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

by

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DECLARATION

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

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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

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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 geselekteerde stamme vir gebruik in die vervaardiging van aangesuurde melk.

deur

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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-tagtig 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 geselekteerde 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 gegroei 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 ingewikkelde en omvattende taak van suurselnavorsing, veral ten opsigte van mesofiele kulture vir gefermenteerde melkprodukte.

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