IMPACT OF PROCESSING TEMPERATURES ON SURVIVAL OF MICROBIAL CONTAMINANTS FROM PASTEURISED MILK

PHOLISA DUMALISILE

Thesis presented in partial fulfilment for the degree of

MASTER OF SCIENCE IN FOOD SCIENCE

Department of Food Science
Faculty of Agricultural and Forestry Sciences
University of Stellenbosch

Study Leader: Professor T.J. Britz
Co-study Leader: Dr. R.C. Witthuhn

August 2004

DECLARATION

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

_____ _____

Pholisa Dumalisile Date

ABSTRACT

Milk has been identified as having the potential of being a carrier of human pathogens, and it is thus essential to eliminate or reduce the likelihood of milk borne contamination. This problem of milk contamination is generally solved by the process of pasteurisation which is achieved by heating the "raw" material for a sufficient period of time to destroy any pathogenic and spoilage bacteria which may be present at a temperature of below 100°C. Presently, there are two basic methods of pasteurisation in use in the dairy industry, the LTLT and the HTST methods, where the applied heat treatment is considered sufficient to ensure public safety and adequate keeping quality. In addition to these, there is another method, the "pot" pasteurisation, to be found in Southern Africa that was designed to eliminate potential pathogenic and spoilage bacteria present in raw milk. As far as it is known no thermal studies have been done on the "pot" pasteurisation method. The objectives of this study were to determine the impact of different milk pasteurisation temperature and time combinations on the survival of selected microbes. The accuracy of the "pot" pasteurisation method and how it differs from the other pasteurisation methods was also determined using the same selected microbes.

The six selected microbes were thermally inactivated by using the LTLT, HTST and the "pot" pasteurisation methods at low and high inoculum levels of 10^4 and 10^6 cfu.ml^{-1}. The thermal death curves were constructed for each selected species. The selected microbes included the strains *Bacillus cereus* (S4), *Chryseobacterium meningosepticum* (S5), *Pseudomonas putida* (S6), *Acinetobacter baumannii* (C3), *Escherichia coli* (58) and *Candida lipolytica* (G1). Survivors were enumerated after heating for 0, 5, 10, 15, 20, 25, 30, 35 and 40 min for both the LTLT and HTST pasteurisation methods and after heating for 0, 10, 20 and 30 min for the "pot" pasteurisation method.

The results from this study showed that with the exception of the *B. cereus* strain, the other selected microbes at both high and low concentration levels did not survive the LTLT or the HTST pasteurisation methods. It was found that for all the organisms used in this study, there was a rapid initial death rate just before the required pasteurisation temperatures of 63°, 72° and 90°C were reached, during the "come-up" period. In contrast, the results from the "pot" pasteuriser showed that the

B. cereus (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3) and *E. coli* (58) strains survived the pasteurisation conditions applied.

From these results it was thus concluded that the "pot" pasteuriser under the conditions evaluated in this study, did not pasteurise effectively. Therefore, it is recommended that the manufacturer improves the heating quality of the "pot" pasteuriser. As it was found that only the *B. cereus* (S4) strain survived all the different pasteurisation methods, future research needs to be done to determine at which temperature this heat resistant bacterial strain will be destroyed. This is very important because there is a need to destroy all the spoilage microorganisms that can lead to the deterioration of food products.

UITTREKSEL

Melk is 'n potensiële draer van mikrobes wat patogenies is vir die mens. Dit is dus essensiëel om die besmetting van melk te verlaag of te elimineer. Die probleem van melkbesmetting word opgelos deur die proses van pasteurisasie. Die proses word toegepas deur verhitting van die rou material vir 'n voldoende periode om patogeniese en bederf organismes te vernietig. Temperature onder 100°C word gebruik. In die suiwelbedryf word twee basiese metodes gebruik: die LTLT (lae temperatuur, lang tyd) metode en die HTKT (hoë temperatuur, kort tyd) metode. Albei hittebehandelings is voldoende om publieke veiligheid en 'n genoegsame rakleeftyd te verseker. 'n Derde metode, "pot" pasteurisasie, word in Suidelike Afrika gebruik. Die metode is ontwikkel om potensiële patogene en bederf organismes in rou melk te elimineer. Die probleem is dat daar geen navorsing op die temperatuur eienskappe van die “pot" metode gedoen is nie. Die doelwitte van hierdie navorsing was om die effek van verskillende temperatuur:tyd kombinasies op die oorlewing van sekere mikrobes te bepaal. Die akkuraatheid van die "pot" metode en die manier hoe dit van ander metodes verskil, is ook in ag geneem. Die navorsing is ten alle tye gebaseer op die geselekteerde mikroorganismes.

Die ses geselekteerde spesies van mikrobes is vernietig deur middel van die LTLT, HTKT en "pot" pasteurisasie metodes. Die mikrobes is geïnaktiveer teen lae en hoë inokulums van 10^4 en 10^6 kve.ml^{-1}. Terminale dodings kurwes is opgestel vir elke geselekteerde spesie. Die mikrobes van belang is *Bacillus cereus* (S4), *Chryseobacterium meningosepticum* (S5), *Pseudomonas putida* (S6), *Acinetobacter baumannii* (C3), *Escherichia coli* (58) en *Candida lipolytica* (G1). Die oorlewende mikroorganismes is na hitte behandelings van 0, 5, 10, 15, 20, 25, 30, 35 en 40 minute vir beide die LTLT en die HTKT pasteurisasie metodes en na hitte behandelings van 0, 10, 20, en 30 minute vir die "pot" pasteurisasie metode getel.

Die resultate van die navorsing dui aan dat, behalwe vir *B. cereus*, die geselekteerde mikrobes teen beide lae en hoë konsentrasies nie die LTLT en die HTKT metodes oorleef het nie. Daar is gevind dat, vir al die organismes, vinnige aanvanklike dodingstempos teenwoordig was net voor die noodsaaklike pasteurisasie temperatuur van 63°, 72° en 90°C bereik is, gedurende die "come-up" periode. Inteenstelling hiermee het die resultate van die "pot" metode bewys dat *B.*

cereus (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3) en *E. coli* (58) stamme die pasteurisasie toestande oorleef het.

Uit die resultate is ’n gevolgtrekking gemaak dat die "pot" pasteurisasie metode nie effektief was nie. Daar word dus aanbeveel dat die vervaardiger die verhittings-kwaliteit van die "pot" pasteurisasie apparaat verbeter. Aangesien net die *B. cereus* (S4) stam al drie pasteurisasie metodes oorleef het, moet toekomstige navorsing gedoen word om die vernietigings temperatuur van dié hittebestande stam te bepaal. Die navorsing is van belang weens die behoeftes om alle bederf mikroorganismes wat tot die agteruitgang van voedsel produkte kan lei, te vernietig.

dedicated to my parents and my sister, Noluvo

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following persons and institutions for their contributions to the completion of this research:

Professor T.J. Britz, Study Leader and Chairman of the Department of Food Science, University of Stellenbosch, for his expect guidance, willing assistance and valuable criticism in the preparation of this thesis and throughout the course of my study;

Dr. R.C. Witthuhn, Co-study Leader and Senior Lecturer at the Department of Food Science, for valuable advice and positive criticism during the course of my research and fulfilment of the requirement of this thesis;

National Research Foundation (Grand Holder Bursary), the Department of Food Science, Stellenbosch University and Student Affairs for financial support throughout my post-graduate studies;

Mr. G.O. Sigge, Mrs. L. Mass and Mr. E. Brooks for technical assistance and answering the endless questions;

Ms. Michelle Cameron, Mrs. Corné Lamprecht and Ms. Kelly Wainwright for always being willing to answer questions and for valuable advice;

Mrs. M.T. Reeves and Mrs. J. Van Wyk for help with administration;

My fellow post-graduate students and friends, for their support and help throughout my studies;

My family for their continual love and support; and

The Almighty God for giving me strength and courage to successfully complete this research.

CONTENTS

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

CHAPTER 1

INTRODUCTION

Milk is, from a nutritional standpoint, the most nearly perfect food. Due to its nutrient content, it is valued in the manufacturing of most dairy products (Charley & Weaver, 1998). The rich nutritional composition of milk, combined with the fact that it is a liquid made up of an emulsion of fat droplets suspended in an aqueous solution of protein, mineral salts and vitamins, makes it an excellent growth medium for a variety of microorganisms (Pyke, 1964; Fox & Cameron, 1982).

Milk quality depends on the strictest sanitary control practises employed in the dairy industry (Potter & Hotchkiss, 1995). Although milk is practically free of bacteria during milking of a clean and healthy cow, it is almost impossible to maintain it in this condition (Fox & Cameron, 1982). Bacteria from the milk container, the milker, the milking machine or the atmosphere may contaminate the milk (Fox & Cameron, 1982). The potential danger of milk as a carrier of human pathogens is established and it is essential to eliminate or reduce the likelihood of milk borne contamination. From a practical point of view, this problem is generally solved by applying the pasteurisation process (Corash, 1951).

The pasteurisation process is essentially the application of a sufficient amount of heat to a product for a sufficient period of time to destroy any pathogenic and most spoilage bacteria which may be present (Corash, 1951; Dubos, 1998). The term pasteurisation was derived from the research of the famous French scientist, Louis Pasteur, whose experiments in 1870 showed that the heating of wine greatly improve the keeping quality (Harvey & Hill, 1967; Holsinger *et al.,* 1997).

In establishing the standards for pasteurisation of milk, early investigators had to take into consideration factors such as: the minimum temperature and time of exposure necessary to destroy the most heat resistant types of harmful microorganisms; adequate factors needed beyond the minimum in order to prevent unforeseen or abnormal conditions; effect of the treatment on the flavour and appearance of the product; effect of the treatment upon the nutritive quality; and the economic feasibility of the process (Corash, 1951). Presently, there are two

methods of pasteurisation generally acceptable for the processing of milk, the holding method or the Low Temperature Long Time (LTLT) and the High Temperature Short Time (HTST) pasteurisation methods (Corash, 1951; Fox & Cameron, 1982; Caudill, 1993).

The custom of preserving milk by heat may be as old as the cow and the use of fire (Holsinger *et al.,* 1997). In South Africa, milk pasteurisation was introduced as a public health measure in order to destroy the most heat resistant, non-spore forming human pathogens (*Mycobacterium paratuberculosis* and *Coxiella burnetti*) likely to be present in raw milk (Stauffer, 1993; Grant *et al*., 1996). The regulations relating to milk and dairy products were drafted by the Minister of Health in terms of section 15 (1) of the Foodstuffs, Cosmetics and Disinfectant Act, 1972 (Act No. 54 of 1972). According to these regulations, raw milk with a plate count of more than 200 000 cfu. ml^{-1} may not be consumed by humans, and no person is allowed to sell pasteurised milk with a standard plate count exceeding 50 000 $cfu.ml^{-1}$ (Anon., 2002). The quality of raw milk is the most important factor that determines the final quality of pasteurised milk (Van Twisk, 1997).

The adequacy of pasteurisation is vital to ensure the safety of pasteurised milk and milk products (Nelson, 1981). The standard plate count of pasteurised milk and related products may be seen as an index of good manufacturing practises. If the raw product is of satisfactory microbial quality, the processing is carried out efficiently, protection from environmental contamination is satisfactory and holding temperatures and times are such that growth of microbes cannot occur to any significant extent, the microbial count will be low. However, even a low count does not ensure that the product will be free from pathogenic organisms. In dairy products it is generally considered that the rate at which spoilage develops depends on the initial microbial number, the tempo at which the microbes may grow at the holding temperature used, and the ability to cause an organoleptically detectable change in the product. Theoretically, the presence of only one cell is capable of causing off-flavour and taste in time. Thus the longer the product is kept the more microbes will grow resulting in spoilage (Nelson, 1981; Holsinger *et al*., 1997).

Research has at times reported the low quality of raw milk in South Africa. Lund *et al.* (1992) in a study on proteolytic and lipolytic psychrotrophic

Enterobacteriaceae in pasteurised milk and dairy products found a high percentage of psychrotrophic coliforms in the pasteurised milk and cream samples. Lindsay *et al*. (2000), during a study on the physiology of dairy-associated *Bacillus* sp., isolated four dominant *Bacillus* strains from the alkaline wash solutions in a dairy plant and it was suggested that these *Bacillus* strains might be the cause of pre- and post-pasteurisation spoilage of milk and dairy products. Among a large collection of bacterial isolates from South African dairy products, a novel *Chryseobacterium* taxon (DNA group 3) was delineated in a polyphase taxonomic study (Hugo *et al*., 2003). The post-pasteurisation spoilage of milk through contact surfaces of milk pipelines and processing equipment constitutes the main direct source of the contamination of pasteurised milk (Koutzayiotis, 1992).

Cronjé (2003) in a study on the production of Kepi grains using pure cultures, also identified the presence of microbes in pasteurised and even in "double" pasteurised milk. These "milk isolates" included strains of: *Pediococcus* sp.; *Acinetobacter* sp.; two strains of *Lactococcus lactis* ssp. *lactis; Candida lipolytica; Candida guilliermondii; Chryseobacterium meningosepticum*; *Pseudomonas putida*; and four isolates related to the *Bacillus cereus* group.

The shelf-life of traditionally pasteurised milk is between 7 and 10 days, provided the product is stored at or below 6°C (Buys, 2001). Spoilage results in a shorter milk shelf-life and decreases the quality of the final dairy product. The presence of these "milk isolates" in pasteurised milk causes a problem for the food industry resulting in the dairy products deteriorating before the stated expiry date. It is not only the keeping quality of milk that is important in milk processing, but also the consumer that demands the highest standards in milk production (Vassen, 2003). Therefore, Cronjé (2003) after isolating bacterial and yeast contaminants from pasteurised milk strongly recommended that research be done to evaluate the effectiveness of pasteurisation, to assimilate data to use in reconsidering efficiency of the pasteurisation parameters and to highlight post-pasteurisation contamination of South African milk.

According to the literature, attempts to destroy specific bacterial strains in raw milk have failed (Doyle *et al.,* 1987; Knabel *et al*., 1990; Grant, 1998; Lund *et al.,* 2002). The survival of these contaminants in pasteurised milk, followed by spoilage of dairy products necessitates research on pasteurisation. Whether their

survival of the pasteurisation process is due to their survival ability, process temperature/time variations or the pasteurisation method used, is not known. With these facts in mind, a more practical solution for the pasteurisation of milk may play a role to determine the survival/death temperatures of these microbes.

The main objective of this study was to determine the impact of different pasteurisation temperatures on the survival of selected microbes. The impact of "pot pasteurisation" and the survival of different bacterial contaminants originally isolated from pasteurised milk was also determined.

REFERENCES

Anonymous. (2002). Foodstuffs, Cosmetics and Disinfectant Act and Regulations. Act No. 54 of 1972, G.N.R. 1555/1997. Johannesburg, South Africa: Lex Patria Publishers.

Buys, E. (2001). Good hygiene practices for extended shelf-life milk. *South African Society of Dairy Technology: Dairy Symposium,* Gordons Bay, Cape Province. Pp. 1-5.

Caudill, V. (1993). Engineering: plant design, processing and packaging. In: *Dairy Science and Technology Handbook* (edited by Y.H. Hui), Volume 2. Pp. 312-317. New York: VCH Publishers, Inc.

Charley, H. & Weaver, C. (1998). *Foods: A Scientific Approach*, 3rd Ed. Pp. 308-335. New Jersey: Prentice-Hall, Inc.

Corash, P. (1951). Milk and milk products. In: *The Chemistry and Technology of Food and Food Products* (edited by M.B. Jacobs), Volume 3. Pp. 2219-2226. New York: Interscience Publishers.

Cronjé, M.C. (2003). Production of kepi grains using pure cultures as starters. *M.Sc*. *Thesis*, University of Stellenbosch, Stellenbosch, South Africa.

Doyle, M.P., Glass, K.A., Beery, J.T., Garcia, G.A., Polland, D.J. & Schultz, R.D. (1987). Survival of *Listeria monocytogenes* in milk during high temperature short time pasteurisation. *Applied and Environmental Microbiology*, **53**, 1433-1438.

Dubos, R. (1998). *Pasteur and Modern Science* (edited by T.D. Brock). Pp. 3-27. Washington D.C.: ASM Press.

Fox, B.A. & Cameron, A.G. (1982). Food spoilage, preservation and hygiene. In: *Food Science - A Chemical Approach,* 4th ed. Pp. 315-316. London: Hodder & Stoughton.

Grant, I.R. (1998). Letter to the editor: Does *Mycobacterium paratuberculosis* survive current pasteurisation conditions? *Applied and Environmental Microbiology*, **64**, 2760-2761.

Grant, I.R., Ball, H.J. & Rowe, M.T. (1996). Inactivation of *Mycobacterium paratuberculosis* in cow's milk at pasteurisation temperatures. *Applied and Environmental Microbiology,* **62**, 631-636.

Harvey, C. & Hill, H. (1967). *Milk: Production and Control*, 4th ed. Pp. 363-373. London: Lewis & Co. Ltd.

Holsinger, V.H., Rajkowski, K.T. & Stabel, J.R. (1997). Milk pasteurisation and safety: a brief history update. *Revue Scientifique et Technique Office International des Epizooties*, **16**, 441-451.

Hugo, C.J., Segers, P., Hoste, B., Vancanneyt, M. & Kersters, K. (2003). *Chryseobacterium joostei* sp. *nov*., isolated from the dairy environment. *International Journal of Systematic and Evolutionary Microbiology*, **53**, 771-777.

Knabel, S.J., Walker, H.W., Hartman, P.A. & Mendonca, A.F. (1990). Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurisation. *Applied and Environmental Microbiology*, **56**, 370-376.

Koutzayiotis, C. (1992). Bacterial biofilms in milk pipelines. *South African Journal of Dairy Science,* **24**, 19-21.

Lindsay, D., Brozel, V.S., Mostert, J.F. & Holy, A. (2000). Physiology of dairy associated *Bacillus* sp. over a wide pH range. *International Journal of Food Microbiology*, **54**, 49-62.

Lund, A.M., Mostert, J.F., Carelsen, M.A. & Lategan, B. (1992). Proteolytic and lipolytic psychrotrophic *Enterobacteriaceae* in pasteurised milk and dairy products. *South African Journal of Dairy Science*, **24**, 7-10.

Lund, B.M., Gould, G.W. & Rampling, A.M. (2002). Pasteurisation of milk and the heat resistance of *Mycobacterium avium* subsp. *paratuberculosis*: a critical review of the data. *International Journal of Food Microbiology*, **77**, 135-145.

Nelson, F.E. (1981). The microbiology of market milk. In: *Dairy Microbiology* (edited by R.K. Robinson) Volume 1. Pp. 188-201. London: Applied Science Publishers.

Potter, N.N. & Hotchkiss, J.H. (1995). *Food Science*, 5th ed. Pp. 140-286. New York: Chapman & Hall.

Pyke, M. (1964). *Food Science and Technology*. Pp. 5-87. London: William Clowes & Sons LTD.

Stauffer, J.E. (1993). Quality assurance and dairy processing. In: *Dairy Science and Technology Handbook* (edited by Y.H. Hui), Volume 2. Pp. 12-25. New York: VCH Publishers, Inc.

Van Twisk, P. (1997). Consumer and processor quality concerns. *Food Industries of South Africa*, **50**, 9.

Vassen, P. (2003). The pH and freezing point values of milk in the Western and Southern Cape, and factors affecting these values. *M.Sc. Thesis*, University of the Free State, Bloemfontein, South Africa.

CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

Milk is one of the most complete nutritional foods and also an excellent growth medium for a vast range of organisms, including a number of pathogenic bacteria (Proudlove, 1989). Although milk should be practically free of bacteria at the time it is obtained from a clean and healthy cow, it is almost impossible to maintain it in this condition. Bacteria from the milk container, the milker, the milking machine or the air may contaminate the milk, where these organisms find congenial conditions in which to flourish (Fox & Cameron, 1982). Faecal contamination of milk can occur during the milking process, and this depends on the hygiene practises during teat preparation before the attachment of the milking cluster (Grant *et al.,* 2002a). The potential danger of milk as a carrier of human infection is well established and it is, therefore, important to eliminate or to reduce contamination to the lowest possible degree (Proudlove, 1989).

It is well known that bacterial exposure to temperatures above the range for normal cell growth leads to progressive loss of bacterial viability. When bacteria are exposed for a short period from lower to higher temperatures within or slightly above their normal growth range, a degree of protection against the lethal effects of a subsequent shift to a higher temperature, an acquired thermotolerance, is achieved. The impact of heat shock and thermotolerance may be important because certain foods are thermally processed to ensure food safety (Farber & Brown, 1990; Bunning *et al.,* 1992). Therefore, efficient heat treatment of milk is essential to eliminate pathogenic and spoilage bacteria (Proudlove, 1989). From a practical point of view, this problem is best solved by the process of pasteurisation (Corash, 1951). Pasteurisation is essentially the application of a sufficient amount of heat to a product for a sufficient period of time to destroy any pathogenic bacteria which may be present (Corash, 1951; Dubos, 1998). Pasteurisation is now used all over the world and has found application to wine, beer, vinegar, milk, and countless other perishable beverages, foods and organic products (Stauffer, 1993; Dubos, 1998).

The term pasteurisation was derived from the research of the famous French scientist, Louis Pasteur, whose experiments in 1870 showed that the

heating of wine greatly improved the keeping quality. The distinction for demonstrating the value of the pasteurisation method, however, is credited to an Italian biologist, Lazzaro Spallanzani who, in 1768, conserved food by means of heat (Harvey & Hill, 1967). The employment of heat as a means of food preservation was also investigated by Wilhelm Scheele in 1783, while in 1795, Nicholas Appert (Harvey & Hill, 1967), the inventor of canning, applied the process to milk. Forty years before Pasteur conducted his experiments, William Dewes also observed that if milk was heated to boiling point and cooled quickly, the tendency to spoil was reduced. Although these scientists forestalled Pasteur in the application of heat as a means of food preservation, posterity owes the present process of pasteurisation to him. During the early part of his career, he paid considerable attention to the problem of bacterial growth in milk and between 1857 and 1862 he proved that milk became sour owing to the multiplication of bacteria which, he believed, obtained entrance from the atmosphere. He demonstrated that the application of heat to milk would destroy many of the organisms and souring would be postponed. However, the microbial destruction achieved by the practise of heating milk was not recognised until after the work of Pasteur (Holsinger *et al*., 1997).

Pasteurisation was first applied in the dairy business in the 1880s in Germany and Denmark (Holsinger *et al.,* 1997; Dubos, 1998). The first commercial pasteuriser was constructed in Germany in 1882 and as a result, pasteurisation on a commercial scale quickly became common practise in Denmark and Sweden in the mid-1880s. The first person to pasteurise milk was the German chemist, Soxhlet, who published his work in 1886 (Holsinger *et al*., 1997).

Initially there was strong resistance to the pasteurisation of milk because the flavour and colour is easily altered during the heating process (Corash, 1951; Fellows, 1996). Appropriate pasteurisation methods were developed and experience proved the positive effect on public health. In establishing standards for the pasteurisation of milk, early investigators had to take into consideration factors including the minimum temperature and time of exposure necessary to destroy the most heat resistant bacteria, adequate safety factors needed beyond the minimum in order to protect against unforeseen abnormal conditions, effect of the treatment upon the flavour and appearance of the milk, effect of the treatment upon the quality of milk, and the economic feasibility of the process (Corash,

1951).

Milk is the most strictly controlled of all food commodities in the United States and many other countries. Upon receipt of milk at a processing plant, several inspections and tests may be run to control the quality of the incoming product. These tests commonly include the determination of the bacterial counts, especially “total” viable counts, coliform and yeast counts. Bacterial counts play a major role in the sanitary quality of milk on which milk grading is largely based (Potter & Hotchkiss, 1995).

In 1924, the U.S. Public Health Service (Stauffer, 1993) introduced the Standard Milk Ordinance to assist states and municipalities in managing safe milk supply. This model regulation is now known as the Grade “A” Pasteurised Milk Ordinance (PMO). This PMO is available for adoption by the more than 15 000 states, countries and local health jurisdictions. The PMO requires that only Grade "A" milk and milk products may be sold to the final consumer or to restaurants, grocery stores or similar establishments (Stauffer, 1993). In the PMO, pasteurisation is defined as the process of heating every particle of milk in properly designed and operated equipment to a specified temperature and held at that temperature for a given length of time as indicated in Table 1.

The *Grade “A” Pasteurised Milk Ordinance Recommendations* of the U.S. Public Health Service/Food and Drug Administration (Potter & Hotchkiss, 1995) provides an excellent guide to the setting of microbiological and sanitary standards. Many cities and states have adopted their milk regulations after this code was instated. According to this Ordinance (Potter & Hotchkiss, 1995), Grade “A” raw milk for pasteurisation may not exceed a bacterial plate count of 100 000 $cfu.ml^{-1}$ on milk from individual producers or 300 000 per ml on blended milk; and Grade “A” pasteurised milk may not exceed a total bacterial count of 20 000 per ml or a zero coliform count per 10 ml milk (Potter & Hotchkiss, 1995).

