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Incidence and characterisation of *Bacillus sporothermodurans* isolated from UHT milk

by

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DECLARATION

I hereby declare that the thesis submitted at the University of Pretoria for the award of a PhD degree is my work and has not been submitted by me for a degree at any other university or institution of higher education.

Frederick Tawi Tabit

May 2010

DEDICATION

I dedicate this work to the Almighty God, the creator of heaven and earth and to my father, Peter Akwayi Tabit; my mother, Theresia Omah Tabit and my son, Nkosi Tawi Tabit.

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ABSTRACT

Bacillus sporothermodurans, which was first detected in UHT milk in Germany in 1990, can affect the stability and shelf life of contaminated commercial UHT milk and cause economic losses. Due to the unusual thermal resistance of *B. sporothermodurans* spores, *B. sporothermodurans* can survive UHT treatment and proceed to grow in stored products causing instability because of their proteolytic activity (Huemer *et al*., 1998). This study was conducted to determine the level of *B. sporothermodurans* contamination in South African dairies and to understand the mechanism of *B. sporothermodurans* spore destruction in order to investigate ways of inactivating these spores without severe heating. The objectives were to determine the presence of *Bacillus sporothermodurans* in retail UHT milk along with milk from different points of a processing line and isolates from UHT milk in South Africa, UP20A and a reference strain of *B. sporothermodurans*, DSM 10599 from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Germany were characterised using PCR. The effect of chilling, pre-heating, UHT, reprocessing and H_2O_2, individually and in combination, on the survival of *B. sporothermodurans* was also investigated in broth. Spores were heated at 130 °C for 4, 8 and 12 min in order to investigate the mechanism of structural damage and survival.

The adopted real time (RT) PCR with SYBR Green method was effective for the confirmation of *B. Sporothermodurans.* The structural damage on heated spores was determined using the Transmission Electron Microscopy (TEM), while High Performance Liquid Chromatography (HPLC) and spectrophotometry were used to quantify Dipicolinic acid (DPA) and soluble proteins released from the filtrate of heated spore suspensions. All statistical analyses were done using STATISTICA. *B. sporothermodurans* was detected in retail UHT milk packs from only one processor. The combination of chilling and UHT was more effective in eliminating *B. sporothermodurans* spores to a non detectable level than UHT treatment alone. H_2O_2 was also effective in eliminating *B. sporothermodurans* spores, from 6.31 to 1.64 log cfu/ml after 15 min of exposure and. The release of DPA during wet heat treatment coincides with visible signs of structural damage and significant inactivation of

spores. Visible signs of spore structural damage emanate at different rates. The amount of protein release seems to be strain specific.

This research is the first to detect the presence of *B. sporothermodurans* in UHT milk in South Africa and to determine the effect of UHT processing stresses on its survival. These results can be used to design processing parameters so as to effectively eliminate *B. sporothermodurans* spores during UHT processing. Similarly, this research is the first in which RT PCR with SYBR Green has been used to characterise *B. sporothermodurans* as well as to determine the effect of wet heat treatment on the structure of *B. sporothermodurans* spores. This research aims to contribute to the insight regarding the mechanisms of destruction of *B. sporothermodurans* spores by wet heat.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES