakterieë in rou aangesuurde melk. Die teenwoordigheid van *Escherichia coli* en *Staphylococcus aureus* in sommige van die produkte het voorts die belangrikheid beklemtoon van higiëne tydens vervaardiging van suiwelprodukte op klein skaal. Hierdie studie het ook lig gewerp op die ingewikkelse en omvattende taak van suurselfnavorsing, veral ten opsigte van mesofiele kulture vir gefermenteerde melkprodukte.

## TABLE OF CONTENTS

### ABSTRACT

### UITTREKSEL

LIST OF FIGURES.....	i
LIST OF TABLES.....	i
LIST OF APPENDICES.....	ii
1. INTRODUCTION.....	1
1.1 Objectives.....	2
2. LITERATURE REVIEW.....	3
2.1 Classification of fermented milks.....	3
2.1.1 <i>Type of fermenting organisms</i> .....	3
2.1.2 <i>Pre- and/or post-fermenting processes</i> .....	4
2.1.3 <i>The type of milk used</i> .....	5
2.2 Significance of fermented milks.....	5
2.2.1 <i>Nutritional value and role in the diet</i> .....	5
2.2.2 <i>Therapeutic qualities</i> .....	7
2.2.3 <i>Alleviation of lactose intolerance</i> .....	8
2.2.4 <i>Preservation</i> .....	9
2.2.5 <i>Social values</i> .....	10
2.2.6 <i>Improved flavour, variety and acceptability</i> .....	12
2.2.7 <i>Source of income</i> .....	12
2.3 The manufacturing processes of traditional fermented milk.....	13
2.4 Characteristics of traditional fermented milks.....	20
2.4.1 <i>Chemical characteristics</i> .....	20
2.4.2 <i>Microbiological characteristics</i> .....	22

2.5	Food Safety.....	25
2.6	Modernisation and industrialisation of African fermented milks.....	27
2.7	Starter function.....	28
2.7.1	<i>Acid production</i> .....	28
2.7.2	<i>Gas production</i> .....	30
2.7.3	<i>Flavour production</i> .....	31
2.7.3.1	Compounds involved in flavour production.....	31
2.7.3.2	Factors determining and influencing diacetyl production and destruction.....	33
2.7.3.3	Metabolism problems in relation to culture use and balance of cultures.....	37
3.	MATERIALS AND METHODS.....	39
3.1	Collection of samples.....	39
3.2	Microbiological analysis.....	40
3.2.1	<i>Enumeration of micro-organisms</i> .....	40
3.2.2	<i>Isolation, cultivation and preservation of lactic acid bacteria</i> .....	40
3.2.3	<i>Identification of the lactic acid bacteria to genus level</i> .....	40
3.2.4	<i>Identification of the lactic acid bacteria to species level</i> .....	43
3.2.4.1	Lactococci.....	43
3.2.4.2	Leuconostocs.....	43
3.2.4.3	Identification using the API 50 CH system.....	45
3.2.5	<i>Detection of pathogens</i> .....	45
3.3	Evaluation of the technologically important properties of lactic acid bacteria....	45
3.3.1	<i>Acid production</i> .....	45
3.3.2	<i>Gas production</i> .....	46
3.3.3	<i>Production of volatile organic compounds by selected strains</i> .....	47

3.3.3.1 Preparation of the fermented milk samples for determination of volatile organic compounds.....	47
3.3.3.2 Determination of volatile organic compounds.....	48
3.3.4 <i>Growth characteristics of selected strains in reconstituted non-fat milk.....</i>	48
3.4 Evaluation of selected strains for their suitability as starter cultures for fermented milk products.....	49
3.4.1 <i>Preliminary evaluation of selected strains using "bench-top" sensory evaluation.....</i>	49
3.4.2 <i>Evaluation of three selected strains for their suitability as starter cultures for fermented milk products.....</i>	50
3.4.2.1 Obtaining commercial "Maas" products.....	50
3.4.2.2 Training and vocabulary development for sensory evaluation using four commercial products.....	50
3.4.2.3 Manufacturing of four fermented milk products using three selected strains of lactic acid bacteria.....	51
3.4.2.4 Sensory evaluation of four commercial and four experimental fermented milk products.....	52
3.4.2.5 Determination of pH, viscosity and flow properties of the fermented milk products.....	53
3.4.2.6 Statistical analysis.....	53
4. RESULTS.....	54
4.1 Collection of samples.....	54
4.2 Microbiological analysis.....	55
4.2.1 <i>Enumeration of micro-organisms.....</i>	55
4.2.2 <i>Identification of the lactic acid bacteria to genus level.....</i>	55
4.2.3 <i>Identification of the lactic acid bacteria to species level.....</i>	60
4.2.3.1 Lactococci.....	60