In South Africa, milk pasteurisation was introduced as a public health measure in order to destroy the most heat resistant, non-spore forming human pathogens (*Mycobacterium paratuberculosis* and *Coxiella burnetti*) likely to be present in raw milk (Grant *et al*., 1996a). These regulations relating to milk and dairy products were drafted by the Minister of Health in terms of section 15(1) of the Foodstuffs, Cosmetics and Disinfectant Act, 1972 (Anon., 2002).

Table 1. Pasteurisation conditions (Caudill, 1993; Stauffer, 1993).

Temperature (°C)	Holding time
63°	30 min
72°	15 s
89°	1.0 s
90°	0.5 s
94°	0.1 s
96°	0.05 s
100°	0.01 s

According to these regulations, raw milk with a plate count of more than 200 000 cfu.ml^{-1} may not be consumed by people; and no person is allowed to sell pasteurised milk with a standard plate count exceeding 50 000 cfu.ml^{-1} (Anon., 2002). Furthermore, no person shall sell or use raw milk intended for further processing which on application of the modified Eijkmann test and the VRB MUG agar method, is found to contain any *Escherichia coli* in 0.01 ml of raw milk when the Eijkmann test is used, or any *E. coli* in 1.0 ml of raw milk if the VRB MUG method is used (Anon., 2002).

B. THE EFFECT OF HEAT ON MILK

In its early stages, pasteurisation was prompted mainly by commercial considerations and the maintenance of large volumes of milk in “sweet condition” was the primary aim (Harvey & Hill, 1967). Medical and public health authorities later became interested in the process as a means of preventing infection with animal and human diseases, and they specified suitable heating temperatures and periods of retention. These views were not generally popular amongst distributors who were concerned mainly with improving the keeping quality of their milk as cheaply as possible, irrespective of time and temperature.

Heated milk according to Harvey & Hill (1967), undergoes certain alterations and these changes are influenced by two factors: the period during which the milk is exposed to the heating agent and the temperature reached during the process. This usually leads to the following changes:

i. The milk loses its viscosity;

ii. A skin commences to form on the liquid surface when the milk is in contact with air at temperatures between 60° and 70°C and complete formation takes place when boiling point is reached. This skin contains a proportion of all the constituents in the milk but consists mainly of lactalbumin. Formation does not occur when the milk is treated in a closed vessel;

iii. CO_2 is driven off and the bicarbonates are partially decomposed causing a slight increase in acidity, while the constituent calcium and magnesium salts are precipitated;

iv. Milk which has been heated to a temperature of 73° - 78°C for 30 min will

not coagulate with rennet. The reason for this is that heated milk fails to coagulate due to the precipitation of calcium salts, which render the casein in milk less easily coagulable;

v. Lecithin and nuclein are decomposed;

vi. Above a temperature of 62° - 67°C the cream line is affected and between 10 – 20% reduction may occur although no cream is removed from the milk;

vii. Several enzymes are degraded if milk is held at a temperature of 79° - 84°C. It does not appear that these milk enzymes are of great importance in terms of human nutrition;

viii. The diffusible calcium percentage is reduced;

ix. The heating of milk may produce a noticeable taste to persons who have sensitive palates;

x. At pasteurisation temperatures the freezing-point of milk is raised slightly and the acidity slightly reduced;

xi. Phosphates are precipitated by prolonged heating and certain vitamins (C, B, A and D) may be destroyed; and

xii. Heated milk forms a finer curd in the stomach than raw milk.

C. STANDARD PASTEURISATION METHODS

There are two basic methods of pasteurisation currently in use, the holding process commonly known as the “Batch” or “Low Temperature Long Time” (LTLT) pasteurisation method and the “High Temperature Short Time” (HTST) pasteurisation method (Stauffer, 1993; Potter & Hotchkiss, 1995). In both methods heat treatment sufficient for public safety and adequate keeping quality of the product is ensured (Stauffer, 1993).

Batch Pasteurisation is the oldest method of pasteurisation and has the advantage of simplicity. Raw milk is commonly pumped into a steam heated jacketed vat, brought to temperature, held for the prescribed time, and then pumped over a plate type cooler prior to bottling (Fig. 1). Every particle of milk, by law must be held at 63°C for 30 min (Corash, 1951; Stauffer, 1993; Potter &

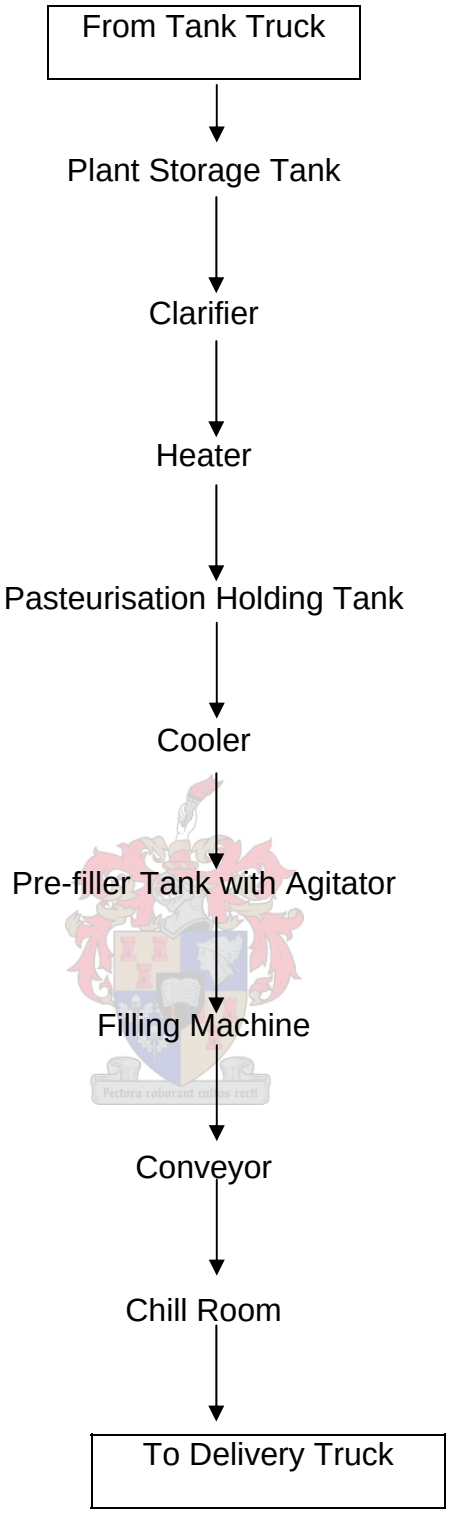


Figure 1. A simple flow chart of the equipment used in pasteurising milk by the holding method (Corash, 1951).

Hotchkiss, 1995). The temperature of the heating medium is maintained by circulating hot water between the walls or in the coils until the pasteurising temperature is reached. The milk is maintained at this temperature for at least 30 min and is then cooled. Cooling may be performed in the same vat or by a separate cooler. Each jacketed vat should be provided with an agitator to prevent stratification of the heated milk and to assure that every particle is sufficiently heated (Corash, 1951). All equipment used in batch pasteurisation should be of sanitary design and precautions must also be taken to avoid possible leaks from valves and fittings (Stauffer, 1993).

In order to determine whether or not the milk has been held for a sufficient period of time, it is necessary to know the filling and the emptying times of the pasteurising vat. For example, the recording of the temperature starts as soon as milk level touches the thermometer, which is usually in the lower part of the pasteuriser. If only 30 min of holding is allotted from this point on, it is clear that the milk added after the bulb is covered will not have been kept at the temperature for the full 30 min (Corash, 1951).

Batch pasteurisers are manually operated and are dependent upon an individual to let the milk into the vat, to supervise its holding for the required period of time, and to open the outlet valve for emptying at the end of the pasteurisation period. It is possible to hook-up a series of two, three, or more individual batch pasteurisers and operate them as a unit (Corash, 1951). Since they are manually operated, their successful use depends upon the skill of the operator. Improper pasteurisation may easily occur because of underheating and underholding. This may also result in the costly loss of time from the standpoint of economical plant operation since this requires more labour (Corash, 1951).

In addition to destroying common pathogens, batch pasteurisation also inactivates the enzyme lipase, which otherwise could quickly cause the milk to become rancid. Batch pasteurisation is still widely practised in some parts of the world especially in laboratories, but it has largely been replaced by the High Temperature Short Time continuous pasteurisation technology (Potter & Hotchkiss, 1995).

High Temperature Short Time (HTST) pasteurisation is a continuous process that possesses several advantages over batch pasteurisation and is the most important operation used in the commercial processing of milk (Caudill, 1993; Cerf & Griffiths, 2000). The minimum temperature and time relationships for pasteurisation of milk are based on the thermal death time studies using heat resistant micro-organisms as inoculum (Caudill, 1993). This type of pasteurisation unit consists of a heater, a holding tube, and a cooler. Since the period of exposure to pasteurisation temperatures is very short in this type of unit, it is extremely important that effective controls be used to make sure that no milk, which has been insufficiently heated or held, will reach the bottle fillers. This is accomplished by means of a flow diversion valve and specific controlling mechanisms (Corash, 1951).

Pasteurisation of liquid milk is mostly achieved commercially by the HTST process. In commercial continuous flow HTST pasteurisation different particles of milk receive slightly different treatments because of differences in flow velocities and other effects like the type of heat exchanger. An empirical approach of ensuring that all particles receive a certain minimum treatment is practised (Franklin, 1965; Grant *et al*., 1998). Evidence suggests that HTST pasteurisation, particularly at minimum levels, is less effective than the holder pasteurisation method in reducing populations of thermoduric micro-organisms. However, the extensive use of HTST pasteurisation has markedly reduced contamination problems with thermophilic bacteria (Nelson, 1981).

Many types of heat exchangers are suitable for application in the HTST process, but the plate heat exchangers presently have universal use. The detailed design of a plant varies but the requirements, as given in Table 2, for a successful operation are common to all types of pasteurisation plants (Varnam & Sutherland, 1994).

D. SURVIVAL OF ORGANISMS

Efficient pasteurisation is the only means by which a milk retailer is able to “guarantee” that the milk is free from any disease-producing organisms. It may be argued that if milk was produced at all times in a cleanly fashion, free from all

Table 2. Requirements for successful operation of a pasteurisation plant (Varnam & Sutherland, 1994).

Requirement 1.	Application of correct thermal process.
Solution	Use of thermostatic control to ensure heating medium at correct temperature.
	Use of correct positive control to ensure flow rate through holding tube.
	Use of long, thin holding tube to minimise short holding times due to turbulent flow.
	Fitting of automatic flow diversion device to return under heated milk to raw milk buffer tank.
Requirement 2.	Prevention of cross-contamination within pasteuriser.
Solution	Vent interspaces between seals to atmosphere to provide an immediate visual indication of gasket failure.
	Maintain a positive pressure balance between pasteurised milk and raw milk in the regeneration section.
	Ensure correct positioning of flow diverter and associated pipe work to avoid contamination of pasteurised milk when through-flow resumes after diversion.
Requirement 3.	Cleaning ability.
Solution	Fabricate milk contact surfaces from high grade stainless steel finished, preferably by electro-polishing, to avoid crevices and consequent entrapment of soil.
	Welds, joints, etc., should be finished to the highest possible standard.
	All materials used in construction should withstand contact with cleaning fluids.
Requirement 4.	Limitation of heat damage.
Solution	Minimise temperature difference (1°C is desirable) between heating medium and milk.
	Minimise milk residence time in 'hot' section of pasteuriser.
	Ensure efficiency of cooling section.
Requirement 5.	Economic operation.
Solution	Ensure efficiency of re-generation section.
	Employ maximum possible ratio of heating surface to volume.

harmful and spoilage organisms, heat treatment of any kind would be unnecessary (Harvey & Hill, 1967).

Bovine tuberculosis have been eliminated from the South African dairy herds. However, there are several other bacteria, which when present in raw milk may and do infect the consumer. For example: *Brucella abortus* can cause human beings to be infected with undulant fever; *Br. melitensis,* the cause of Malta fever has also been isolated from milk, while the presence in milk of C*oxiella burnetii* may cause Q-fever. All these organisms should be destroyed by efficient pasteurisation, but *Br. abortus* is still as prevalent as ever and it would appear that more and more cases of brucellosis are being recorded (Harvey & Hill, 1967).

Enteric and salmonella infections, diphtheria, scarlet fever and septic sore throat can all reach epidemic form due to the human infection of milk, the use of polluted water supplies for dairy purposes or, in the case of septic sore throat, infection of the udders of dairy cows with human streptococci (Harvey & Hill, 1967). The data in Table 3 show that if the requirements of time and temperature as required by efficient pasteurisation are rigidly adhered to, the process will destroy the main groups of milk infecting organisms. Higher temperatures for shorter periods are just as destructive as 63°C.

There can be little doubt that pasteurisation destroys the greater proportion of non-pathogenic bacteria (>99%). This can only be achieved with good quality clean raw milk. In contrast, heavily contaminated with a variety of spoilage and other non-pathogenic organisms, presents a different problem.

The presence of non-pathogenic thermophilic (heat-loving) and thermoduric (heat-resistant) organisms in the plant or in the milk may be the cause of considerable difficulty, although they have no impact on the safety of the milk. The presence of thermoduric organisms in the liquid is undesirable and may result in off-flavours or even curdling. They are one of the causes of high bacterial counts in pasteurised milk and they emphasise faults in plant design, operation and cleansing. These organisms are rarely found in bulked supplies of raw milk and present more difficulty during the low-temperature method than when the short-time process is employed. They are capable of rapid increase in numbers at the usual plant temperatures and when they are present, incoming milk becomes

Table 3. Time and temperature requirements for efficient pasteurisation (Harvey & Hill, 1967) at 63°C.

Organisms	At 63°C destroyed in (min):
Mycobacterium tuberculosis	20
Mycobacterium bovis	20
Corynebacterium diphtheria	1
Salmonella typhosus	2
Shigella dysenteriae	10
Brucella abortus	10 - 15
Streptococcus pyogenes	< 30

contaminated in proportion to the length of time the plant is in operation. As the majority of these organisms form endospores, it is extremely difficult to eliminate them from plant surfaces.

A few of the heat resistant organisms found in raw milk are not endospore formers and include *Streptococcus thermophilus*, which is capable of rapid acid production during low-temperature treatment. Certain actinomycetes are also thermophilic and can be spread throughout the plant in several ways: by running the plant for excessively lengthy periods; by the accumulation of milky deposits and milkstone; by "backwaters" in the plant and in pipe-lines; by reheating post-contaminated pasteurised milk; and by the accumulation of foam in the holding section with consequent incomplete discharge of the vessel (Harvey & Hill, 1967). When temperatures in excess of 70°C are employed in the high temperature short time process, thermophilic infection does not present a serious problem. When pasteurised milk has been efficiently cooled and stored at low temperatures, thermophilic activity ceases.

The microbiological quality of the raw milk before processing will have an effect on the final milk quality after pasteurisation. Generally, Gram-negative bacteria such as species of *Pseudomonas, Moraxella*, *Flavobacterium, Acinetobacter* and *Alcaligenes* predominate over Gram-positive bacteria in causing spoilage in pasteurised milks. These heat sensitive bacteria are part of the microbial population of raw milk that can become resident in the dairy plant and contaminate the milk after it has been pasteurised (Vasacanda & Cousin, 1993).

The organisms that survive pasteurisation, but do not grow at pasteurisation temperatures are considered by the dairy industry to be thermoduric (Nelson, 1981). The degree of survival after pasteurisation can range from a fraction (1%) of the original population to an increase in the population, as in the case of refrigerated cultures of *Microbacterium lacticum* (Nelson, 1981). The thermoduric organisms are resistant to heat and can easily withstand commercial pasteurisation. Certain thermoduric organisms form endospores but even so the the vegetative cells can withstand heating for 30 min at 65° - 75°C and some strains will even remain unharmed at 100°C.

A number of non-sporing thermoduric organisms can generally be divided into the following groups:

i. Streptococci such as S*. thermophilus, S. faecalis, S. durans, S. liquefaciens and S. bovis;*

ii. Micrococci such as *Sarcina lutea* and *S. rosea;*

iii. Microbacteria and micrococcus of various types are fairly common in pasteurised milk as a result of an incubation at 82°C necessary for development. They are also extremely heat resistant and form a large proportion of the thermoduric population on unsterile utensils;

iv. Certain types of coliform organisms are heat resistant and have been isolated from both raw and pasteurised milks; and

v. Several species of proteolytic actinomycetes survive dairy processing. They obtain entrance via dust, soil, manure and water (Nelson, 1981).

Listeria monocytogenes has also been reported to be thermoduric and has been found in pasteurised milk and may even survive HTST pasteurisation treatment in a small-scale plate heat exchanger pasteurisation unit (Doyle *et al*., 1987; Knabel *et al*., 1990).

E. THE MYCOBACTERIUM SITUATION

Classification of acid-fast bacilli isolated from raw milk has led to the identification of *Mycobacterium tuberculosis, M. bovis, M. smegmatis, M. avium* and *M. fortuitum,* as well as other acid-fast bacilli such as *Nocardia* (Holsinger *et al.,* 1997). The *M. paratuberculosis* complex also includes *M. africanum, M. bovis* and *M. microti*. Consumption of raw milk contaminated with pathogenic mycobacteria has been associated with human diseases. Although other avenues of environmental exposure, such as contaminated soil or water supplies may account for some cases of human disease caused by this organism, transmission of mycobacteria from raw milk appears to be the most likely route of exposure (Holsinger *et al.,* 1997).

Mycobacterium avium subspp. *paratuberculosis* is the cause of John's disease, a chronic bowel disease of dairy cows and other ruminants that occur world-wide (Grant *et al*., 1999; Grant *et al*., 2002b). The disease has a long

incubation period and clinical signs may not be seen until the animal is 3 to 5 years of age. Animals that are thought to predominate in an infected herd can shed *M. paratuberculosis* in faeces and milk for up to 18 months prior to showing any clinical signs of infection, so a farmer may not be aware that John's disease exists in his herd. Clinically infected animals can shed as many as 5×10^{12} *M. paratuberculosis* cells per day in faeces and these cells can remain viable for several months in the environment (Grant *et al*., 2002a). It has been suggested that this bacterium may also play a role in the etiology of Crohn's disease in humans (Holsinger *et al*., 1997). Current concerns regarding a possible relationship between Crohn's disease and *M. paratuberculosis* have been illustrated by the recent finding that *M. paratuberculosis'* DNA could be detected in pasteurised milk samples purchased from retailers (Stabel *et al.,* 1997).

Pasteurisation temperatures and times were originally selected to ensure the destruction of *M. paratuberculosis* as it has a high temperature resistance and non-spore forming characteristics (Potter & Hotchkiss, 1995; Stabel *et al.,* 1997). In a study to determine whether *M. paratuberculosis* is able to survive milk pasteurisation, it was found that the thermal death curve obtained for *M. paratuberculosis* was of concave shape, exhibiting a rapid initial death rate followed by significant 'tailing' that indicated low levels of *M. paratuberculosis* after pasteurisation (Grant *et al*., 1996b). It is likely that immediately after heat treatment, sub-lethally heat-injured *M. paratuberculosis* cells exist in pasteurised milk, but given sufficient time these cells could recover to fully growth-competent status (Grant *et al.,* 2002b). It was suggested that the "tailing" of the thermal death curves might be the result of the clumping of bacteria or, high levels of spores during heating, increased heat resistance and survival (Grant *et al*., 1996a).

The findings of several laboratory pasteurisation studies (Grant *et al.,* 2002a) have been reported over the past decade, and these suggest that *M. paratuberculosis* is not completely inactivated by the pasteurisation of milk at 72°C for 15 s, the minimum heat treatment required for milk pasteurisation by the European Commission legislation. In 1998, these findings led the United Kingdom dairy industry to voluntarilly adopt an increased holding time for commercial milk pasteurisation of 25 s rather than the 15 s at 72°C, in an effort to increase the lethality of the pasteurisation process (Grant *et al.,* 2002a).

It was found that *M. paratuberculosis* in naturally infected milk samples is

capable of surviving commercial pasteurisation at 73°C for both 15 s and 25 s with or without prior homogenisation if the bacterial cells are present in sufficient numbers before the heat treatment (Grant *et al.,* 2002a). In 1999, Grant *et al*. found that longer holding times at the existing HTST pasteurisation temperature of 72°C were found to be more effective in inactivating high numbers (10^6 cfu.ml^{-1}) of *M. paratuberculosis* in milk. They found that a longer holding time (25 – 35 s) at 72°C was more effective in killing 10^4 - 10^8 cfu.ml^{-1} than at higher heating temperatures (82° - 92°C).

F. OTHER BACTERIA THAT MAY SURVIVE MILK PASTEURISATION

Raw milk held in the refrigerator temperatures for some time shows the presence of several or all bacteria of the genera or group: *Streptococcus, Leuconostoc, Lactobacillus, Propionibacterium,* coliforms*, Proteus, Pseudomonas, and Bacillus*. The pasteurisation process eliminates all except the thermoduric strains, primarily the streptococci and lactobacilli, and spore formers of the genus *Bacillus* (Jay, 1978).

Bacillus - is a common contaminant of raw milk (Svensson *et al.,* 2000). Members of this genus are Gram-positive, aerobic or facultatively anaerobic, catalase positive and endospore forming rods (Seeley *et al*., 1995; White, 2001). *Bacillus cereus* forms endospores that are heat resistant to pasteurisation but the vegetative cells are rapidly killed at 65°C (Holsinger *et al*., 1997). The spores are associated with mastitis and can be easily isolated from the environment*. Bacillus cereus* was isolated from dairy products including raw and even UHT processed milk. The endospores are not the causative agents of the disease but release enterotoxins upon germination. Two distinct symptoms of gastro-enteritis due to the toxins are vomiting and diarrhoea (Holsinger *et al*., 1997).

In addition to being a disease causing agent, *B. cereus* is a major spoilage organism of dairy products. The spores are also contaminants in dry dairy products such as non-fat dry milk, which may be incorporated as food ingredients. The final number of spores present depends on the initial number of spores in the raw milk. The spore content of powders used for the manufacture of infant formula should not exceed 100 per gram (Holsinger *et al*., 1997).

Escherichia coli - is a Gram-negative rod bacterium, which causes diarrhoea in

humans after consumption. The growth temperature for *E. coli* is over a range of 7°C to 48°C with an optimum temperature of 37°C. Some strains are recognised as low temperature pathogens and can even grow at 4° to 5°C and possibly at lower temperatures (Varnam, 1991). Any strain of *E. coli* that causes diarrhoea in humans is considered to be enteropathogenic. *Escherichia coli* are readily isolated from the intestinal tract of warm-blooded animals, including dairy cattle. Raw milk is thus easily contaminated through contact with faecal material. *Escherichia coli* may also be isolated from the milk of mastitic animals. Since *E. coli* does not survive pasteurisation, the presence of coliforms in pasteurised milk is commonly used by dairy plants as an indicator of post-pasteurisation contamination (Holsinger *et al.,* 1997).

***Streptococcus* -** the members of this genus are Gram-positive, catalase negative cocci that often appear as spherical to ovoid forms. They produce small colonies when growing on culture media (Frazier, 1967). They grow within the psychrophillic range while some of them are mesophillic. Streptococci are wide-spread on plants and in dairy products. The presence of some species in foods in large numbers may indicate faecal contamination (Jay, 1978).

***Pseudomonas* -** are short Gram-negative rods that are generally motile by polar flagella. The pseudomonads are strict aerobes though some species can grow anaerobically using nitrate of O_2 as a terminal electron acceptor (Seeley *et al*., 1995). Many psychrophillic species and strains are members of this genus. Psychrophillic bacteria are bacteria that grow well below 20°C and can grow at temperatures just above freezing. Pseudomonads are the most important bacteria in the low temperature spoilage of foods (Jay, 1978; Frazier & Westhoff, 1978). These bacteria are metabolically very versatile and wide-spread in soil, pond water, eggs, milk, fish, shellfish, leafy vegetables and poultry (Seeley *et al*., 1995).

Mycelial fungi - if spores are present, these organisms begin to grow at the surface of the sour milk and raise the pH towards neutrality, thus allowing more proteolytic bacteria such as *Pseudomonas* spp. to grow and bring about the deproteinisation of milk (Jay, 1978).

G. SHELF-LIFE OF PASTEURISED MILK

The shelf-life of pasteurised milk and dairy products mainly depends on the extent

of post-pasteurisation contamination (Vasacanda & Cousin, 1993). Prevention of recontamination is not a discrete process such as pasteurisation, but is of major importance in the production of pasteurised milk that has both a safe and satisfactory shelf-life. During HTST pasteurisation, milk moves through the plant to the final container in closed pipes and tanks. Despite this, recontamination of pasteurised milk does occur with serious potential consequences for public health and spoilage. The possible routes of contamination are summarised in Fig. 2 (Varnam & Sutherland, 1994). The shelf-life of pasteurised milk may be extended by the application of higher pasteurisation temperatures or the LP (lactoperoxidase) system, addition of biopreservatives, use of microfiltration techniques, the electrical or thermisation process (Sarkar, 1999; Marks *et al*., 2001).

Application of the lactoperoxidase (LP) system – The LP system is a naturally-occurring antimicrobial system present in raw milk which is active against both Gram-positive and Gram-negative bacteria to varying extents. LP is heat sensitive but retains the majority of its activity in milk pasteurised at 72°C/15 s. However, as the temperature is increased, the LP activity decreases rapidly until it cannot be detected following treatment at approximately 80°C (Marks *et al*., 2001).

Utilisation of LP-activated milk for the production of pasteurised milk shows positive results in terms of shelf-life of the product. Thus the activation of the LP-system to extend the shelf-life of pasteurised milk is suggested when raw milk is to be stored for more than two days prior to processing (Sarkar, 1999).

Use of biopreservatives – Antimicrobial compounds produced by starter cultures could be incorporated in milk to restrict the growth of spoilage and pathogenic organisms. Nicin, a bacteriocin like compound produced by *Lactococcus lactis* subsp. *lactis*, when incorporated into milk has been reported to induce an extension in the shelf-life of pasteurised milk from 2 - 12 d at 25°C or 32 d at 4°C. Therefore, an extension in the shelf-life of pasteurised milk due to incorporation of nicin is also dependent on the temperature of its storage (Sarkar, 1999).

Application of the bactofugation technique – Bactofugation is a process in which milk is subjected to centrifugal force and the bacteria are removed based on

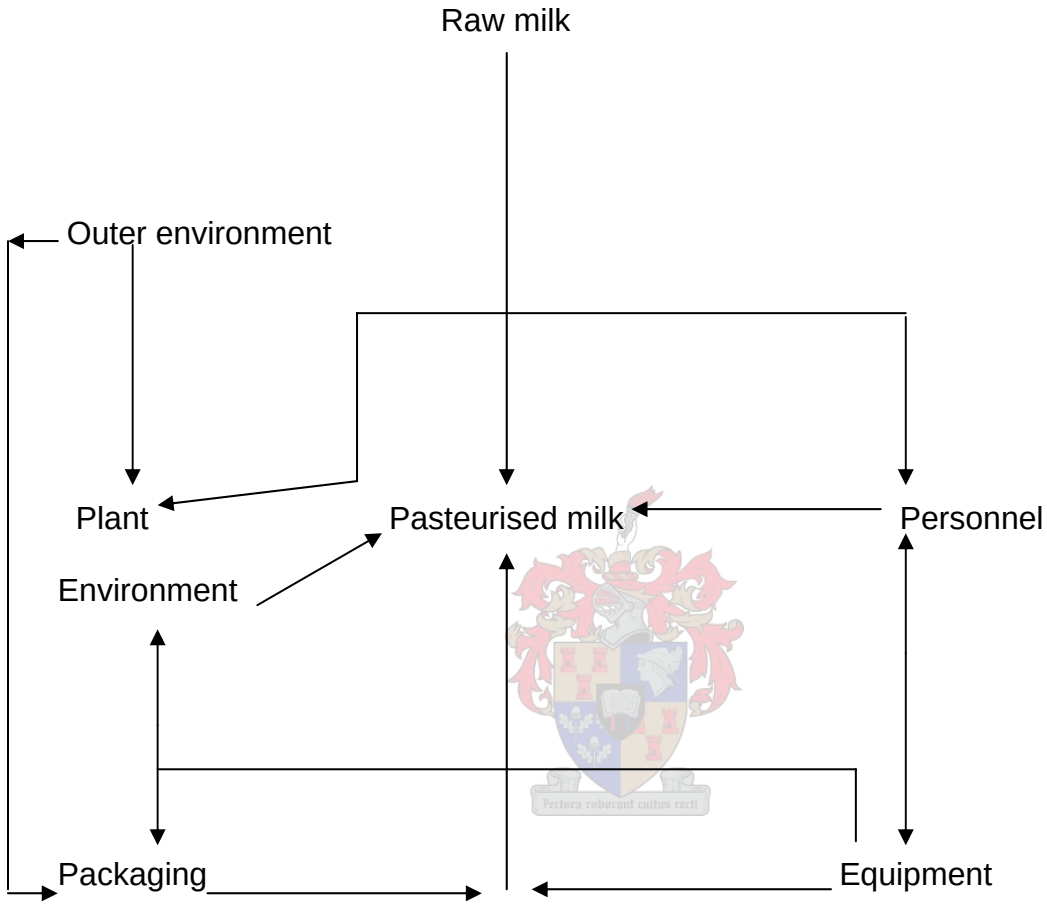
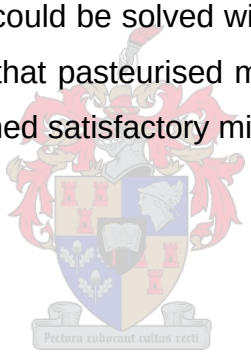


Figure 2. Possible routes of contamination of pasteurised milk (Varnam & Sutherland, 1994).

their density. Bactofugation of milk is reported to give a reduction of 92% in total bacteria, of which 88% may be aerobic spore formers (Sarkar, 1999). A reduction of spore count and an extension in the shelf-life of bactofugated milk by more than 20 h has been reported if recontamination is avoided (Sarkar, 1999).

Use of microfiltration techniques – Microbiological and hygienic quality of traditional products could be improved by installing membrane filtration (MF) techniques. The use of MF-techniques for microbial purification of milk intended for pasteurised milk production would result in an extension in the shelf-life due to the removal of almost all endospores (Sarkar, 1999).

Application of thermisation processes – Thermisation is a process in which an extra heat-treatment is given to milk in addition to the pasteurisation step. Problems with pasteurised milk associated with the growth of psychrotrophs due to extended refrigerated storage could be solved with the introduction of thermisation process. Results have shown that pasteurised milk produced from thermised milk stored for 3 d at 3° - 5°C, retained satisfactory microbiological quality for more than 7 d at 3° - 5°C (Sarkar, 1999).



H. DISCUSSION

The aim of modern milk processing is to produce a food product that appeals to the consumer, is safe and has an acceptable shelf-life as economically as possible (Banks *et al*., 1981). The microbiological spoilage of milk and dairy products depends on the quality of the raw milk used, the contamination during processing and the processing treatments applied (Vasacanda & Cousin, 1993; Hayes & Boor, 2001). Spoilage of pasteurised milk may also be as a result of the growth of organisms that survive pasteurisation (Banks *et al.,* 1981; Bunning *et al*., 1988). Recontamination of milk (post-pasteurisation) may also be a factor that leads to milk deterioration in finished foods. However, the presence of psychrotrophs in cold raw milk (pre-processing) could be the critical factor in undermining the keeping quality of pasteurised milk and other dairy products (Banks *et al*., 1981; Zall, 1981; Knabel *et al*., 1990; Waak *et al.,* 2002).

Cronjé (2003) identified the presence of microbes in pasteurised and

double pasteurised milk. The isolated bacterial cultures were found to strongly contribute to the deterioration of milk. This resulted in a shorter shelf-life of the milk and the quality of the final dairy product. The survival of these contaminants in pasteurised milk with subsequent spoilage of any further produced dairy products necessitates research on pasteurisation temperature/time combinations, in order to produce dairy products that are safe and have a long shelf-life.

I. REFERENCES

Anonymous. (2002). *Foodstuffs, Cosmetics and Disinfectant Act and Regulations.* Act no. 54 of 1972, G. N. R. 1555/1997. Johannesburg, South Africa: Lex Patria Publishers.

Banks, W., Dalgleish, D.G. & Rook, J.A.F. (1981). Milk and milk processing. In: *Dairy Microbiology* (edited by R.K. Robinson), Volume 1. Pp. 1-34. London: Applied Science Publishers.

Bunning, V.K., Donnelly, C.W., Peeler, J.T., Briggs, E.H., Bradshaw, J.G. Crawford, R.G., Beliveau, C.M. & Tierney, J.T. (1988). Thermal inactivation of *Listeria monocytogenes* within bovine milk phagocytes. *Applied and Environmental Microbiology*, **54**, 364-370.

Bunning, V.K., Crawford, R.G., Tierney, J.T. & Peeler, J.T. (1992). Thermotolerance of heat shocked *Listeria monocytogenes* in milk exposed to High-Temperature Short-Time pasteurisation. *Applied and Environmental Microbiology*, **58**, 2096-2098.

Caudill, V. (1993). Engineering: plant design, processing & packaging. In: *Dairy Science and Technology Handbook* (edited by Y.H. Hui), Volume 2. Pp. 312-317. New York: VCH Publishers, Inc.

Cerf, O. & Griffiths, M.W. (2000). Letter to the editor. *Letters in Applied Microbiology*, **30**, 341-344.

Corash, P. (1951). Milk and milk products. In: *The Chemistry and Technology of Food and Food Products* (edited by M.B. Jacobs), Volume 3. Pp. 2219-2226. New York: Interscience Publishers.

Cronjé, M.C. (2003). Production of kepi grains using pure cultures as starters. *M.Sc Thesis*, University of Stellenbosch, Stellenbosch, South Africa.

Doyle, M.P., Glass, K.A., Beery, J.T., Garcia, G.A., Pollard, D.J. & Schultz, R.D. (1987). Survival of *Listeria monocytogenes* in milk during High-Temperature Short-Time pasteurisation. *Applied and Environmental Microbiology*, **53**, 1433-1438.

Dubos R. (1998). *Pasteur and Modern Science* (edited by T.D. Brock). Pp. 3-27. Washington D.C.: ASM Press.

Farber, J.M. & Brown, B.E. (1990). Effect of prior heat shock on heat resistance of *Listeria monocytogens* in meat. *Applied and Environmental Microbiology*, **56**, 1584-1587.

Fellows, P.J. (1996). Pasteurisation. In: *Food Processing Technology, Principles and Practice*. Pp. 210-220. England: Woodhead Publishing Limited.

Fox, B.A. & Cameron, A.G. (1982). Food spoilage, preservation and hygiene. In: *Food Science - A Chemical Approach*, 4th ed. Pp. 315-316. London: Hodder & Stoughton.

Franklin, J.G. (1965). A simple laboratory scale HTST milk pasteuriser. *Journal of Dairy Research*, **32**, 281-289.

Frazier, W.C. (1967). *Food Microbiology*, 2nd ed. Pp. 58-94. New York: McGraw-Hill Book Company.

Frazier, W.C. & Westhoff, D.C. (1978*). Food Microbiology*, 3rd ed. Pp. 17-64. New York: McGraw-Hill Book Company.

Grant, I.R., Ball, H.J., Neill, S.D. & Rowe, M.T. (1996a). Inactivation of *Mycobacterium paratuberculosis* in cow's milk at pasteurisation temperatures. *Applied and Environmental Microbiology*, **62**, 631-636.

Grant, I.R., Ball, H.J. & Rowe, M.T. (1996b). Thermal inactivation of several *Mycobacterium spp*. in milk by pasteurisation. *Letters in Applied Microbiology*, **22**, 253-256.

Grant, I.R., Ball, H.J. & Rowe, M.T. (1998). Effect of High-Temperature Short-Time (HTST) pasteurisation on milk containing low numbers of *Mycobacterium paratuberculosis*. *Letters in Applied Microbiology*, **26**, 166-170.

Grant, I.R., Ball, H.J. & Rowe, M.T. (1999). Effect of higher pasteurisation temperatures, and longer holding times at 72°C, on the inactivation of *Mycobacterium paratuberculosis* in milk. *Letters in Applied Microbiology*, **28**, 461-465.

Grant, I.R., Hitchings, E.I., McCartney, A., Ferguson, F. & Rowe, M.T. (2002a). Effect of commercial-scale High Temperature Short Time pasteurisation on the viability of *Mycobacterium paratuberculosis* in naturally infected cows' milk. *Applied and Environmental Microbiology*, **68**, 602-607.

Grant, I.R., Ball, H.J. & Rowe, M.T. (2002b). Incidence of *Mycobacterium paratuberculosis* in bulk raw and commercially pasteurised cows' milk from approved dairy processing establishments in the United Kingdom. *Applied and Environmental Microbiology*, **68**, 2428-2435.

Harvey, C. & Hill, H. (1967). *Milk: Production and Control*, 4th ed. Pp.363-373 London: Lewis & Co Ltd.

Hayes, M.C. & Boor, K. (2001). Raw milk and fluid milk products. In: *Applied Dairy Microbiology*, 2nd ed. (edited by E.H. Marth & J.L. Steele). Pp. 59-79. New York: Marcel Dekker, Inc.

Holsinger, V.H., Rajkowski, K.T. & Stabel, J. R. (1997). Milk pasteurisation and safety: a brief history and update. *Revue Scientifique et Technique Office International des Epizooties*, **16**, 441-451.

Jay, J.M. (1978). *Modern Food Microbiology*, 2nd ed. Pp. 14-19. New York: D Van Nostrand Company.

Knabel, S.J., Walker, H.W., Hartman, P.A. & Mendonca, A.F. (1990). Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurisation. *Applied and Environmental Microbiology*, **56**, 370-375.

Marks, N.E., Grandison, A.S & Lewis, M.J. (2001). Challenge testing of the lactoperoxidase system in pasteurised milk. *Journal of Applied Microbiology*, **91**, 735-741.

Nelson, F.E. (1981). The microbiology of market milk. In: *Dairy Microbiology* (edited by R.K. Robinson), Volume 1. Pp. 188-201. London: Applied Science Publishers.

Potter, N.N. & Hotchkiss, J.H. (1995). *Food Science*, 5th ed. Pp.140-286. New York: Chapman & Hall.

Proudlove, R.K. (1989). Dairy products. In: *The Science and Technology of Foods,* 2nd ed. Pp. 106-107. London: Forbers Publications Limited.

Sarkar, S. (1999). Techniques to extend the shelf life of pasteurised milk. *Indian Food Industry,* **18**, 174-177.

Seeley, H.W., Van Dmark, P.J. & Lee, J.L. (1995). Microbes in action. In: *A Laboratory Manual of Microbiology*, 4th ed. Pp 251-264. New York: W. H. Freeman & Co.

Stabel, J.R., Steadham, E.M. & Bolin, C.A. (1997). Heat inactivation of *Mycobacterium paratuberculosis* in raw milk: are current pasteurisation conditions effective? *Applied and Environmental Microbiology*, **63**, 4975-4977.

Stauffer, J.E. (1993). Quality assurance and dairy processing. In: *Dairy Science and Technology Handbook* (edited by Y.H. Hui), Volume 2. Pp. 12–25. New York: VCH Publishers, Inc.

Svensson, B., Eneroth, A., Brendehaug, T., Molin, G. & Christiansson, A. (2000). Involvement of pasteuriser in the contamination of milk by *Bacillus cereus* in a commercial dairy plant. *Journal of Dairy Research*, **67**, 455-460.

Varnam, A.H. (1991). Control of pathogenic micro-organisms in milk. In: *Foodborne Pathogens: An Illustrated Text*. Pp. 447-458. London: Wolfe Publishing Ltd.

Varnam, A.H. & Sutherland, J.P. (1994). *Milk and Milk Products Technology*, *Chemistry and Microbiology*, 1st ed. Pp.89-99. London: Chapman & Hall.

Vasacanda, P.C. & Cousin, M.A. (1993). Dairy microbiology and safety. In: *Dairy Science and Technology Handbook* (edited by Y.H. Hui), Volume 2. Pp. 378-381. New York: VCH Publishers, Inc.

Waak, E., Tham, W. & Danielsson-Tham, M.L. (2002). Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. *Applied and Environmental Microbiology*, **68**, 3366-3370.

White, C.H. (2001). Testing of milk and milk products. In: *Applied Dairy Microbiology*, 2nd ed. (edited by E.H. Marth & J.L. Steele). Pp. 645-655. New York: Marcel Dekker, Inc.

Zall, R.R. (1981). Control and destruction of microorganisms. In: *Dairy Microbiology* (edited by R.K. Robinson), Volume 1. Pp. 77-89. London: Elsevier Applied Science Publishers.

CHAPTER 3

IMPACT OF PROCESSING TEMPERATURES ON SURVIVAL OF MICROBIAL CONTAMINANTS FROM PASTEURISED MILK

Abstract

In this study thermal inactivation of six selected microbes was studied by using the LTLT, HTST and the "pot" pasteurisation methods at low and high inoculum concentration levels of 10^4 and 10^6 cfu.ml^{-1}. The selected microbes included strains of *Bacillus cereus* (S4), *Chryseobacterium meningosepticum* (S5), *Pseudomonas putida* (S6), *Acinetobacter baumannii* (C3), *Escherichia coli* (58) and the *Candida lipolytica* (G1). Survivors were enumerated after heating for 0, 5, 10, 15, 20, 25, 30, 35 and 40 min for both the LTLT and HTST pasteurisation methods and after heating for 0, 10, 20 and 30 min for the "pot" pasteurisation method. The thermal dearth curves were constructed for each selected species. The results showed that with the exception of the *B. cereus* strain other selected microbes at both high and low concentration levels did not survive the LTLT and HTST pasteurisation methods. In contrast, the results from the "pot" pasteuriser showed that *B. cereus* (S4)*, Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3) and *E. coli* (58) strains survived pasteurisation conditions applied. From these results it was thus concluded that the "pot" pasteuriser under the conditions evaluated does not pasteurise effectively, and it is thus recommended that the manufacturer of the "pot" improves the heating quality of the "pot" in order to fulfil the purpose of the function of the "pot" pasteuriser.

Introduction

Milk is considered to be the most nearly perfect food and is especially valued for manufacturing of most dairy products as a result of its nutrient content (Charley & Weaver, 1998). However, because of its high nutritional value it must also be considered an excellent growth medium for a variety of microorganisms (Fox & Cameron, 1982). Thus, efficient heat treatment of milk is essential to eliminate any potential pathogenic and spoilage bacteria that may occur. This problem was

practically solved by the pasteurisation process originally designed by Louis Pasteur. The pasteurisation process is applied by heating the raw milk to a temperature of less than 100°C for a sufficient period of time to destroy any pathogenic bacteria which may be present (Corash, 1951; Dubos, 1998).

Presently, there are two basic methods of pasteurisation in use in the dairy industry, the LTLT and the HTST methods, where the applied heat treatment is considered sufficient to ensure public safety and adequate keeping quality of the dairy product (Grant *et al*., 1996b). In addition to these two methods, there is another method that was introduced a few years ago specifically to accommodate the new South African entrepreneurs who cannot afford to purchase the larger pasteurisers available on the market. This new or cheaper technology is locally known as the "pot" pasteurisation method and was designed to eliminate potential pathogenic and spoilage bacteria possibly present in raw milk so as to produce a good quality final product (Central Melk Melkmasjiendienste, Middelburg, S.A.).

Cronjé (2003) in a study on the production of Kepi grains using pure cultures as starters, identified the presence of microbes in pasteurised and even in "double" pasteurised milk. The "milk isolates" included strains of: *Acinetobacter* sp.; *Candida lipolytica; Chryseobacterium meningosepticum; Pseudomonas putida;* and four isolates related to the *Bacillus cereus* group. The presence of these "milk isolates" in pasteurised milk causes a problem for the food industry resulting in dairy products deteriorating before the expiry date. Whether their survival of the pasteurisation process is due to their survival ability, process temperature/time variations or the pasteurisation method used, is not known. With these facts in mind, Cronjé (2003), after isolating these bacterial and yeast contaminants from pasteurised milk, strongly recommended that research be done to evaluate the effectiveness of pasteurisation, to assimilate data to use in reconsidering efficiency of the pasteurisation parameters and to highlight post-pasteurisation contamination in South African dairies. The aim of this study was to determine the impact of different milk pasteurisation temperature and time combinations on the survival of selected microbes. The accuracy of the "pot" pasteurisation method and how it differs from the other pasteurisation methods will also be determined using the same selected microbes.

Materials and methods

Organisms

Bacillus cereus (S4 = 1335), *Chryseobacterium meningosepticum* (S5 = 1336), *Pseudomonas putida* (S6 = 1337), *Acinetobacter baumani* (C3 = 1334), *Escherichia coli* (58 = ATCC11775) and *Candida lipolytica* (G1 = 1342) were obtained from the University of Stellenbosch Food Science Culture Collection (USFSCC). These strains were originally isolated from pasteurised and double pasteurised milk purchased as pasteurised milk from local supermarkets (Cronjé, 2003). Reference cultures of *Staphylococcus aureus* (29 = ATCC112600), *Bacillus coagulans* (60 = ATCC7050), *Listeria monocytogenes* (1273 = ATCC15313), *Lactobacillus acidophilus* (1348 = ATCC1348) and *Lactococcus lactis* (315) were also obtained from the USFSCC.

Milk samples

The milk samples used in this study were full cream UHT milk produced by one company and purchased from a local supermarket and were found to contain no viable microbial counts on plate count agar (PCA) (Merck), incubated at 30°C for 24 h.

Growth studies and culture conditions

Stock cultures of *B. cereus* (1335), *Chr. meningosepticum* (1336), *P. putida* (1337), *E. coli* (58), *A. baumanii* (1334), *B. coagulans* (60), *L. monocytogenes* (1273), *S. aureus* (29) and *Can. lipolytica* (1342) were grown on nutrient broth (Merck) and incubated at 30°C for 24 h, with the exception of the *L. monocytogenes* (1273) which was grown in nutrient broth and incubated at 35°C for 48 h. Stock cultures of the *Lb. acidophilus* and *Lc. lactis* strains were grown in MRS broth (Merck) and incubated at 35°C for 24 h. Strain purity was confirmed by streaking the cultures on either PCA or MRS plates at either 30°C or 35°C.

The identification of the *E. coli* strain (58) was done using the API 20E system (API System S.A., La alme le Grottes, 38390 Montalieu Vercieu, France), the *Can. lipolytica* strain using the Rapid ID 32C system, the *B. cereus* strain using the API 50CHB system and the *Chryseobacterium, Acinetobacter* and *Pseudomonas* strains using the API 20NE system. The morphology of the strains was microscopically

investigated after Gram-staining and the catalase and oxidase tests (Merck) were also done on all the strains.

Preparation of inoculum

Serial dilutions (10^{-1} to 10^{-8}) of all the cultures used in the study were done and samples were taken to determine the optical density at 500 nm, using a spectrophotometer (Spectronic 20, Genesys, Spectronic Instruments, USA). The serial dilutions were done in sterile saline solution (SSS) (0.85% (m/v) NaCl) and plated on PCA using the plate method. These plates were incubated at 30°C for 24 h and the number of colonies were determined. From the optical densities (OD) and the number of colonies (cfu.ml^{-1}), standard growth curves (OD versus cfu.ml^{-1}) were used to obtain standard solutions that would yield either 10^4 or 10^6 cfu.ml^{-1} suspensions when added to UHT milk samples.

Pasteurisation

The inoculated milk was pasteurised in the laboratory by three methods as follows:

"Pot" pasteurisation method - Aliquots (2 L) of inoculated UHT milk heated in a "pasteurisation pot" (8 L pasteurisation pot, Central Melk Melkmasjiendienste, Middelburg, S.A.) that was set at 80°C. Milk samples were inoculated with *B. cereus* (1335), *Chr. meningosepticum* (1336), *P. putida* (1337), *E. coli* (58), *A. baumanii* (1334) and *Can. lipolytica* (1342) strains, at a concentration of 10^6 cfu.ml^{-1}. The milk was held until the pasteurisation temperature was attained (35 min "come-up" time) and then held at this temperature for a further 5, 10, 15, 20, 25 and 30 min, as recommended by the manufacturer (Central Melk Melkmasjiendienste, Middelburg, S.A.). After the appropriate holding times, the milk samples were cooled to 4°C and assessed for survival of the inoculated strain.

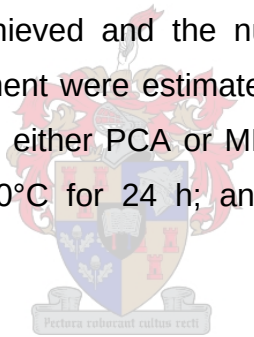
Low Temperature Long Time (LTLT) method - Aliquots (10 ml) of the inoculated milk heated in sterile vials (20 ml volumes) were sealed with aluminium caps and completely immersed in a waterbath adjusted at 63°C. The waterbath temperature during heating was monitored by means of a temperature probe (Manual Thermometer 638Pt, Crison Instruments, S.A.). The vials were immersed until an

internal vial pasteurisation temperature of 63°C was attained (3 min "come-up" time) and they were held at this temperature for 5, 10, 15, 20, 25, 30, 35 and 40 min. After each appropriate holding time, a vial was transferred to an ice-waterbath and cooled to below 10°C, prior to sampling. When the vials reached the desired temperature they were aseptically opened in a laminar flow cabinet (LAMINAIRE, NICO'S Instrumentation c.c., Sanlamhof, S.A.) and plated on PCA.

High Temperature Short Time (HTST) method - The same procedure was followed as done for the LTLT pasteurisation method except that the waterbath was operating at either 72° or 90°C, instead of 63°C. The time necessary for the inoculated milk to attain the temperature of either 72° or 90°C was 2 min ("come-up" time). Sealed milk vials were removed after 0, 0.5, 1, 2, 5 and 10 min.

Assessment of pasteurisation survival

Initial inoculum levels achieved and the number of the microbial colonies surviving the specific heat treatment were estimated as follows: milk samples were serially diluted in SSS; plated on either PCA or MRS using the pour plate method; the plates were incubated at 30°C for 24 h; and the number of colonies were determined.



Results and discussion

As a result of the large volume of data generated during the study, and to simplify the discussion section, the "complete" data is given at the end of this chapter as Appendix A.