4.2.3.2 Leuconostocs.....	60
4.2.3.3 Identification using the API 50 CH system.....	61
4.2.4 <i>Detection of pathogens</i> .....	61
4.3 Evaluation of the technologically important properties of lactic acid bacteria....	61
4.3.1 <i>Acid production</i> .....	61
4.3.2 <i>Gas production</i> .....	63
4.3.3 <i>Production of volatile organic compounds by selected strains</i> .....	63
4.3.4 <i>Growth characteristics of selected strains in reconstituted non-fat milk</i> .....	63
4.4 Evaluation of the sensory and objective characteristics of fermented milk products.....	66
4.4.1 <i>Sensory evaluation of products made with selected strains of lactic acid bacteria</i> .....	66
4.4.2 <i>Sensory evaluation of four commercial and four experimental fermented milk products using a trained sensory panel</i> .....	66
4.4.3 <i>Objective physical characteristics of the fermented milk products</i> .....	70
5. DISCUSSION.....	73
6. GENERAL CONCLUSIONS.....	86
7. REFERENCES.....	88

## LIST OF FIGURES

Figure 2.1:	Population percentages in South Africa (RSA Statistics in brief, 1997).....	10
Figure 3.1:	Scheme for presumptive identification of <i>Leuconostoc</i> spp.....	44

## LIST OF TABLES

Table 2.1:	Countries, product names and manufacturing steps of some of the traditional fermented milk products (and by-products) found in Africa....	15
Table 2.2:	Chemical composition of some traditional African fermented milks.....	21
Table 2.3:	Microbiological information on some African traditional fermented milks.....	23
Table 3.1:	Traditional fermented milk samples collected in South Africa and Namibia.....	39
Table 3.2:	Methodology for microbiological analysis of indigenous fermented milks.....	41
Table 3.3:	Differential characteristics of lactic acid bacteria.....	42
Table 3.4:	Preparation of samples for the determination of volatile organic compounds.....	47
Table 3.5:	Preparation of fermented milk products with seven selected strains for evaluation of flavour acceptability.....	49
Table 3.6:	Reference standards for selected descriptors.....	51
Table 3.7:	Composition and percentage inoculum of starter cultures used in the manufacturing of four fermented milk products.....	52
Table 4.1:	Traditional fermented milk samples collected in South Africa and Namibia.....	54
Table 4.2:	Microbiological counts (per ml) obtained from traditional fermented milk samples.....	56
Table 4.3:	Average microbiological counts of products manufactured in clay pots and calabashes.....	57

Table 4.4:	Summary of the results of the secondary phenotypical tests.....	58
Table 4.5:	Distribution of genera in products manufactured in clay pots and calabashes.....	60
Table 4.6:	Results of the biochemical reactions of 10 selected lactic acid bacterial strains tested with the API 50 CH identification system.....	62
Table 4.7:	Gas production by 110 <i>Leuconostoc</i> strains in skim milk with and without added sodium citrate after 16, 48 and 144 h at 22 °C.....	64
Table 4.8:	The production of volatile organic compounds by some lactic acid bacteria in skim milk after 16 h at 22 °C.....	64
Table 4.9:	The ability of nine selected lactic acid bacterial strains to coagulate milk at 22 and 30 °C using 1, 10 and 20 % (v/v) inoculum sizes.....	65
Table 4.10:	Sensory evaluation and pH of ten experimental fermented milk products manufactured with selected lactic acid bacterial strains.....	67
Table 4.11:	Average sensory scores for 19 attributes describing four commercial (C1, C2, C3, C4) and four experimental fermented milk products (E1, E2, E3, and E4).....	68
Table 4.12:	Correlation values and probability values of selected attributes for commercial and experimental fermented milk products.....	69
Table 4.13:	Mean values for the physical characteristics of both commercial and experimental fermented milk products.....	71
Table 4.14:	Correlation values and probability values of selected sensory attributes and objective physical characteristics.....	72

## LIST OF APPENDICES

Appendix A:	Score card for sensory evaluation.....	111
	Definition of terms.....	113