Confirmation of microbe identity

The yeast isolate (G1) used in this study was identified as a member of the species *Candida lipolytica* (API identification = 99%). The results of the rapid ID 32C used to confirm the identification of the isolate are given in Table A1 of the Appendix.

The Gram-positive, rod-shaped isolate (S4) used in this study was identified as a member of the *Bacillus cereus* group with the API 50CHB system (Table A2) (API identification = 87.9%). This isolate was catalase positive and oxidase negative and showed endospore formation.

The two other Gram-negative rods (S5 and S6) were identified using the API 20NE data base (Table A3) as *Chryseobacterium meningosepticum* (API identification = 99.9%) and *Pseudomonas putida* (API identification = 99.6%), respectively. Both these rod-shaped bacterial isolates were catalase positive, oxidase negative and negative for endospore formation.

The identification of the cocco/bacilli-shaped isolate C3 was confirmed as a member of *Acinetobacter baumannii* (API identification = 99.9%) with the API 20NE data base. The results of the API 20NE are given in Table A3 of the appendix. This *Acinetobacter baumannii* strain was found to be catalase positive, oxidase negative, Gram-negative and showed no endospore formation.

The reference cultures, *Lactococcus lactis* (315) and *Staphylococcus aureus* (29) were found to be Gram-positive cocci. Both of these cultures were catalase positive, oxidase negative and showed no endospore formation.

Bacillus coagulans (60) was identified as a Gram-positive rod that was catalase and oxidase positive with endospore formation. *Lactobacillus acidophilus* (1348) and *Listeria monocytogenes* (1273) were both identified as Gram-positive rods that were oxidase negative with no endospores being formed. The difference between these two rods was that, *Lb. acidophilus* (1348) was catalase negative and *L. monocytogenes* (1273) was catalase positive.

Milk status before heat treatment

In all cases, the full cream UHT milk used in the study showed no viable counts on PCA that had been incubated at 30°C. For the subsequent studies, the full cream UHT milk was inoculated with a standardised inoculum to give specifically either a 10^4 or a 10^6 cfu.ml^{-1} inoculation level. The inoculated UHT milk was symbolised as M+I (M = milk with no viable counts, and I = specific inoculum level) in the Tables and Figures to follow.

The data in Fig. A1 of the Appendix show all the standard curves of the bacteria examined in the study. From each standard curve, a point of intersection where an OD of 500 nm corresponds with the specific number of colonies (cfu.ml^{-1}) was chosen and the suspension diluted to obtain the required cfu yield.

During the study it was difficult to obtain the same specific data point values for the standard curves. Even though the experiments were repeated at least five

times, it was difficult to get the same values for the same OD values. There was always a small variation in the values that were obtained.

"Come-up" time

The "come-up" times were found to be very important during the pasteurisation treatments used in this study. For the LTLT pasteurisation (63°C) the "come-up" time was 3 min and for HTST (72°C) pasteurisation the "come-up" time was 2 min. These "come-up" times are considered to be long when compared to other reports on pasteurisation treatments (Lund *et al*., 2002; McCormick *et al*., 2003). The explanation for these long "come-up" times was because of the equipment used for the pasteurisation method, the waterbath method used in this experiment took a longer time than a plate heat exchanger used by Doyle *et al*. (1987). These long "come-up" times resulted in some of the study organisms dying off before the required starting temperature was reached, indicating the temperature sensitivity of some of the strains examined.

Low Temperature Low Time (LTLT = Holder method) pasteurisation method

The thermal death/survival curves obtained after applying the LTLT pasteurisation treatment (63°C for 30 min) to the UHT milk inoculated with the "milk isolates" and *E. coli* (58) at a 10^4 cfu.ml^{-1} inoculation level, are shown in Fig. 1. The exact numbers of survivors and the time of death of these organisms are shown in Table 1. The death/survival curves of each strain studied are individually shown in Fig. A2 - A10 of the Appendix.

The data obtained in the study showed that five of the six organisms (Table 1) did not survive the pasteurisation process. The exception, *Bacillus cereus* strain S4, survived pasteurisation at 63°C for 30 min. After the 30 min pasteurisation treatment, the cell reduction of this organism was less than one log reduction (Fig. 1 - 4). Even after a further 10 min pasteurisation time had been added, it was found that this strain still survived pasteurisation for 40 min at 63°C. The survival of the *B. cereus* strain at longer pasteurisation times showed that the endospores of this organism are resistant to pasteurisation. In order to destroy these endospores, sterilisation at a temperature of more than 100°C, would probably be a better method to use.

When the initial concentration of the bacterial strains in the milk substrate was increased to the 10^6 cfu.ml^{-1} level (Table 2), the *B. cereus* strain (S4) again survived

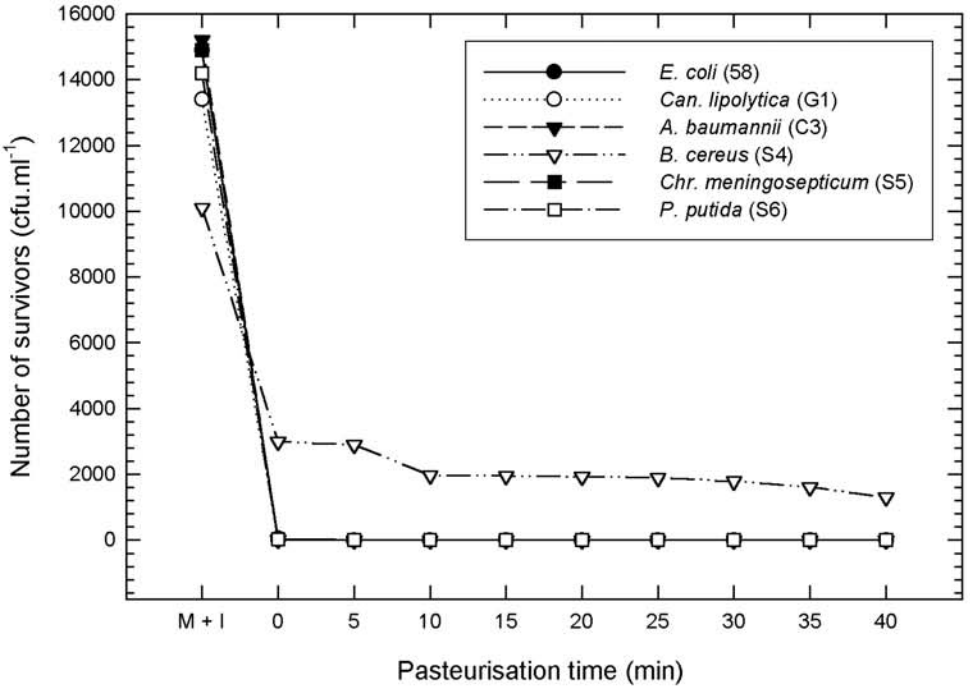


Figure 1. Impact of pasteurisation time at 63°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1.0 X 10^4 (average of quadruplicates).

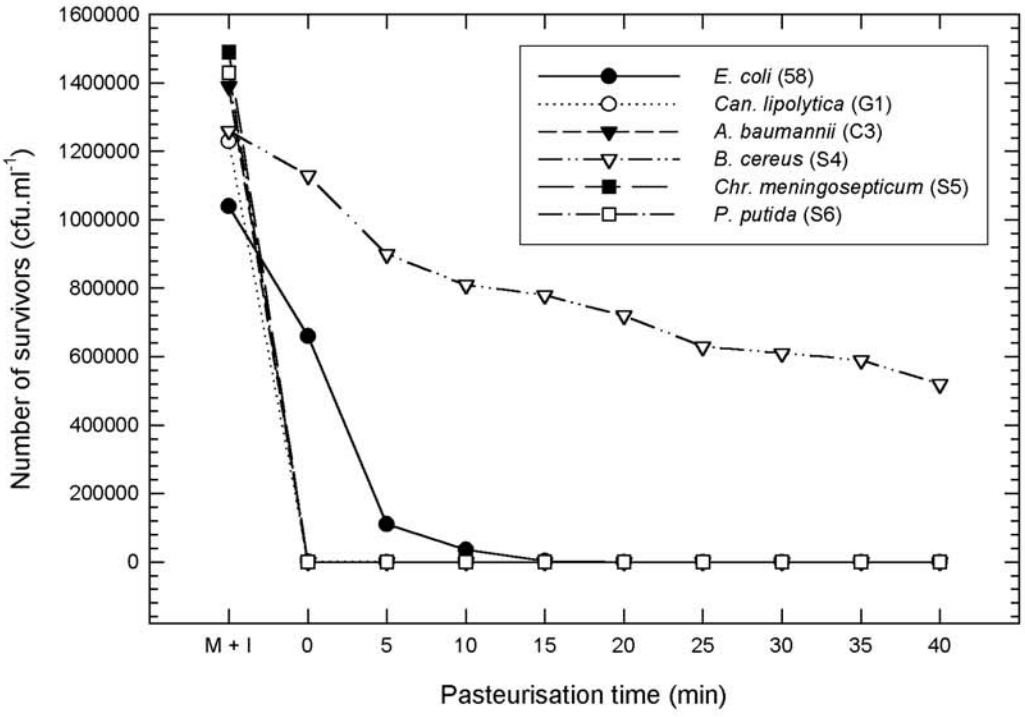


Figure 2. Impact of pasteurisation time at 63°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1.0 X 10^6 (average of quadruplicates).

Table 1. Impact of LTLT pasteurisation at 63°C on the "milk isolates" and *E. coli* (58) at $1x10^4$ $cfu.ml^{-1}$ levels. The values in brackets are the minimum and maximum values of four repeats and the values "not in brackets" are the averages.

Time (min)	***E. coli (58)***	***Can. lipolytica* (G1)**	***A. baumannii* (C3)**	***B. cereus* (S4)**	***Chr. meningosepticum* (S5)**	***P. putida* (S6)**
M	0	0	0	0	0	0
M + I	14 500 (13 200-15 800)	13 300 (13 000-13 500)	14 700 (14 000-15 200)	10 100 (9 700-10 600)	14 000 (12 900-14 900)	14 100 (13 800-14 400)
0 at 63°C (after 3min come-up time)	61 (45-73)	22 (19-25)	11 (8-16)	3 500 (3 000-4 200)	19 (17-22)	29 (26-32)
5	4 (3-6)	0 (0-0)	0 (0-0)	2 880 (2 500-3 400)	8 (6-12)	11 (9-13)
10	0 (0-0)	0 (0-0)	0 (0-0)	2 200 (1 970-2 430)	0 (0-0)	2 (1-4)
15	0 (0-0)	0 (0-0)	0 (0-0)	2 110 (1 950-2 280)	0 (0-0)	0 (0-0)
20	0 (0-0)	0 (0-0)	0 (0-0)	2 070 (1 930-2 240)	0 (0-0)	0 (0-0)
25	0 (0-0)	0 (0-0)	0 (0-0)	2 000 (1 900-2 200)	0 (0-0)	0 (0-0)
30	0 (0-0)	0 (0-0)	0 (0-0)	1 810 (1 790-1 840)	0 (0-0)	0 (0-0)
35	0 (0-0)	0 (0-0)	0 (0-0)	1 590 (1 570-1 619)	0 (0-0)	0 (0-0)
40	0 (0-0)	0 (0-0)	0 (0-0)	1 410 (1 310-1 480)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

Table 2. Impact of LTLT pasteurisation at 63°C on the "milk isolates" and *E. coli* (58) at $1x10^6$ cfu.ml^{-1} levels. The values in brackets are the minimum and maximum values of four repeats and the values "not in brackets" are the averages.

Time (min)	***E. coli* (58)**	***Can. lipolytica* (G1)**	***A. baumannii* (C3)**	***B. cereus* (S4)**	***Chr. meningosepticum* (S5)**	***P. putida* (S6)**
M	0	0	0	0	0	0
M + I	1 030 000 ($99x10^4$-$106x10^4$)	1 220 000 ($118x10^4$-$124x10^4$)	1 290 000 ($121x10^4$-$139x10^4$)	1 190 000 ($114x10^4$-$126x10^4$)	1 390 000 ($130x10^4$-$149x10^4$)	1 430 000 ($140x10^4$-$145x10^4$)
0 at 63°C (after 3min come-up time)	650 000 ($60x10^4$-$67x10^4$)	1 390 ($12.3x10^2$-$14.5x10^2$)	114 (109-117)	1 110 000 ($106x10^4$-$115x10^4$)	129 (127-132)	136 (133-138)
5	115 000 ($110x10^3$-$118x10^3$)	72 (69-75)	57 (49-61)	970 000 ($90x10^4$-$100x10^4$)	68 (64-71)	51 (47-53)
10	37 000 $35x10^3$-$39x10^3$)	4 (2-6)	10 (7-11)	880 000 ($81x10^4$-$95x10^4$)	17 (15-20)	8 (5-10)
15	2 600 ($24x10^2$-$28x10^2$)	0 (0-0)	0 (0-0)	840 000 ($78x10^4$-$89x10^4$)	0 (0-0)	2 (0-3)
20	9 (5-11)	0 (0-0)	0 (0-0)	810 000 ($72x10^4$-$87x10^4$)	0 (0-0)	0 (0-0)
25	0 (0-0)	0 (0-0)	0 (0-0)	7500 00 ($63x10^4$-$82x10^4$)	0 (0-0)	0 (0-0)
30	0 (0-0)	0 (0-0)	0 (0-0)	730 000 ($61x10^4$-$79x10^4$)	0 (0-0)	0 (0-0)
35	0 (0-0)	0 (0-0)	0 (0-0)	680 000 ($59x10^4$-$76x10^4$)	0 (0-0)	0 (0-0)
40	0 (0-0)	0 (0-0)	0 (0-0)	630 000 ($52x10^4$-$70x10^4$)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

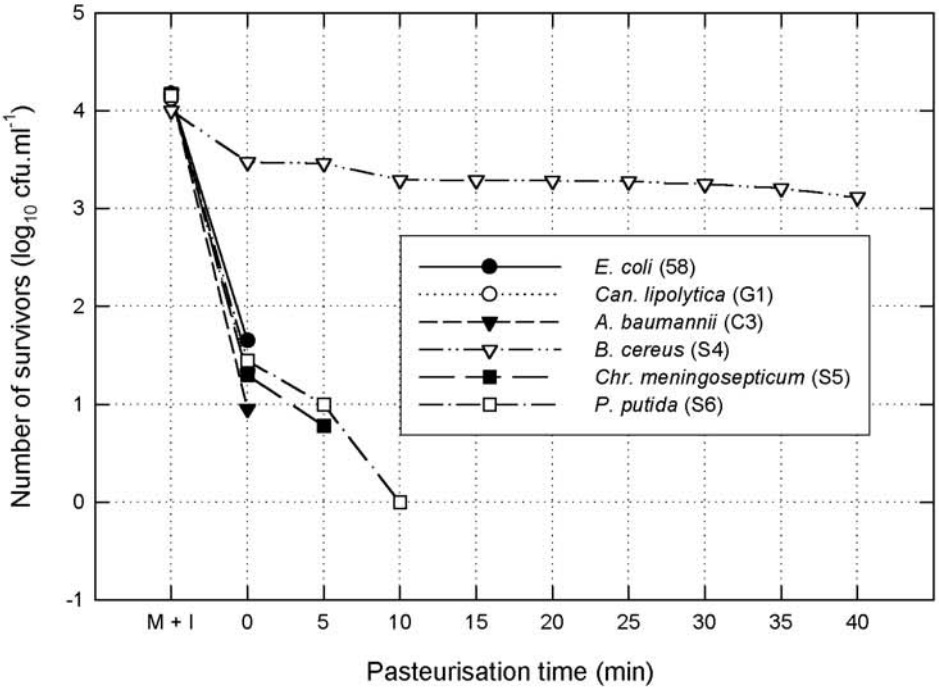


Figure 3. Impact of pasteurisation time at 63°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1.0 X 10^4 (average of quadruplicates).

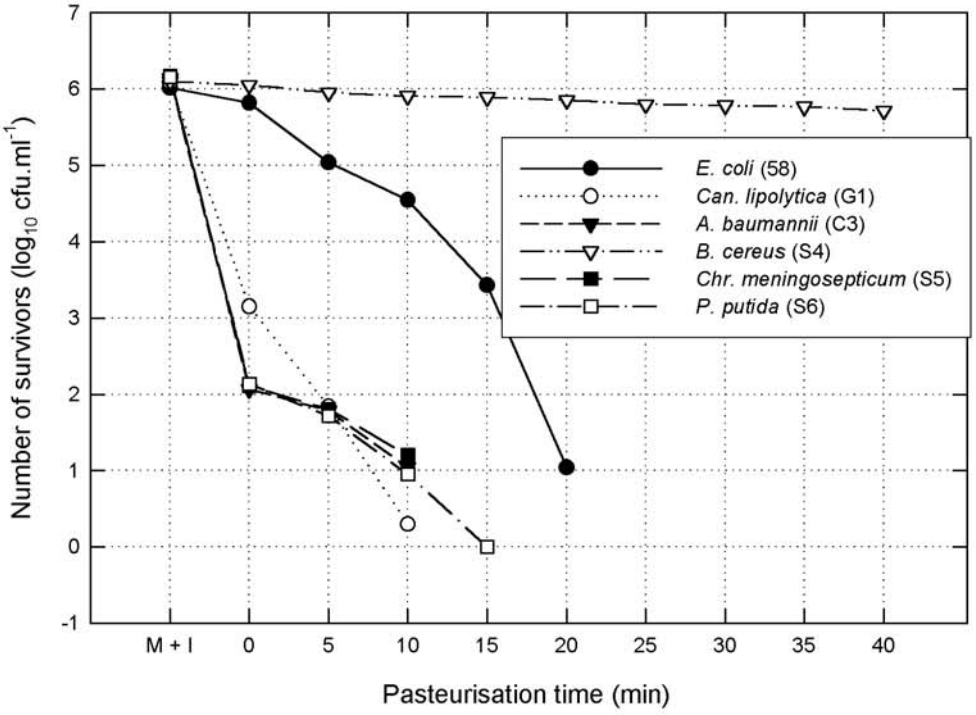


Figure 4. Impact of pasteurisation time at 63°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1.0 X 10^6 (average of quadruplicates).

40 min pasteurisation and a log reduction of less than 1 was found. After 25 min pasteurisation, the *E. coli* (58) strain could not be detected in the milk. The *P. putida* (S6) strain survived for 20 min, followed by the *Chr. meningosepticum* (S5), *A. baumannii* (C3) and *Can. lipolytica* (G1) strains at 15 min.

It was found that for all the organisms used in the study, there was a rapid initial death rate just before the required pasteurisation temperature of 63°C was reached (M + I to time 0). The rapid initial death rate was followed by a significant tailing, which could be ascribed to the survival of heat tolerant or heat resistant cells of the *B. cereus* (S4) strain. In other organisms the rapid initial death rate was followed by a gradual death rate. This means, the presence of these bacteria in pasteurised milk could only have been caused by post-pasteurisation contamination or the improper pasteurisation of milk in the dairy plant.

The decimal reduction times (D-values) were calculated using the data from the death curves of the five organisms that did not survive pasteurisation. D-values are defined as the time required to achieve 1 log reduction in the bacterial population at a designated temperature (Juneja, 2003; McCormick, 2003). However, in this study the "come-up" times were also included in the D-value calculations since the lethality of many bacterial cells would be impacted by the "come-up" time. For the 10^4 cfu.ml^{-1} inoculum levels, $D_{63°C}$ values of the five organisms ranged from 1.6 to 3.6 min (see Fig. 3). The $D_{63°C}$ values noted when using the 10^6 cfu.ml^{-1} inoculum level (see Fig. 4) were larger than those of 10^4 cfu.ml^{-1}. The higher levels ranged from 2.6 to 4 min. This phenomena could possibly be ascribed to the protection offered by high numbers and/or cell clumping.

Reference cultures

Reference cultures of the species *Lc. lactis* (315) and *Lb. acidophilus* (1348) were used in this study because they may play a major role in milk spoilage (Nelson, 1981). A *Bacillus coagulans* (60) reference strain was also included as it is an aerobic endospore former and a second *Bacillus* strain was thought to be necessary to compare with the *Bacillus cereus* (S4) strain as they are of the same genus and have many similar characteristics. The *L. monocytogenes* (1273) and *Staph. aureus* (29) strains were included as it has been reported in the literature that they may survive pasteurisation and are potential pathogens (Doyle *et al*., 1987; Knabel *et al*., 1990).

In the literature it has been shown that the species *Lc. lactis* and *Lb. acidophilus* are capable of growing at low temperatures but are sensitive to heat treatments at 63°C (Sorhaug & Stepaniak, 1997). In this study it was found that both these bacterial strains did not survive pasteurisation at 63°C for 30 min at both the 10^4 and 10^6 cfu.ml^{-1} inoculum levels (Fig. 5 and 6, and Tables 3 and 4 for the complete data set) (Fig. A4 and A5 of the Appendix). It was thus concluded that their presence in pasteurised milk could possibly be due to post-pasteurisation contamination. Their extreme sensitivity to heat was clearly shown in this study as they could, at both inoculum levels of 10^4 and 10^6 cfu.ml^{-1}, not survive even the "come-up" time of 3 min that was applied to reach the recommended 63°C. This implies that even if these bacteria are present at high concentration before LTLT pasteurisation treatment, they will not survive pasteurisation at 63°C.

Listeria monocytogenes (1273) was included in this study as, according to literature, it has been reported to survive LTLT pasteurisation and even HTST pasteurisation (Doyle *et al*., 1987; Knabel, 1990, Bunning *et al*., 1992). In this study the *L. monocytogenes* (1273) strain did not survive pasteurisation at 63°C for 30 min but reached a zero value after 15 min at the 1 x 10^4 cfu.ml^{-1} inoculum and 20 min at the 1 x 10^6 cfu.ml^{-1} inoculum level (Fig. 5 and 6) (Fig A4 and A5 of the Appendix). The precise values are given in Tables 3 and 4. *Listeria monocytogenes* is of concern to the food industry because it is one of the bacteria known as a disease causing microbe/pathogen. Ingestion of food contaminated with *L. monocytogenes* may cause listeriosis, a disease with symptoms of diarrhoea and a mild fever that usually appears in one to more weeks after ingestion (Varnam, 1991). Listeriosis may also result in death in severe cases. The presence of *Listeria* has been associated with the dairy products, especially raw milk (Varnam, 1991).

The data from the study also showed that *Staphylococcus aureus* did not survive pasteurisation at 63°C for 30 min (Fig. 5 and 6) (Fig A4 and A5 of the Appendix) (Tables 3 and 4 for the complete data set). However, the strain did survive for 15 and 20 min at the 10^4 and 10^6 inoculum levels, respectively. This organism is known to be "heat resistant" when the bacterial culture is stored at room temperature, but the data from this study showed that the organism does not survive the LTLT pasteurisation method.

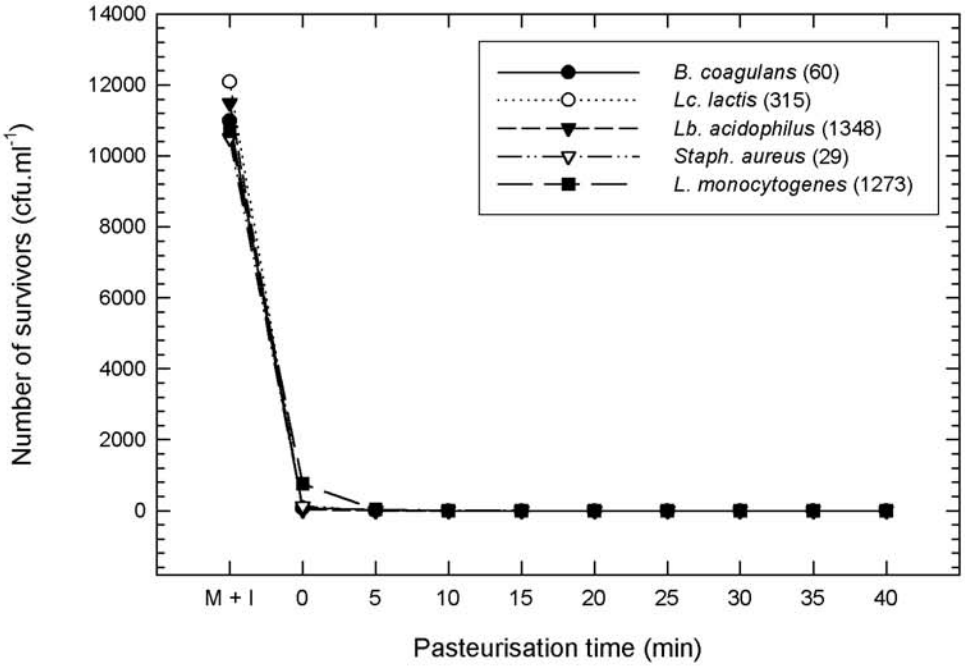


Figure 5. Impact of pasteurisation time at 63°C on the sensitivity of *B. coagulans* (60), *Lc. lactis* (315), *Lb. acidophilus* (1348), *Staph. aureus* (29) and *L. monocytogenes* (1273) with an average starting inoculum of 1.0 X 10^4 (average of triplicates).

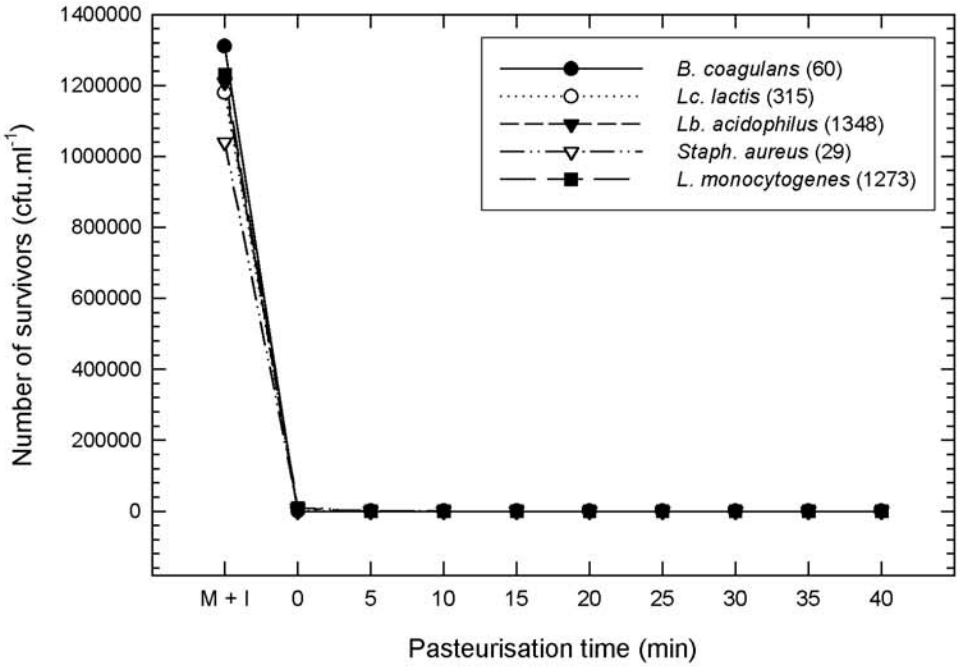


Figure 6. Impact of pasteurisation time at 63°C on the sensitivity of *B. coagulans* (60), *Lc. lactis* (315), *Lb. acidophilus* (1348), *Staph. aureus* (29) and *L. monocytogenes* (1273) with an average starting inoculum of 1.0 X 10^6 (average of triplicates).

Table 3. Impact of LTLT pasteurisation at 63°C on the reference culture at 1 X 10^4 cfu.ml^{-1} levels. The values in brackets are the minimum and maximum values of the triplicates and the values "not in brackets" are the averages.

Time (min)	***B. coagulans* (60)**	***Lc. lactis* (315)**	***Lb. acidophilus* (1348)**	***Staph. aureus* (29)**	***L. monocytogenes* (1273)**
M	0	0	0	0	0
M + I	11 100 (10 700-11 500)	12 200 (11 900-12 700)	12 200 (11 500-12 800)	10 900 (10 500-11 200)	11 400 (10 700-12 100)
0 at 63°C (after 3min come-up time)	79 (74-89)	56 (49-64)	31 (29-34)	132 (129-136)	813 (760-850)
5	16 (12-20)	0 (0-0)	5 (3-7)	9 (7-11)	35 (30-42)
10	0 (0-0)	0 (0-0)	1 (0-2)	4 (3-4)	6 (4-9)
15	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
20	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
25	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
30	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
35	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
40	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

Table 4. Impact of LTLT pasteurisation at 63°C on the reference cultures at 1 X 10^6 cfu.ml^{-1} levels. The values in brackets are the minimum and maximum values of triplicates and the values "not in brackets" are the averages.

Time (min)	***B. coagulans* (60)**	***Lc. lactis* (315)**	***Lb. acidophilus* (1348)**	***Staph. aureus* (29)**	***L. monocytogenes* (1273)**
M	0	0	0	0	0
M + I	1 230 000 ($116x10^4$-$131x10^4$)	1 250 000 ($118x10^4$-$131x10^4$)	1 210 000 ($119x10^4$-$124x10^4$)	1 090 000 ($104x10^4$-$114x10^4$)	1 240 000 ($118x10^4$-$131x10^4$)
0 at 63°C (after 3min come-up time)	188 (179-208)	380 (360-410)	113 (108-118)	9 700 ($91x10^2$-$102x10^2$)	10 300 ($97x10^2$-$110x10^2$)
5	67 (60-74)	27 (21-32)	28 (23-32)	700 (680-720)	600 (570-620)
10	6 (4-8)	5 (2-7)	6 (4-7)	81 (79-83)	66 (64-73)
15	0 (0-0)	0 (0-0)	1 (0-1)	26 (22-30)	3 (2-4)
20	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
25	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
30	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
35	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
40	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

Data from the pasteurisation treatment showed that the *B. coagulans* (60) did not survive the pasteurisation at 63°C for 30 min at both the high (10^6) and low (10^4) concentrations (Tables 3 and 4) (Fig. 5 and 6) (Fig. A4 and A5 of the Appendix), but the strain did survive for 10 and 15 min at the two inoculum levels. These results support the findings that, endospores of *B. coagulans* are less heat resistant than the endospores of *B. cereus* (Sorhaug & Stepaniak, 1987; Palop *et al.,* 1999) and could be destroyed by the LTLT heat treatment. This means that *B. coagulans* should not be a problem in efficiently pasteurised milk.

High Temperature Short Time (HTST) pasteurisation method at *72°C*

The thermal survival/death curves obtained for the "milk isolates" at the HTST (72°C for 10 min) pasteurisation at the two different inoculum concentrations are shown in Fig. 7 and 8, and the individual graphs are shown in Fig. A6 and A7 of the Appendix. The precise values of the obtained data are given in Tables 5 and 6. *Escherichia coli, Can. lipolytica* and all the reference cultures with the exception of *B. coagulans* (60) were not included in the HTST pasteurisation treatment because previous results with the LTLT heat treatment showed that they were not even resistant to the low temperature treatment.

From the data obtained for the thermal curves, only the *B. cereus* strain (S4) survived the HTST pasteurisation treatment at 72°C for 10 min as applied in this study. This bacterial strain was so resistant to this specific heat treatment that not even 1 log reduction of its initial concentration at both the 10^4 and 10^6 cfu.ml^{-1} inoculum levels was reached. The other four bacterial strains, *A. baumannii* (C3), *Chr*. *meningosepticum* (S5), *P. putida* (S6) and *B. coagulans* (60) did not survive the HTST pasteurisation treatment at 72°C.

Of the reference cultures only the *B. coagulans* was selected to be evaluated at higher temperatures so as to compare it with the *B. cereus* strain. The other reference strains were sensitive to the HTST pasteurisation treatment in as much that at both inoculum levels of 10^4 and 10^6 cfu.ml^{-1} they could not survive even the "come-up" time of 2 min that was applied to reach the recommended 72°C. The "come-up" time of 2 min applied in this study was longer than the usual "come-up" time that would industrially be applied for the HTST pasteurisation treatment which is normally 55 sec (Doyle *et al*., 1987). From the data obtained, it was concluded that the longer "come-up" time was the main reason for the death of these organisms.

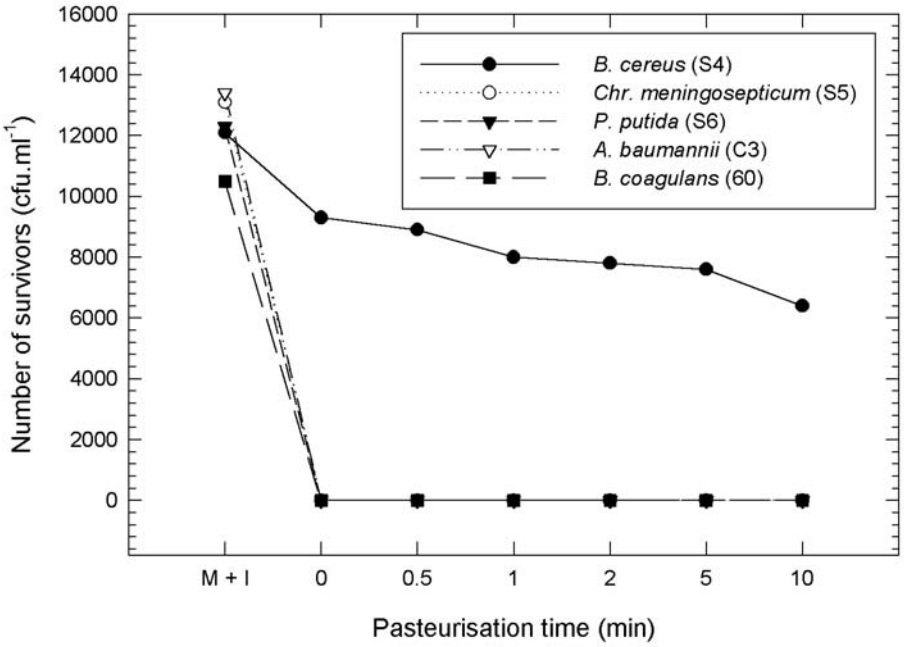


Figure 7. Impact of pasteurisation time at 72°C on survival of *B. c*ereus (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3) and *B. coagulans* (60) with an average starting inoculum of 1.0 x 10^4 (average of triplicates).

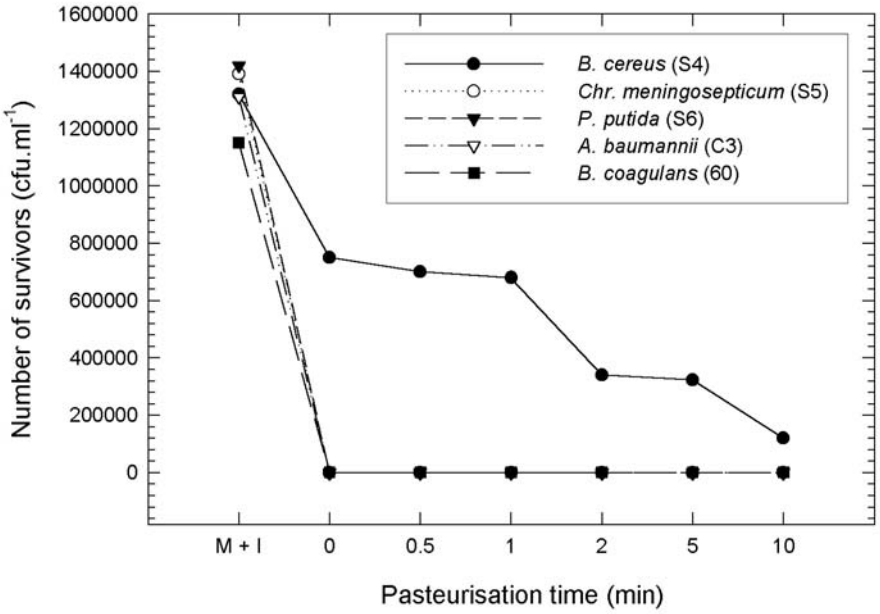


Figure 8. Impact of pasteurisation time at 72°C on survival of *B. c*ereus (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3) and *B. coagulans* (60) with an average starting inoculum of 1.0 x 10^6 (average of triplicates).

Table 5. Impact of HTST pasteurisation at 72°C on the "milk" bacterial isolates and *B. coagulans* (60) at 1 X 10^4 cfu.ml^{-1} levels. The values in brackets are the minimum and maximum values of triplicates and the values "not in brackets" are the averages.

Time (min)	***A. baumannii* (C3)**	***B. cereus* (S4)**	***Chr. meningosepticum* (S5)**	***P. putida* (S6)**	***B. coagulans* (60)**
M	0	0	0	0	0
M + I	12 600 (12 000-13 400)	12 000 (11 700-12 300)	12 600 (12 100-13 100)	12 400 (11 900-13 00)	11 300 (10 500-12 100)
0 at 72°C (after 3min come-up time)	0 (0-0)	9 400 (9 200-9 600)	0 (0-0)	0 (0-0)	0 (0-0)
0.5	0 (0-0)	8 900 (8 800 9 000)	0 (0-0)	0 (0-0)	0 (0-0)
1	0 (0-0)	8 100 (7 900-8 300)	0 (0-0)	0 (0-0)	0 (0-0)
2	0 (0-0)	7 700 (7 600-7 800)	0 (0-0)	0 (0-0)	0 (0-0)
5	0 (0-0)	7 500 (7 300-7 600)	0 (0-0)	0 (0-0)	0 (0-0)
10	0 (0-0)	6 500 (6 300-6 800)	0 (0-0)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

Table 6. Impact of HTST pasteurisation at 72°C on the "milk" bacterial isolates and *B. coagulans* (60) at 1 X 10^6 cfu.ml^{-1} levels. The values in brackets are the minimum and maximum values of triplicates and the values "not in brackets" are the averages.

Time (min)	***A. baumannii* (C3)**	***B. cereus* (S4)**	***Chr. meningosepticum* (S5)**	***P. putida* (S6)**	***B. coagulans* (60)**
M	0	0	0	0	0
M + I	1 220 000 ($109x10^4$-$131x10^4$)	1 300 000 ($124x10^4$-$134x10^4$)	1 320 000 ($126x10^4$-$139x10^4$)	1 360 000 ($128x10^4$-$142x10^4$)	1 150 000 ($104x10^4$-$125x10^4$)
0 at 72°C (after 3min come-up time)	0 (0-0)	760 000 ($73x10^4$-$81x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
0.5	0 (0-0)	710 000 ($69x10^4$-$74x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
1	0 (0-0)	650 000 ($62x10^4$-$68x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
2	0 (0-0)	360 000 ($33x10^4$-$40x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
5	0 (0-0)	217 000 ($201x10^3$-$232x10^3$)	0 (0-0)	0 (0-0)	0 (0-0)
10	0 (0-0)	127 000 ($121x10^3$-$133x10^3$)	0 (0-0)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

High Temperature Short Time (HTST) pasteurisation method at 90°C

The thermal heat treatment curves obtained for the "milk isolates" at the HTST (90°C for 10 min) pasteurisation treatment at the two different inoculum concentrations are shown in Fig. 9 and 10. The individual graphs are shown in Fig. A8 and A9 of the Appendix. The same strains that had been tested in the pasteurisation at the 72°C for 10 min treatment were evaluated in this method.

In this method only the *B. cereus* (S4) strain survived the pasteurisation temperature of 90°C for 10 min. But in this case of the 10^6 cfu.ml^{-1} concentration level the bacterial cells were found to decrease by 1 log reduction, and it was thus concluded that the higher the temperature, the better the reduction was. At the 10^4 cfu.ml^{-1} level the cell reduction was found to be less than 1 log, which was similar to the previous method applied. The other bacterial strains did not survive the HTST pasteurisation treatment of 90°C for 10 min at both the 10^4 and 10^6 cfu.ml^{-1} concentration levels. It was also found that they did not survive the "come-up" time of 2 min, again illustrating their heat sensitivity to the higher temperatures.

Survival profile

All the "milk isolates" used in this study, at both the high (90°C) and lower (72°C) HTST pasteurisation treatments, exhibited linear thermal death curves except for the *Bacillus cereus* strain which was more heat resistant. The thermal survival curve obtained for the *B. cereus* was concave in shape, exhibiting a rapid initial death rate, followed by significant "tailing" which indicated survival after pasteurisation.

Grant *et al.* (1996a) while working on the inactivation of *Mycobacterium paratuberculosis* in cow's milk at pasteurisation temperatures, suggested that the tailing of thermal death curves may be the result of the clumping of bacterial cells during heating. If that was the case, then the initial rapid drop in numbers of these bacterial cells observed was not representing death but instead, aggregation of cells into clumps (as can be seen in Fig. 11, micrograph of the *Staph. aureus* strain).

In the case of the *B. cereus* (S4) strain used in this study, the survival may rather be ascribed to the formation of heat resistant endospores as shown in Fig. 12. The endospores of *B. cereus* in pasteurised milk survived the heat treatment, germinated and then formed colonies. Since *B. cereus* endospores survive the heat treatment

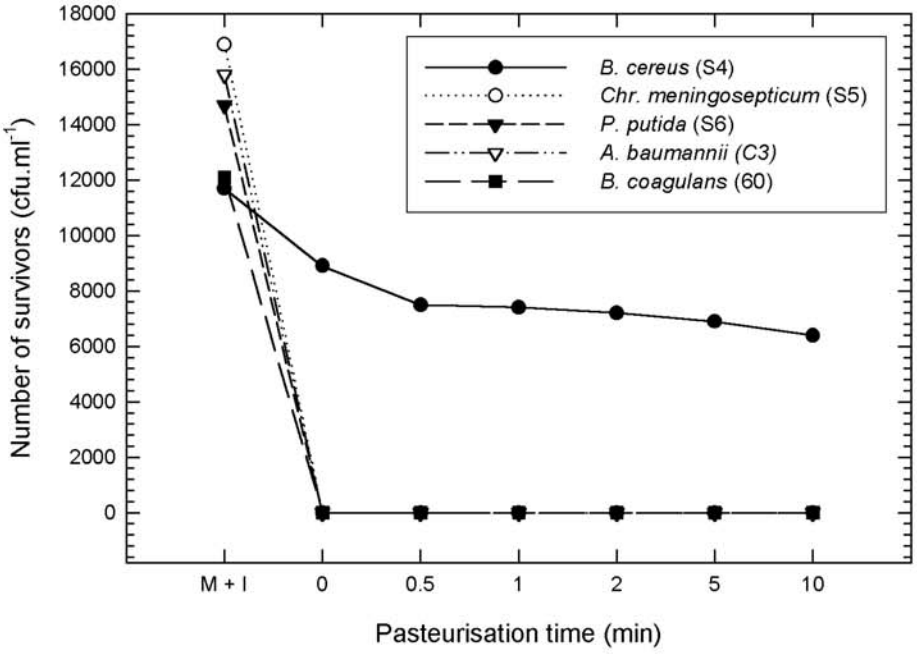


Figure 9. Impact of pasteurisation time at 90°C on survival of *B. c*ereus (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3) and *B. coagulans* (60) with an average starting inoculum of 1.0 x 10^4 (average of triplicates).

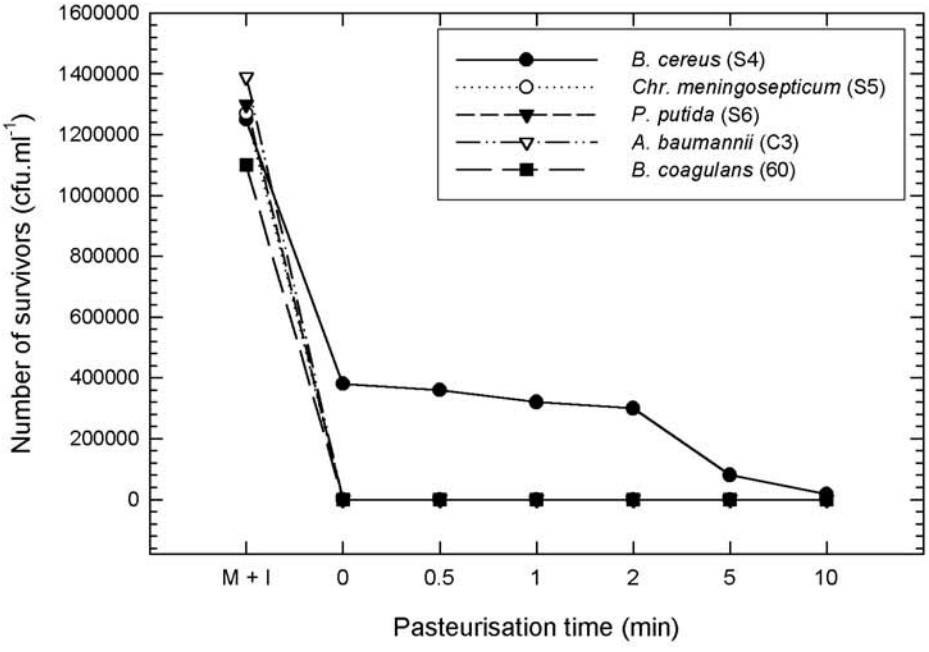


Figure 10. Impact of pasteurisation time at 90°C on survival of *B. c*ereus (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3) and *B. coagulans* (60) with an average starting inoculum of 1.0 x 10^6 (average of triplicates).

Table 7. Impact of HTST pasteurisation at 90°C on the "milk" bacterial isolates and *B. coagulans* (60) at 1 X 10^4 cfu.ml^{-1} levels. The values in brackets are the minimum and the maximum values of triplicates and the values "not in brackets" are the averages.

Time (min)	***A. baumannii* (C3)**	***B. cereus* (S4)**	***Chr. meningosepticum* (S5)**	***P. putida* (S6)**	***B. coagulans* (60)**
M	0	0	0	0	0
M + I	14 300 (12 900-15 800)	11 900 (11 700-12 300)	14 300 (12 900-16 900)	13 400 (12 500-14 700)	11 500 (10 900-12 100)
0 at 90°C (after 3min come-up time)	0 (0-0)	9 000 (8 900-9 300)	0 (0-0)	0 (0-0)	0 (0-0)
0.5	0 (0-0)	7 800 (7 500-8 100)	0 (0-0)	0 (0-0)	0 (0-0)
1	0 (0-0)	7 600 (7 500-7 900)	0 (0-0)	0 (0-0)	0 (0-0)
2	0 (0-0)	7 400 (7 000-8 100)	0 (0-0)	0 (0-0)	0 (0-0)
5	0 (0-0)	6 900 (6 700-7 200)	0 (0-0)	0 (0-0)	0 (0-0)
10	0 (0-0)	6 400 (6 300- 6 600)	0 (0-0)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

Table 8. Impact of HTST pasteurisation at 90°C on the "milk" bacterial isolates and *B. coagulans* (60) at 1 X 10^6 cfu.ml^{-1} levels. The values in brackets are the minimum and maximum values of triplicates and the values "not in brackets" are the averages.

Time (min)	***A. baumannii* (C3)**	***B. cereus* (S4)**	***Chr. meningosepticum* (S5)**	***P. putida* (S6)**	***B. coagulans* (60)**
M	0	0	0	0	0
M + I	1 280 000 ($120x10^4$-$139x10^4$)	1 200 000 ($117x10^4$-$125x10^4$)	1 210 000 ($111x10^4$-$127x10^4$)	1 230 000 ($118x10^4$-$130x10^4$)	1 200 000 ($110x10^4$-$126x10^4$)
0 at 90°C (after 3min come-up time)	0 (0-0)	390 000 ($37x10^4$-$42x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
0.5	0 (0-0)	350 000 ($33x10^4$-$37x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
1	0 (0-0)	330 000 ($31x10^4$-$35x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
2	0 (0-0)	280 000 ($25x10^4$-$30x10^4$)	0 (0-0)	0 (0-0)	0 (0-0)
5	0 (0-0)	75 000 ($71x10^3$-$80x10^3$)	0 (0-0)	0 (0-0)	0 (0-0)
10	0 (0-0)	17 600 ($171x10^2$-$180x10^2$)	0 (0-0)	0 (0-0)	0 (0-0)

M = milk before inoculation
I = inoculum

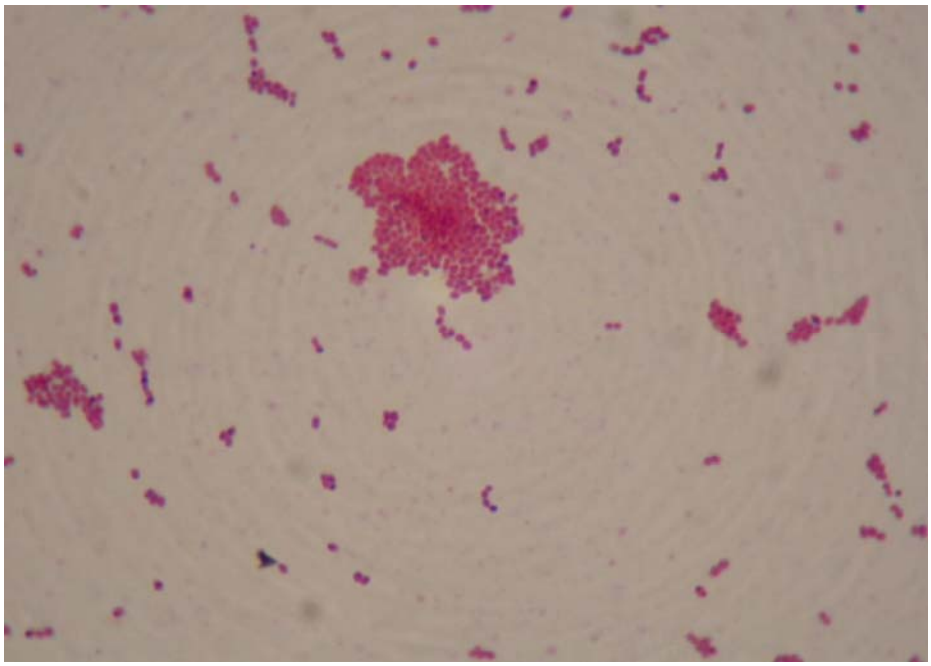


Figure 11. Micrograph showing the aggregation of *Staph. aureus* cells into clumps

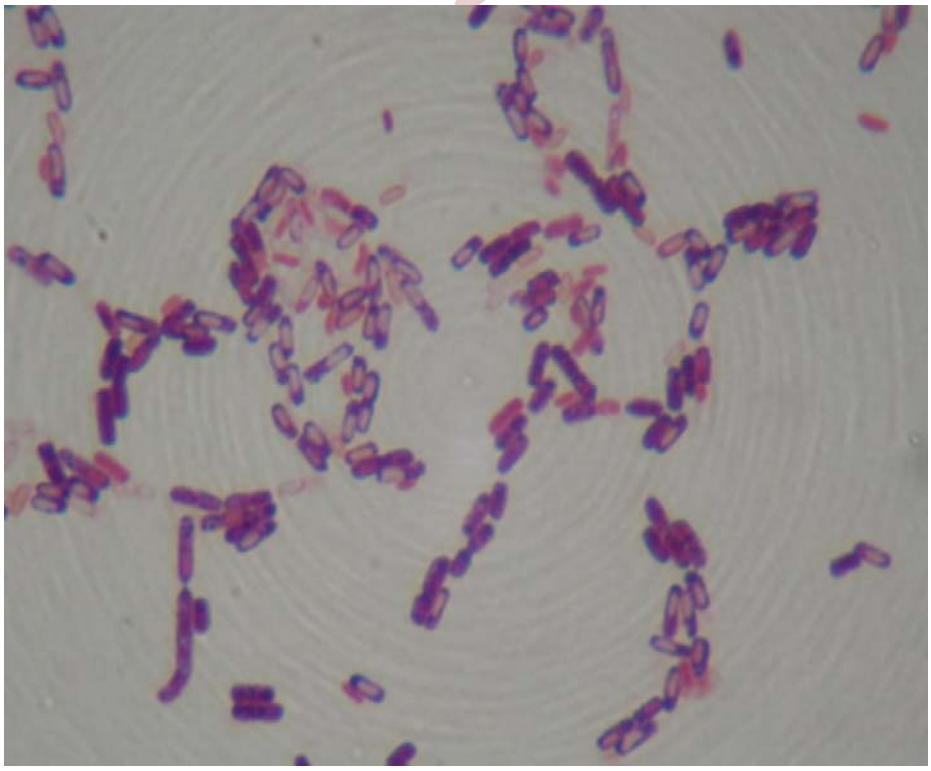


Figure 12. Micrograph showing the endospores of *B. cereus* cells

and after germination will multiply even in refrigerated milk stored at 6°C, it is difficult to determine if these types of endospore formers are a recontaminating source of the milk along the process line. Considering that they are present in the milk during the whole production process and that they are highly heat resistant, there could be other endospore contamination sources, for example, dead ends, pockets and traps where these bacteria can form biofilms in the system (Eneroth *et al.,* 1999; Eneroth *et al.*, 2001). Most dairies have effective "cleaning-in-place" systems but they might occasionally fail to disinfect hidden reservoirs. So, it is important for each dairy plant to regularly monitor for the presence of the thermo-resistant endospores and set shorter number of cycles or time periods between cleaning liquid changes, as endospores can survive in both alkali and acid solutions which, over time, would lead to an increase in the numbers and subsequent spoilage of the milk.

"Pot" Pasteurisation application

Milk pasteurisation equipment is an essential necessity for any farmer or small food producer doing business in the dairy field. According to the South African regulations relating to milk and dairy products that were drafted by the Minister of Health in terms of section 15(1) of the Foodstuffs, Cosmetics and Disinfectant Act, 1972 (Act No. 54 of 1972), raw milk with a microbial plate count of more than 200 000 cfu.ml^{-1} may not be sold and no person is allowed to sell pasteurised milk with a standard plate count exceeding 50 000 cfu.ml^{-1} (Anon., 2002). Unfortunately, some of the new African entrepreneurs, small scale farmers and food producers cannot afford to purchase the larger pasteurisers available on the market to pasteurise their milk and other dairy products. However, a local company (Central Melk Melkmasjiendienste, Middelburg, S.A.) manufactures smaller and much cheaper pasteurisers known as the "pot pasteuriser" and distributes its products all over Southern Africa. This company advertises the "pot" pasteuriser as an excellent efficient small pasteuriser which is "good value for money".

In a previous study by Cronjé (2003) where the "pot" pasteuriser was used to pasteurise milk for use in the mass production of Kepi grains using pure cultures, it was reported that many spoilage organisms survived this pasteurising method. Several strains, which were also evaluated in this study, were isolated and identified from milk that had been pasteurised using the "pot" pasteurisation method at the conditions prescribed by the manufacturer. The report by Cronjé (2003), that

organisms can survive this method of pasteurisation, made it essential to re-evaluate the pasteurisation status of this method. In the previous sections of this study it was found that most of these organisms do not survive the LTLT or HTST pasteurisation. The exception was the *Bacillus cereus* strain. It is thus also essential that the same organisms be evaluated using the "pot" pasteurisation method to make sure if it is the method of pasteurisation or the organism that has the ability to survive these pasteurisation temperatures.

The data obtained in this section of this study where the "pot" pasteuriser was adjusted to 80°C for 30 min and an inoculum concentration of about 10^6 cfu.ml^{-1} used, is shown in Fig. 13 and Table 9. The thermal survival/death curves are individually shown in Fig. A10 of the Appendix.

With the "pot" pasteurisation method, only the bacteria isolated by Cronjé (2003) from the pasteurised milk and the *E. coli* (58) reference strain were evaluated. They included, the *B. cereus* (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), *A. baumannii* (C3), *Can. lipolytica* (G1) and *E. coli* (58) strains. From the data it was found that only the *Can. lipolytica* strain that did not survive the heat treatment when using this pasteurisation method. This yeast strain survived for only 10 min, indicating its sensitivity to heat. All the other bacteria survived the "pot" pasteurisation method at 80°C for 30 min. In this pasteurisation method the bacteria were so resistant that even though the "come-up" time of 35 min was very long, they still could withstand the high temperature for longer times.

The results obtained in this study showed that the *B. cereus* (S4) and *A. baumannii* (C3) strains decreased by 4 log reductions from the initial inoculum levels of 10^6 cfu.ml^{-1}. The *E. coli* (58), *Chr. meningosepticum* (S5) and the *P. putida* (S6) strain numbers decreased by 5 log reduction during this pasteurisation method.

When the results obtained from the "pot" pasteurisation method are compared with those obtained from the LTLT and HTST pasteurisation methods as discussed in the previous experiments, it is clear that these results differ. The difference is probably caused by the different methods used. In the "pot" method, it is possible that the survival of these bacterial contaminants may be caused by the heat applied that was from one direction. The lid of the "pot" pasteuriser is not transferring the same temperature as it is transferred from the bottom of the "pot", so that is why

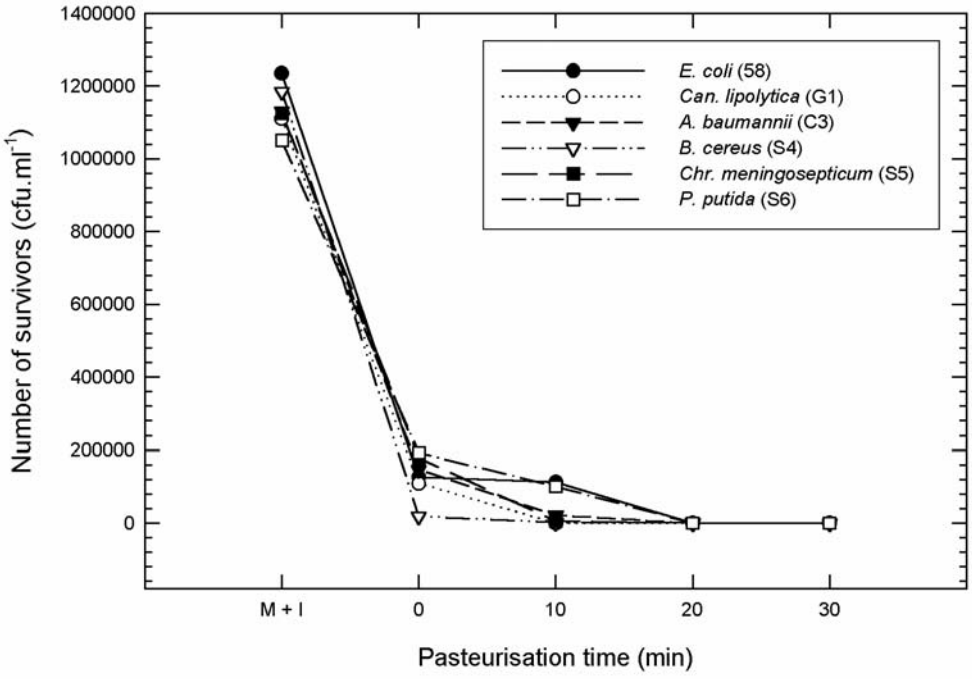


Figure 13. Impact of pasteurisation time in a "pot" pasteuriser at 80°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1 X 10^6 level (average triplicates).

Table 9. Impact of "pot" pasteurisation at 80 °C for 30 min on bacterial strains at 1 X 10^6 cfu.ml^{-1} levels. The values in brackets are the minimum and maximum values of triplicates and the values "not in brackets" are the averages.

Time (min)	***E. coli* (58)**	***Can. lipolytica* (G1)**	***A. baumannii* (C3)**	***B. cereus* (S4)**	***Chr. meningosepticum* (S5)**	***P. putida* (S6)**
M	0	0	0	0	0	0
M + I	1 235 000 ($121x10^4$-$126x10^4$)	1 110 000 ($101x10^4$-$121x10^4$)	1 130 000 ($107x10^4$-$119x10^4$)	1 185 000 ($114x10^4$-$123x10^4$)	1 125 000 ($109x10^4$-$116x10^4$)	1 050 000 ($94x10^4$-$117x10^4$)
0 at 80°C (after 35min come-up time)	127 000 ($124x10^3$-$129x10^3$)	109 750 ($99x10^3$-$121x10^3$)	147 500 ($139x10^3$-$156x10^3$)	19 000 ($16x10^3$-$22x10^3$)	178 500 ($155x10^3$-$202x10^3$)	192 500 ($18.5x10^4$-$20x10^4$)
10	112 000 ($109x10^3$-$115x10^3$)	0 (0-0)	22 500 ($22x10^3$-$23x10^3$)	2 000 ($2.3x10^2$-$2.5x10^2$)	6 300 ($62x10^2$-$64x10^2$)	100 500 ($87x10^3$-$114x10^3$)
20	10 (10-11)	0 (0-0)	63 (60-65)	735 (550-920)	35 (29-41)	13 (12-13)
30	2 (1-2)	0 (0-0)	46 (42-50)	51 (46-56)	4 (3-4)	9 (8-10)

M = milk before inoculation
I = inoculum

the "come-up" time was very long, unlike in the case of LTLT and HTST vials where the same temperature was transferred from all directions.

These results also show the difference in log reduction of strain *B. cereus* (S4) when using the different pasteurisation methods. In the HTST pasteurisation treatment at the 10^6 cfu.ml^{-1} inoculum level, the cells decreased by 1 log reduction while in the "pot" method the cell reduction was 4 logs. This implies that the longer this strain can stay at a higher temperature the more sensitive it becomes.

From this study it is noticeable that the bacteria react differently to different pasteurisation set-ups and to different temperature and time combinations. This "pot" method was used to pasteurise the milk where these isolates were isolated, suggesting that they survived pasteurisation because of the method used and not just because they are resistant to pasteurisation.

Conclusions

The results of this study clearly illustrated that different pasteurisation methods (LTLT, HTST and "pot" pasteurisation) play an important role in the survival/destruction of different bacterial contaminants. Bacterial strains of *E. coli* (58), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5), *P. putida* (S6), a yeast *Can. lipolytica* (G1) and a reference strain *B. coagulans* (60) were pasteurised at different pasteurisation methods. It was found that only the *B. cereus* (S4) strain survived pasteurisation in the LTLT and the HTST pasteurisation treatments while the other bacterial and yeast strains did not survive the pasteurisation. In contrast, the same bacterial strains when treated with the "pot" pasteuriser survived pasteurisation with the exception of the yeast isolate. From these results it is clear that the "pot" pasteuriser does not pasteurise effectively.

In this study it was also found that as the initial concentration of the cfu.ml^{-1} level increases for both the LTLT and HTST pasteurisation treatments, the longer it takes for the bacterial strains to be destroyed. Therefore, the producers of dairy products should be careful of the milk contamination before pasteurisation.

If the bacterial contaminants in this study were isolated from pasteurised milk and in this study it was proved that these contaminants do not survive pasteurisation with the exception of *B. cereus* (S4) strain, this means that their occurrence in the pasteurised milk was because of post-pasteurisation contamination in the dairy plant.

References

Anonymous. (2002). *Foodstuffs, Cosmetics and Disinfectant Act and Regulations*. Act no. 54 of 1972, G.N.R. 1555/1997. Johannesburg, South Africa: Lex Patria Publishers.

Bunning, V.K., Crawford, R.G., Tierney, J.T. & Peeler, J.T. (1992). Thermotolerance of heat shocked *Listeria monocytogenes* in milk exposed to High Temperature Short Time pasteurisation. *Applied and Environmental Microbiology*, **58**, 2096-2098.

Charley, H. & Weaver, C. (1998). *Foods: A Scientific Approach*, 3rd ed. Pp. 308-335. New Jersey: Prentice-Hall Inc.

Corash, P. (1951). Milk and milk products. In: *The Chemistry and Technology of Food and Food Products*. (edited by M. B. Jacobs). Volume 3, Pp.2219-2226. New York: Interscience Publishers.

Cronjé, M.C. (2003). Production of Kepi grains using pure cultures as starters. *M.Sc. Thesis* , University of Stellenbosch, Stellenbosch, South Africa.

Doyle, M.P., Glass, K.A., Beery, J.T., Garcia, G.A., Pollard, D.J. & Schultz, R.D. (1987). Survival of *Listeria monocytogenes* in milk during high temperature short time pasteurisation. *Applied and Environmental Microbiology*, **53**, 1433-1438.

Dubos, R. (1998). Pasteur and Modern Science (edited by T.D. Brock). Pp. 3-27. Washington D.C.: ASM Press.

Eneroth, A., Christiansson, A., Brendehaug, J. & Molin, G. (1999). Critical contamination sites in the production line of pasteurised milk, with reference to the psychrotrophic spoilage flora. *International Dairy Journal*, **8**, 829-834.

Eneroth, A., Svensson, B., Molin, G. & Christiansson, A. (2001). Contamination of pasteurised milk by *Bacillus cereus* in the filling machine. *Journal of Dairy Research*, **66**, 189-196.

Fox, B.A. & Cameron, A.G. (1982). Food spoilage, preservation and hygiene. In: *Food Science - A Chemical Approach*, 4th ed. Pp. 315-316. London: Hodder & Stoughton.

Grant, I.R., Ball, H.J., Neill, S.D. & Rowe, M.T. (1996a). Inactivation of *Mycobacterium paratuberculosis* in cow's milk at pasteurisation temperatures. *Applied and Environmental Microbiology*, **62**, 631-636.

Grant, I.R., Ball, H.J. & Rowe, M.T. (1996b). Thermal inactivation of several *Mycobacterium spp*. in milk by pasteurisation. *Letters in Applied Microbiology*, **22**, 253-256.

Juneja, V.K. (2003). A comparative heat inactivation study of indigenous microflora in beef with that of *Listeria monocytogenes, Salmonella serotypes* and *Escherichia coli* 0157:H7. *Letters in Applied Microbiology*, **37**, 292-298.

Knabel, S.J., Walker, H.W., Hartman, P.A. & Mendonca, A.F. (1990). Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurisation*. Applied and Environmental Microbiology*, **56**, 370-375.

Lund, B.M., Gould, G.W. & Rampling, A.M. (2002). Pasteurisation of milk and the heat resistance of *Mycobacterium avium* subsp. *paratuberculosis*: a critical review of the data*. International Journal of Food Microbiology*, **77**, 135-145.

McCormick, K., Han, I.Y., Acton, J.C., Sheldon, B.W. & Dawson, P.L. (2003). D- and Z- Values for *Listeria monocytogenes* and *Salmonella typhimurium* in packaged low-fat ready-to-eat turkey bologna subjected to a surface pasteurisation treatment. In: *Journal of Poultry Science* Pp. 1337-1342. Poultry Science Association, Inc.

Nelson, F.E. (1981). The microbiology of market milk. In: *Dairy Microbiology* (edited by R.K. Robinson) Volume 1, Pp. 188-201.

Palop, A., Raso, J., Pagan Rafael, Condon, S. & Sala, F.J. (1999). Influence of pH on heat resistance of spores of *Bacillus coagulans* in buffer and homogenised foods. *International Journal of Food Microbiology*, **46**, 243-249.

Sorhaug, T. & Stepaniak, L. (1997). Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. *Trends in Food Science and Technology*, **8**, 35-40.

Varnam, A.H. (1991). Control of pathogenic micro-organisms in milk. In: *Foodborne Pathogens: An Illustrated Text*. Pp. 447-458. London: Wolfe Publishing Ltd.

APPENDIX A

To Chapter Three

Tables A1 - A3 and Figures A1 - A10 are given in this appendix. The large amount of data generated was placed in a separate appendix to simplify the discussion section of this chapter.

Table A1. Characterisation of the yeast strain (G1 = 1342) originally isolated from pasteurised milk (Cronjé, 2003) using the Rapid ID 32C identification system

Test	Strain G1 (1342)
Galactose	-
Actidione	+
Saccharose	-
N Acetyl glucosamine	+
DL Lactate	+
Arabinose	-
Cellobiose	-
Raffinose	-
Maltose	-
Trehalose	-
2-keto-gluconate	-
Metyhyl-D-glucoside	-
Sorbitol	-
Xylose	-
Ribose	-
Glycogene	+
Rhamnose	-
Palatinose	-
Erythritol	+
Melibiose	-
Glucuronate	-
Melezitose	-
Gentibiose	-
Levulinate	-
Mannitol	+
Lactose	-
Inositol	-
Glucose	+
Sorbose	-
Gluconate	-
Esculine	-

API ID = 99% *Candida lipolytica* (Rapid ID 32C system)

Actidione = cycloheximide

Saccharose = sucrose

Table A2. Characterisation of the Gram-positive endospore forming rod-shaped "milk" bacterial isolate (S4 = 1335), using the API 50CHB identification system.

Test	Strain S4 (1335)
Glycerol	+
Erythritol	-
D-Arabinose	-
L-Arabinose	-
Ribose	+
D-xylose	-
L-xylose	-
Adonitol	-
ß Methyl-xyloside	-
Galactose	+
D-Glucose	+
D-Fructose	+
D-Mannose	-
L-Sorbose	-
Rhamnose	-
Dulcitol	-
Inositol	+
Mannitol	-
Sorbitol	-
α Methyl-D-mannoside	-
α Methyl-D-glucoside	-
N Acetyl glucosamine	+
Amygdaline	-
Arbutine	+
Esculine	+
Salicine	+
Cellobiose	+
Maltose	+
Lactose	+
Melibiose	-
Saccharose	+
Trehalose	+
Inuline	-
Melezitose	-
D-Raffinose	-
Amidon	-
Glycogene	-
Xylitol	-
ß Gentiobiose	-
D-Turanose	+
D-Lyxose	-
D-Tagatose	-
D-Fructose	-
L-Fructose	-
D-Arabitol	-
L-Arabitol	-
Gluconate	+
2 ceto-gluconate	-
5 ceto-gluconate	-

API ID = 87.9% *Bacillus cereus* (API 50CHB system).

Table A3. Characterisation of the Gram-negative rod-shaped ((S5 = 1336) and (S6 = 1337)), and the cocco/bacilli-shaped (C3 = 1334) "milk" bacterial isolates using the API 20 NE identification systems.

Test	Strain S5 (1336)	Strain S6 (1337)	Strain C3 (1334)
NO_3	-	-	-
Tryptophane	-	-	-
Glucose	-	-	-
Arginine	-	+	-
Urea	+	-	-
Esculine	+	-	-
Gelatine	+	-	-
PNPG#	+	-	-
Glucose	+	+	+
Arabinose	-	-	+
Mannose	+	-	-
Mannitol	+	-	-
N-Acetyl-glucosamine	+	-	-
Maltose	+	-	-
ß-gentiobiose	-	+	-
Caprate	-	+	+
Adipate	-	-	+
Malate	-	+	+
Citrate	+	+	+
Phenyl-acetate	-	+	-

S5 API ID = 99.9% *Chryseobacterium meningosepticum* (API 20NE system)

S6 API ID = 99.6% *Pseudomonas putida* (API 20NE system)

C3 API ID = 99.9% *Acinetobacter baumanii* (API 20NE system)

= p-nitro-phenyl-ßD-galactopyranoside

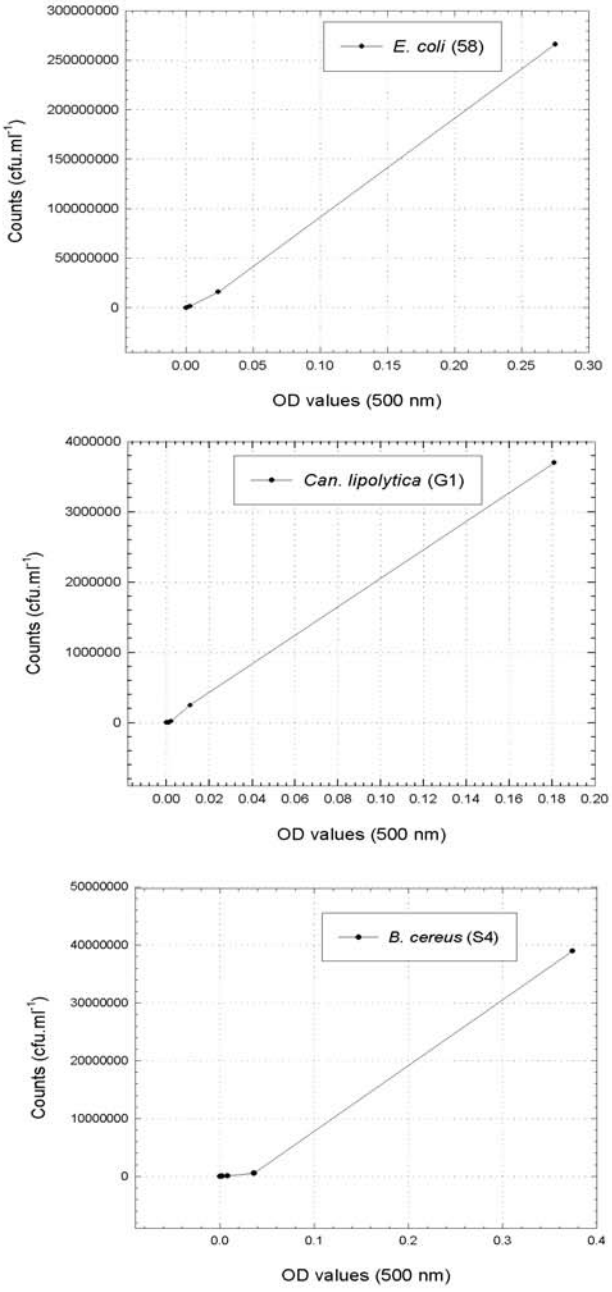
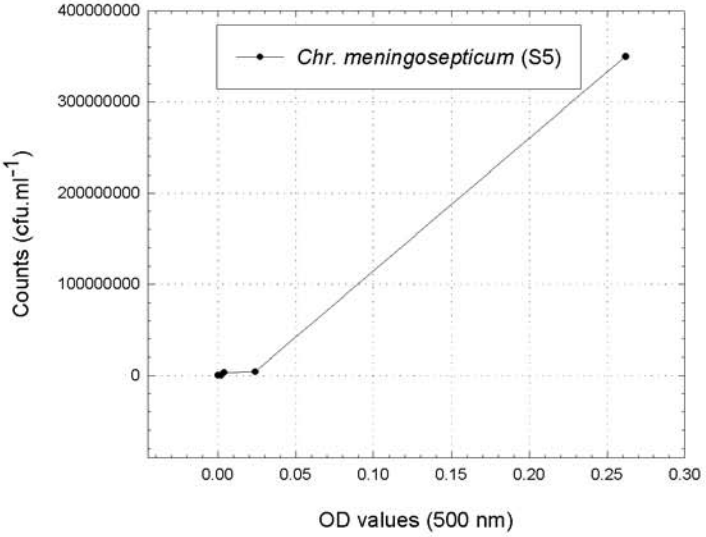
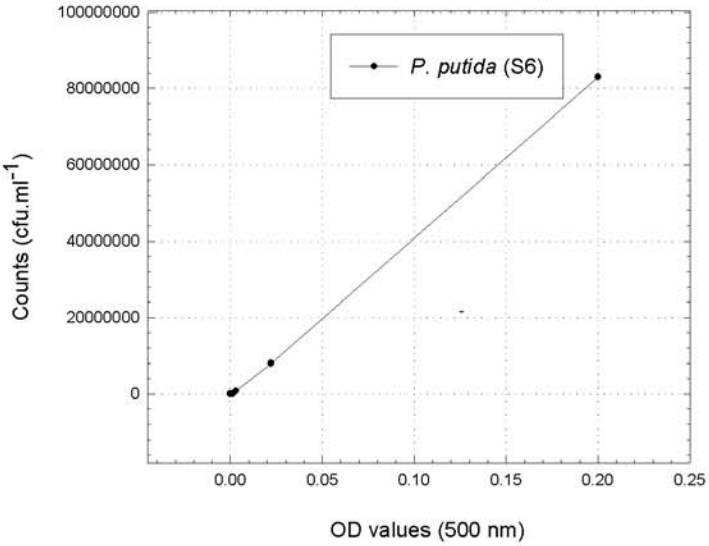


Figure A1. Standard curves of the microorganisms used in the study. Points are average of five repeats.





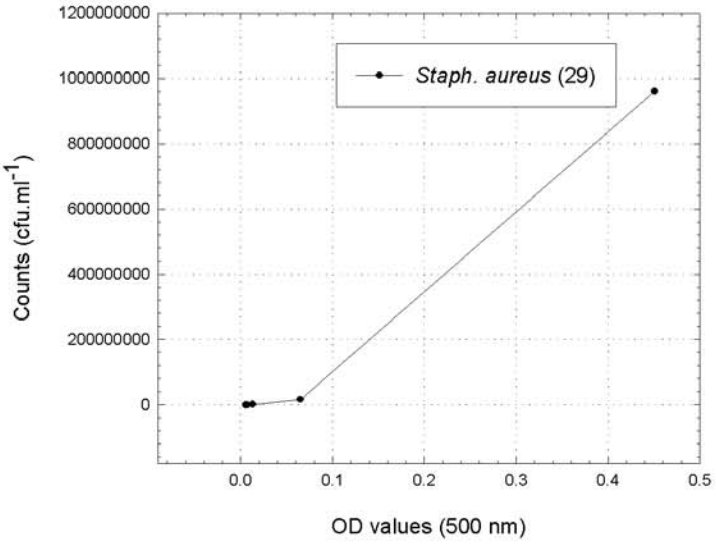


Figure A1. Cont.

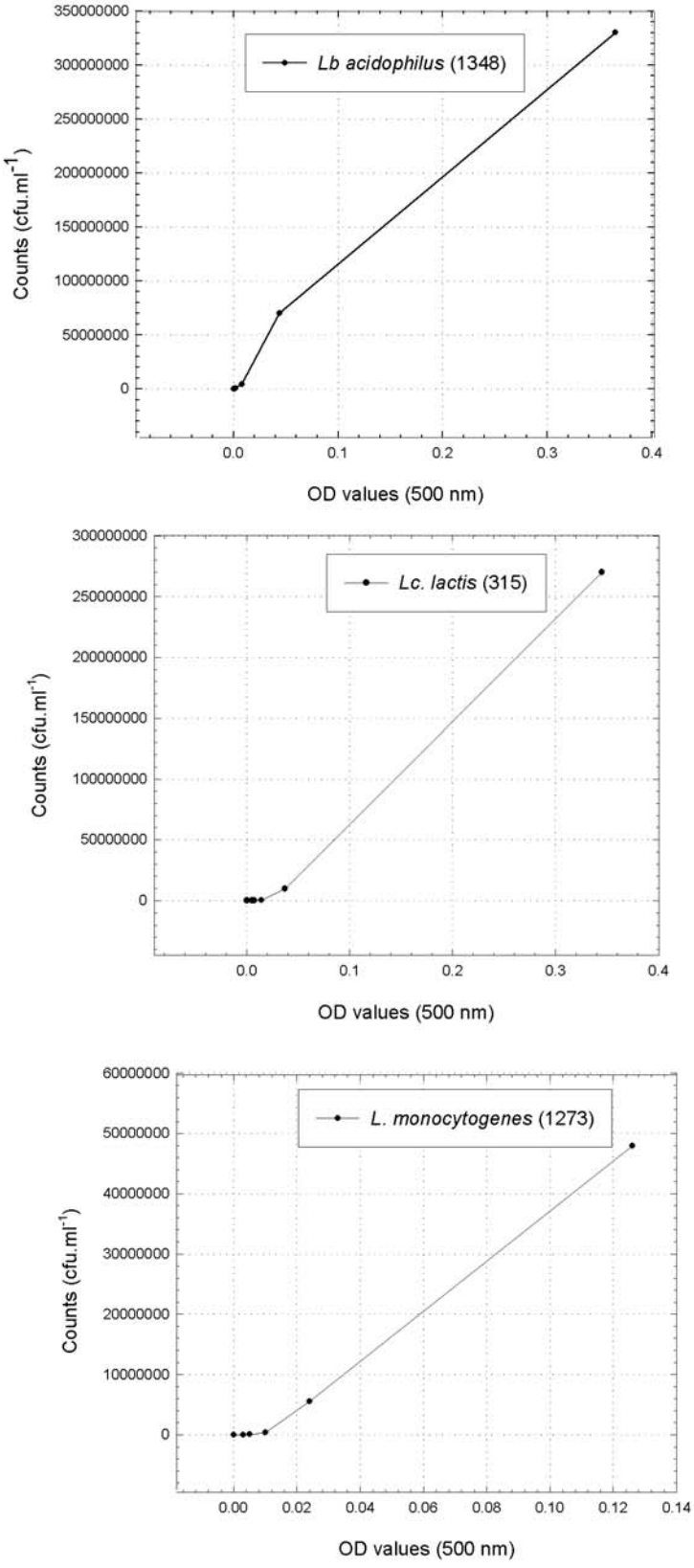
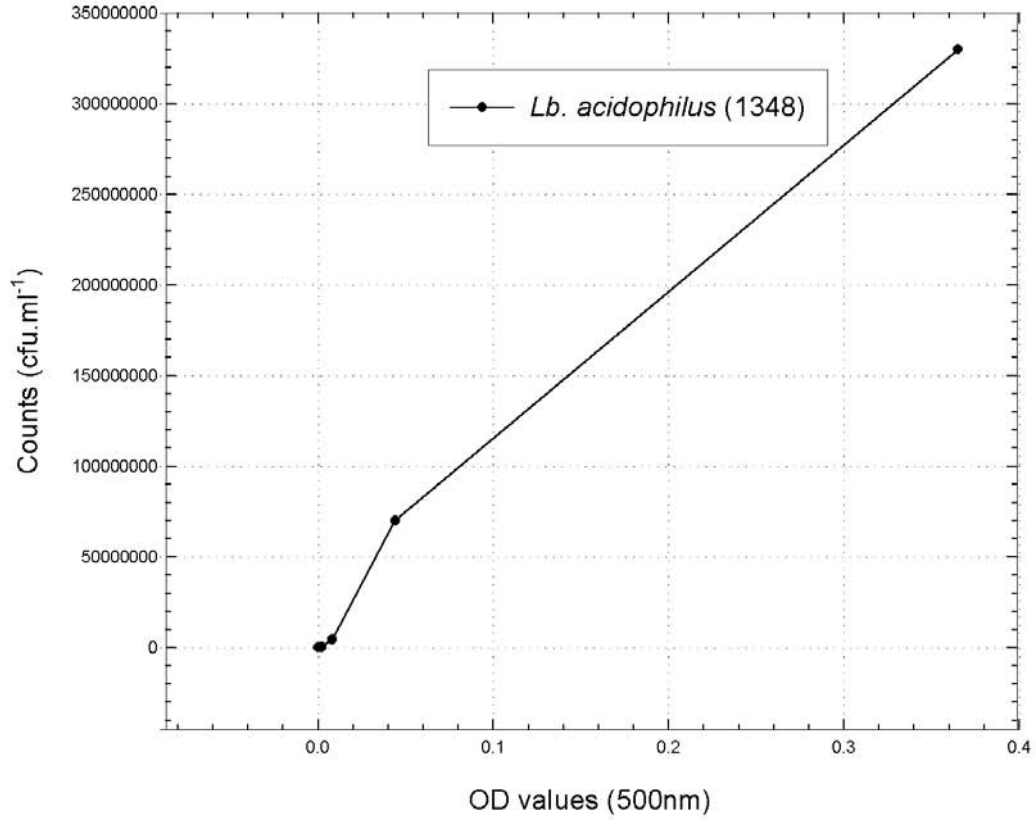


Figure A1. Cont.



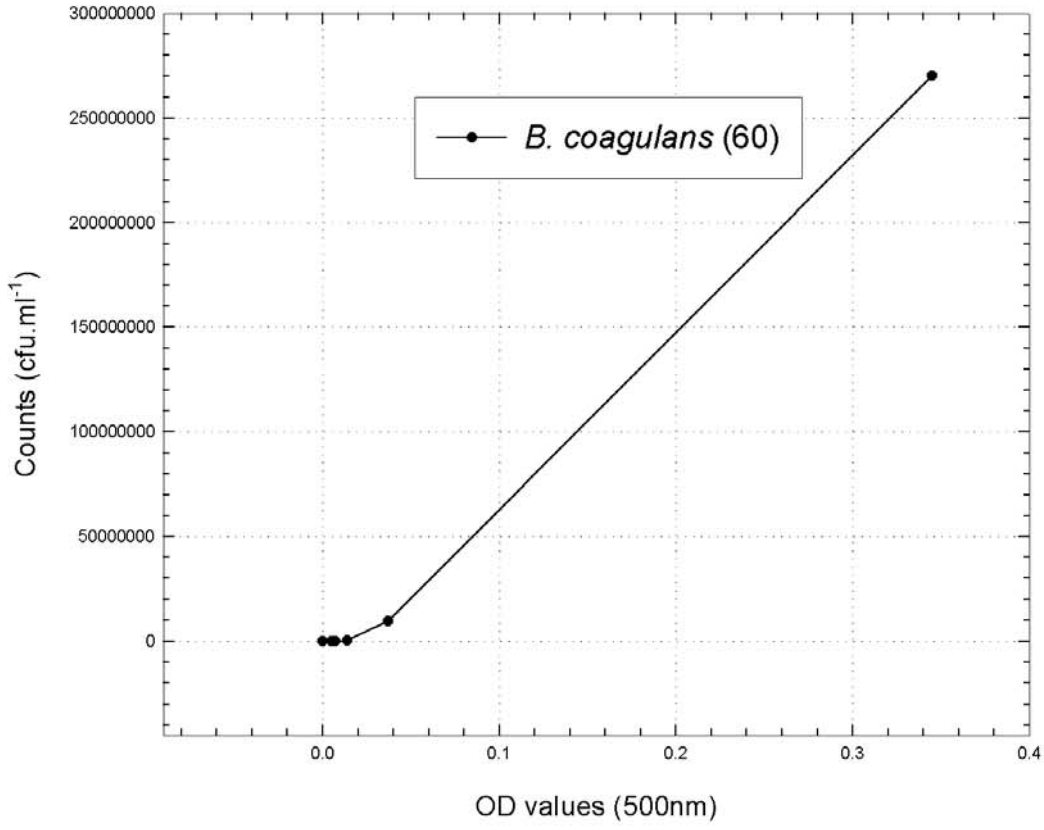


Figure A1. Cont.

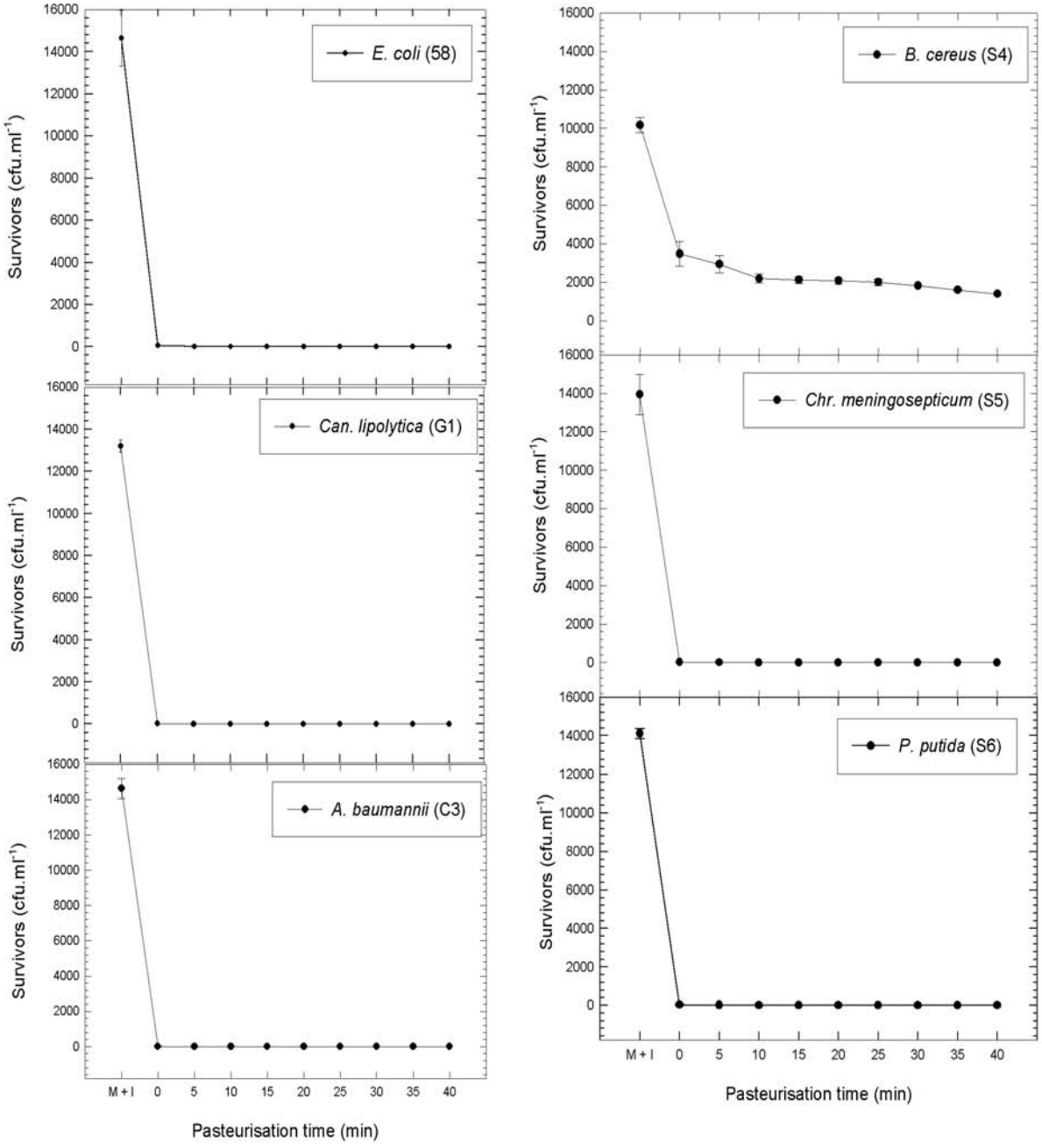


Figure A2. Impact of pasteurisation time at 63°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1.0 X 10^4 (quadruplicate values are shown by standard error bars).

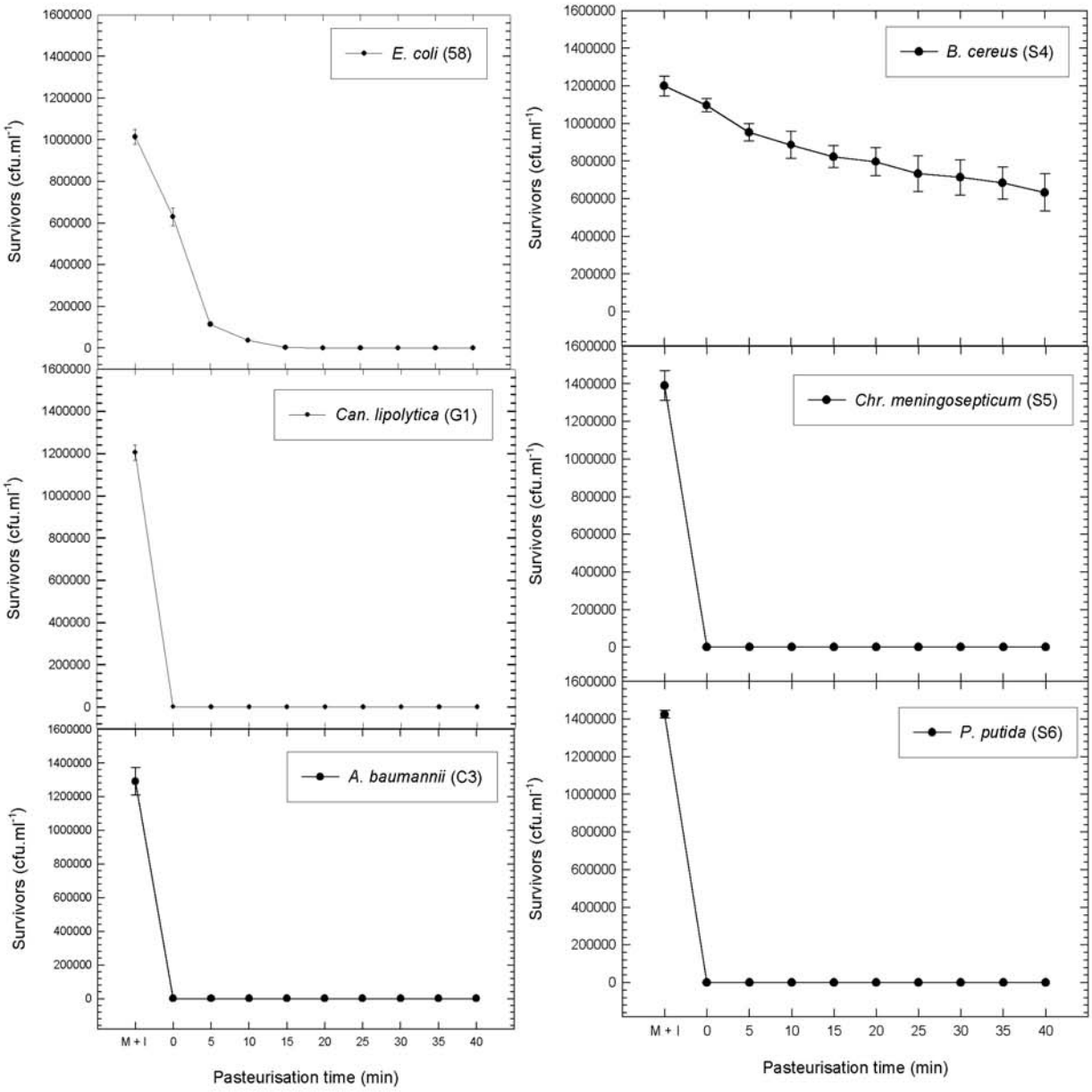


Figure A3. Impact of pasteurisation time at 63°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1.0 X 10^6 (quadruplicate values are shown by standard error bars).

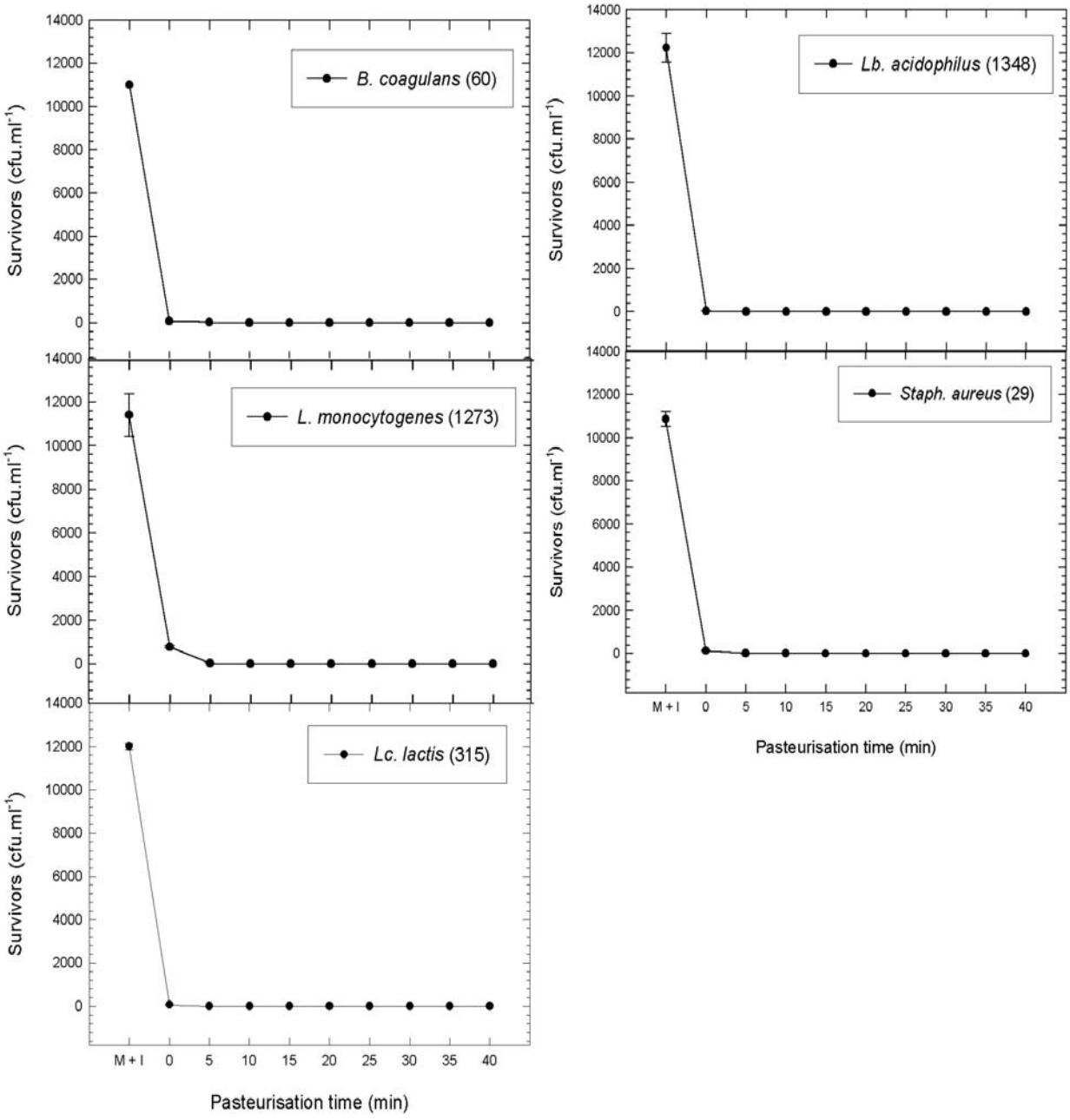


Figure A4. Impact of pasteurisation time at 63°C on the survival of *B. coagulans* (60), *L. monocytogenes* (1273), *Lc. lactis* (315), *Lb. acidophilus* (1348) and *Staph. aureus* (29) with an average starting inoculum of 1.0 X 10^4 (triplicate values are shown by standard error bars).

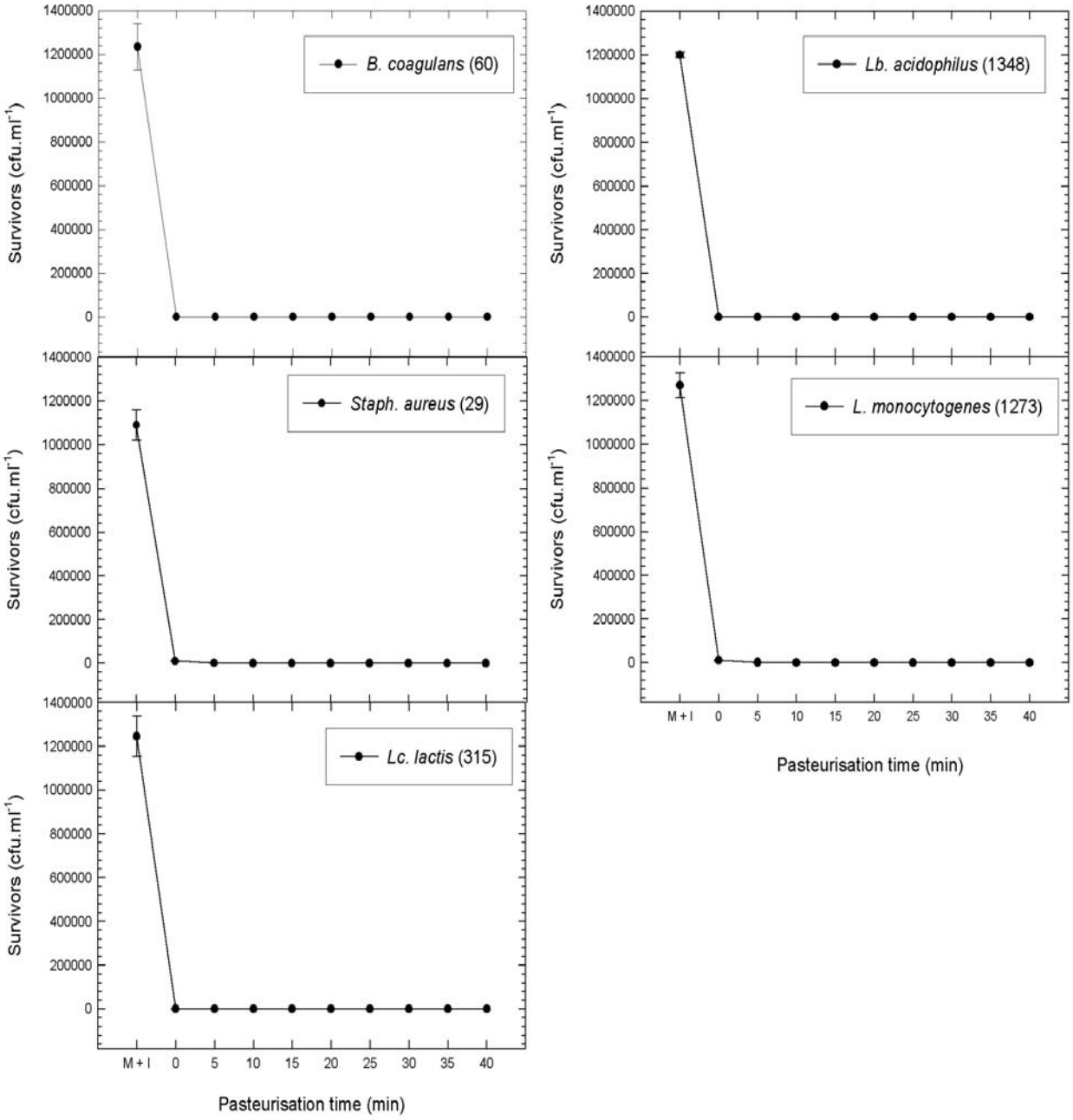


Figure A5. Impact of pasteurisation time at 63°C on the survival of *B. coagulans* (60), *L. monocytogenes* (1273), *Lc. lactis* (315), *Lb. acidophilus* (1348) and *Staph. aureus* (29) with an average starting inoculum of 1.0 X 10^6 (triplicate values are shown by standard error bars).

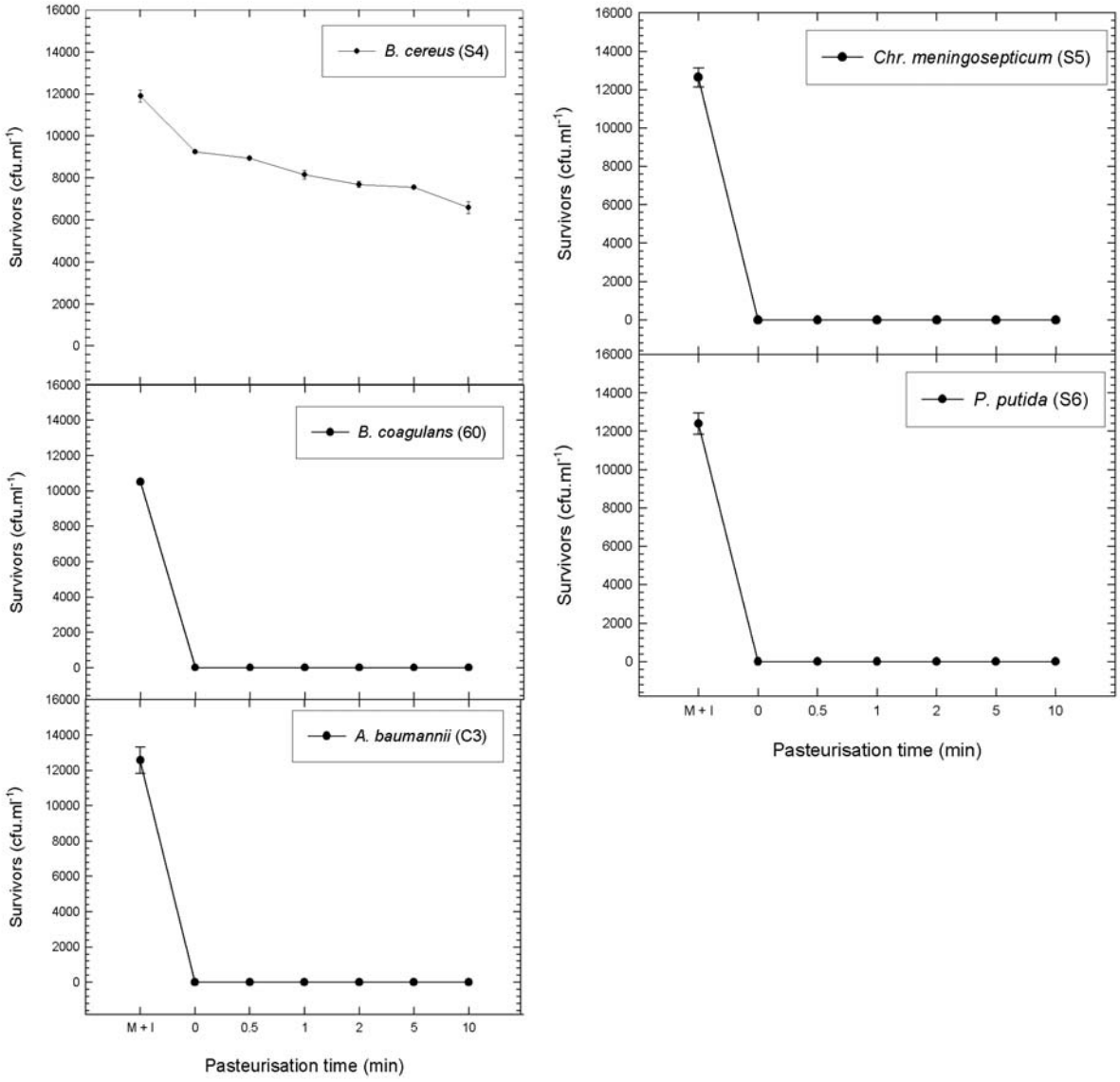


Figure A6. Impact of pasteurisation time at 72°C on the survival of *B. cereus* (S4) and sensitivity of *B. coagulans* (60), *A. baumannii* (C3), *Chr. meningosepticum* (S5), *P. putida* (S6) with an average starting inoculum of 1.0 X 10^4 (triplicate values are shown by standard error bars).

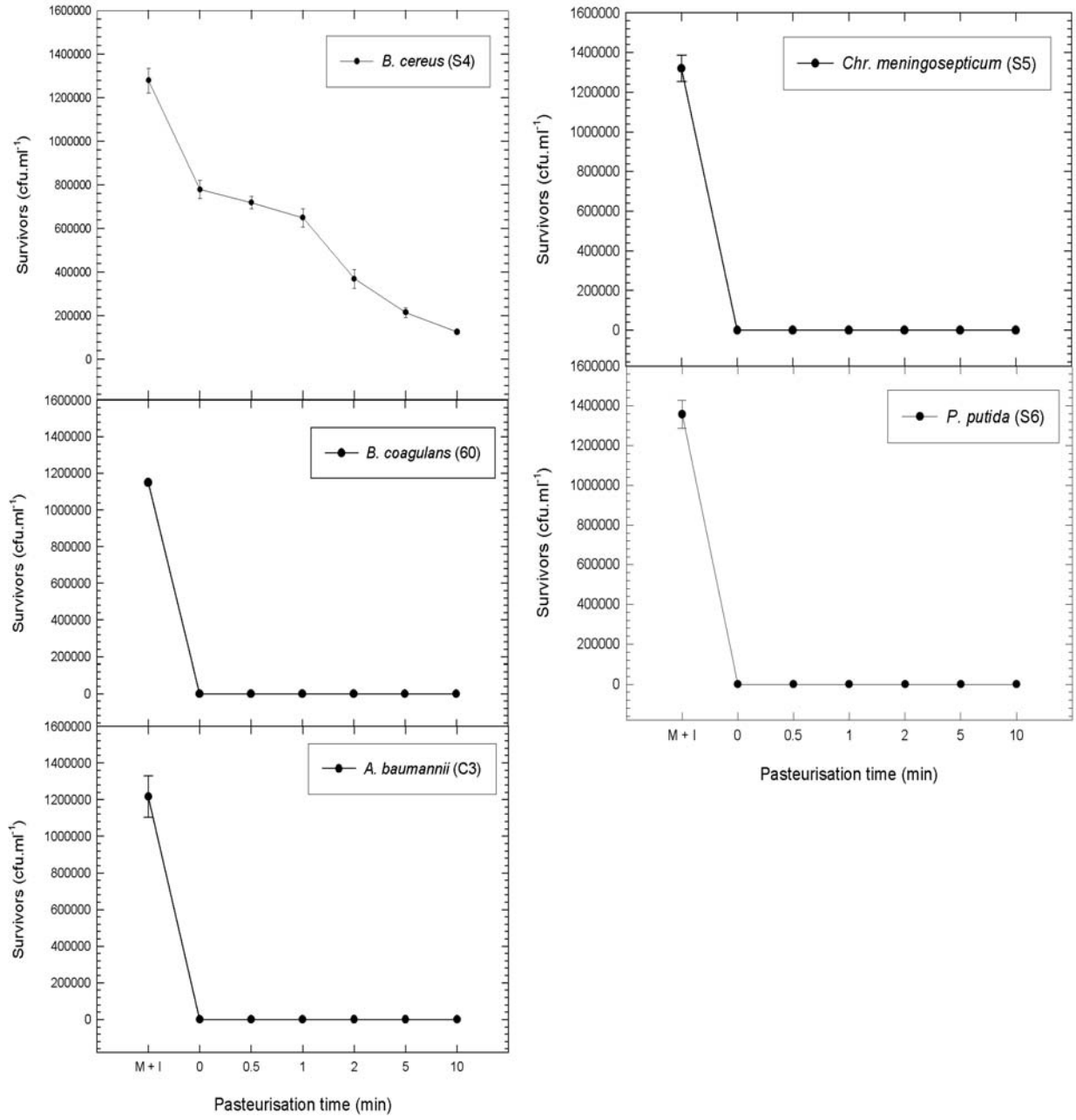


Figure A7. Impact of pasteurisation time at 72°C on the survival of *B. cereus* (S4) and sensitivity of *B. coagulans* (60), *A. baumannii* (C3), *Chr. meningosepticum* (S5), *P. putida* (S6) with an average starting inoculum of 1.0 X 10^6 (triplicate values are shown by standard error bars).

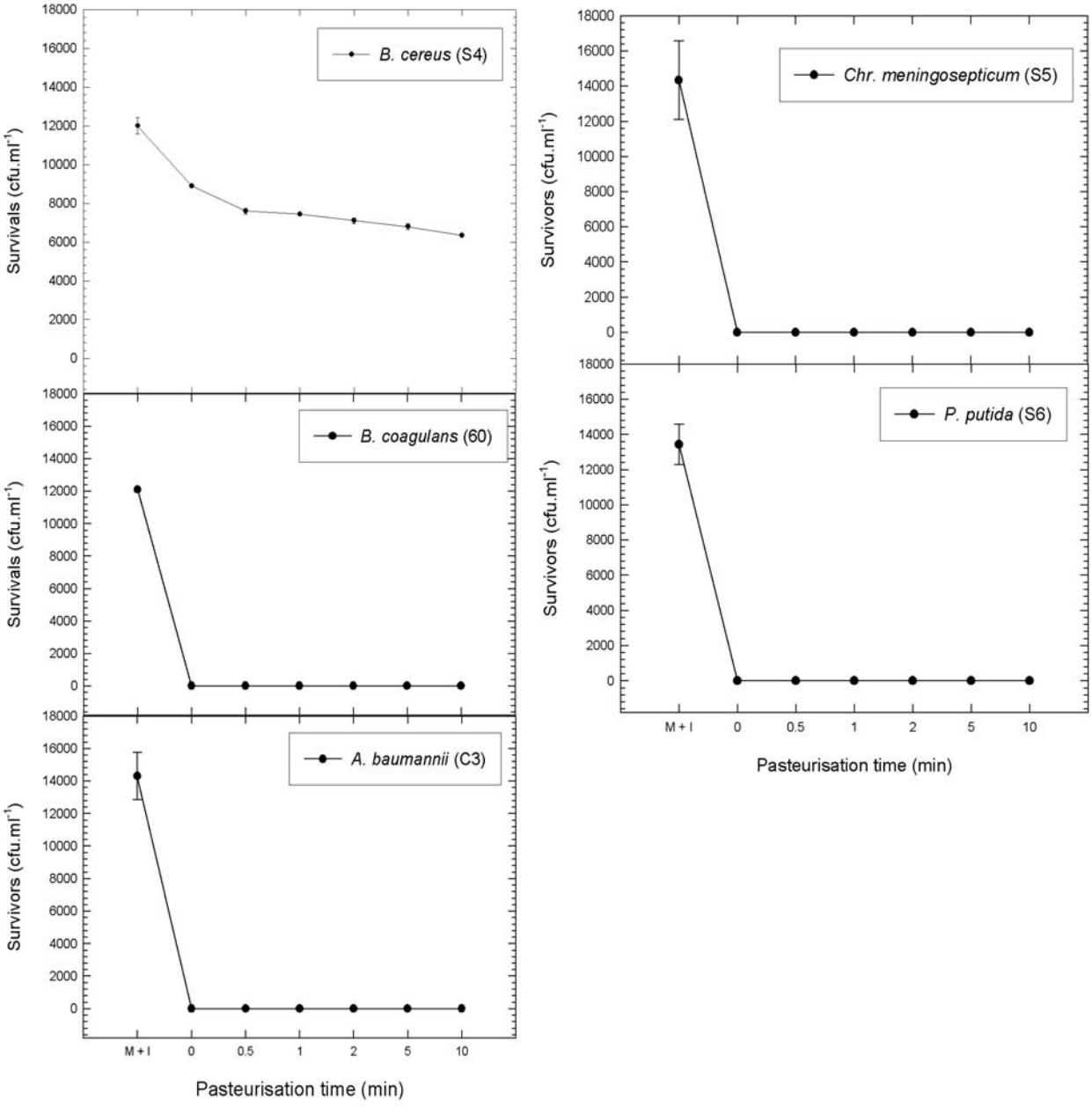


Figure A8. Impact of pasteurisation time at 90°C on the survival of *B. cereus* (S4) and sensitivity of *B. coagulans* (60), *A. baumannii* (C3), *Chr. meningosepticum* (S5), *P. putida* (S6) with an average starting inoculum of 1.0 X 10^4 (triplicate values are shown by standard error bars).

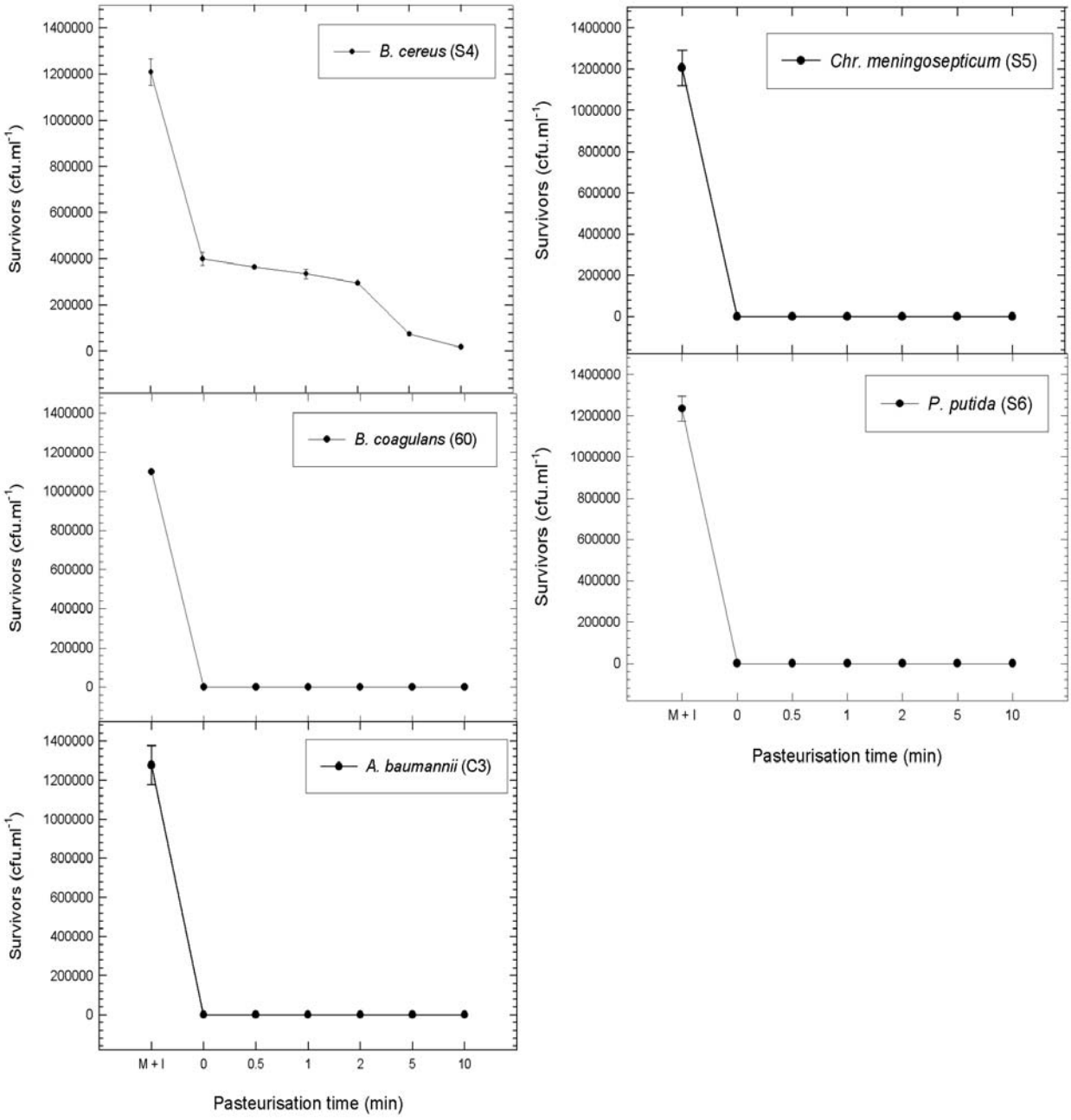


Figure A9. Impact of pasteurisation time at 90°C on the survival of *B. cereus* (S4) and sensitivity of *B. coagulans* (60), *A. baumannii* (C3), *Chr. meningosepticum* (S5), *P. putida* (S6) with an average starting inoculum of 1.0 X 10^6 (triplicate values are shown by standard error bars).

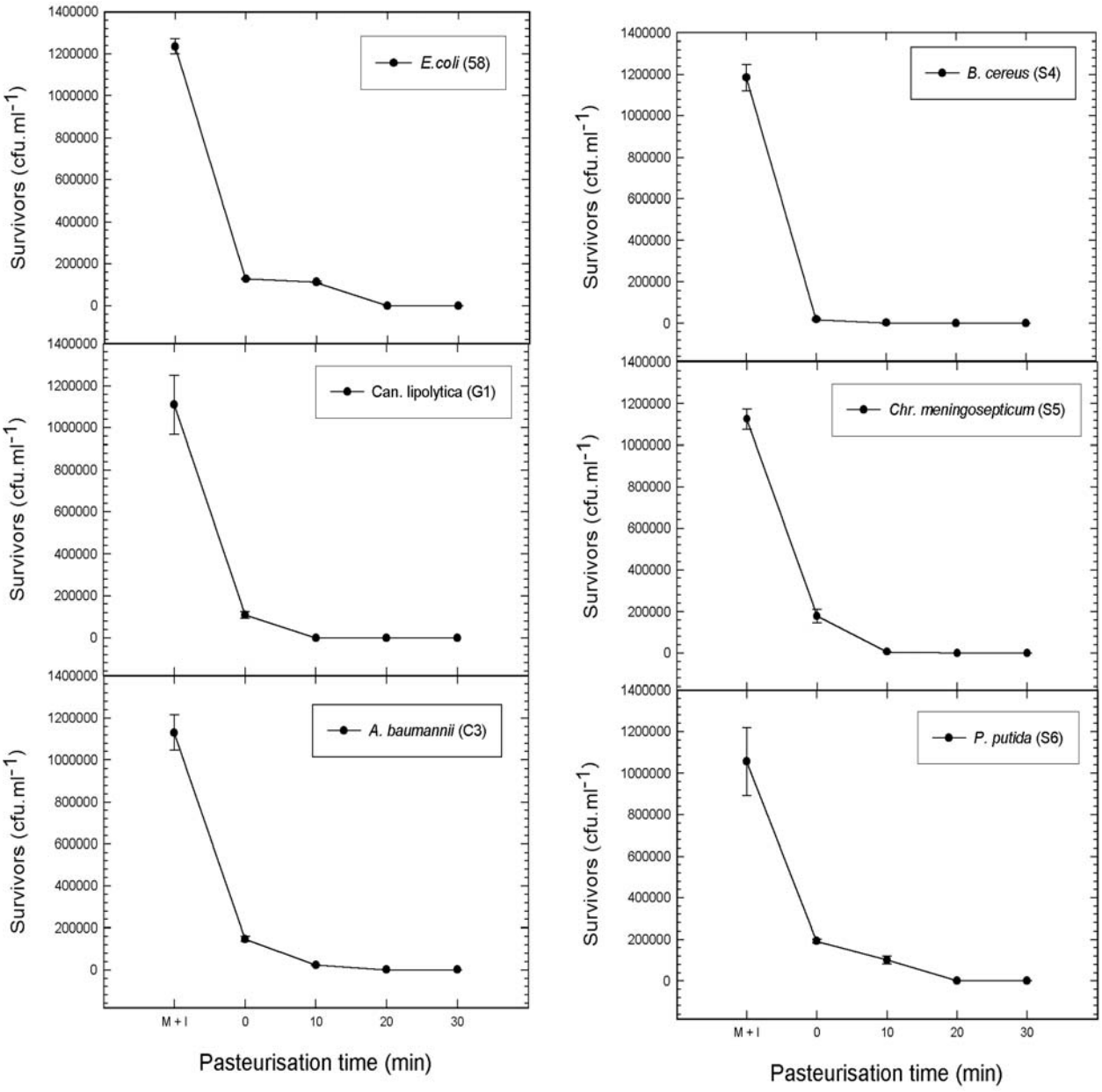


Figure A10. Impact of pasteurisation time at 80°C on the survival of *E. coli* (58), *Can. lipolytica* (G1), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) with an average starting inoculum of 1 X 10^6 (triplicate values are shown by standard error bars)

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

The potential danger of milk as a carrier of human pathogens has been established. Therefore, heat treatment of milk by the process of pasteurisation is essential to eliminate possible pathogenic and spoilage bacteria that might contaminate milk during milking or during the processing phases. In South Africa, milk pasteurisation was introduced as a public health measure in order to destroy the most heat resistant, non-endospore forming human pathogens (*Mycobacterium paratuberculosis* and *Coxiella burnetti*) likely to be present in raw milk (Anon., 2002). The regulations relating to milk and dairy products were drafted by the Minister of Health in terms of section 15 (1) of the Foodstuffs, Cosmetics and Disinfectant Act, 1972 (Act No. 54 of 1972) (Anon., 2002). According to these regulations, raw milk with a plate count of more than 200 000 cfu.ml^{-1} may not be consumed by humans, and no person is allowed to sell pasteurised milk with a standard plate count exceeding 50 000 cfu.ml^{-1} (Anon., 2002).

In the dairy industry there are two basic methods of pasteurisation currently in use, the LTLT and the HTST methods, where the heat treatment is sufficient for public safety and adequate keeping quality of the product is ensured (Stauffer, 1993; Grant *et al*., 1996). In addition to these two methods, there is another method locally known as the "pot" pasteurisation method for which the equipment is much cheaper. This equipment was introduced specifically to accommodate the new South African entrepreneurs, small-scale farmers and food producers who cannot afford to purchase the larger pasteurisers available on the market. According to the instructions of the "pot" pasteuriser it was designed to eliminate the pathogenic and spoilage bacteria from milk to produce a good quality final product.

In a study by Cronjé (2003) on the production of Kepi grains using pure cultures as starters, she identified the presence of microbes in pasteurised milk and even in milk "double" pasteurised that had been treated with the "pot" pasteuriser. From the results of Cronjé (2003), it was strongly recommended that further research be done to evaluate the effectiveness of LTLT, HTST and "pot" pasteurisation, to assimilate data used to determine the efficiency of pasteurisation parameters and to highlight milk post-pasteurisation contamination sources generally found in South

Africa. The question that arose was whether the survival of bacterial contaminants in milk was due to their ability to survive the pasteurisation process, the process temperature/time variations or specifically the pasteurisation method used. Thus the main objective of this study was to determine the impact of different pasteurisation temperature/times on the survival of selected microbes. The accuracy of the "pot pasteurisation" method and how it differs from other pasteurisation methods was also determined using the same selected microbes.

The microbial strains *Escherichia coli* (58), *Candida lipolytica* (G1), *Acinetobacter baumannii* (C3), *Bacillus cereus* (S4), *Chryseobacterium meningosepticum* (S5) and *Pseudomonas putida* (S6) were treated using different pasteurisation methods and each experiment was repeated three or four times to ensure the accuracy of the results. The data of the thermal death/survival curves after the application of the LTLT pasteurisation method showed that only the *B. cereus* (S4) strain survived pasteurisation at 63°C for 30 min. For this strain a log reduction of less than one was found with the low inoculum concentration (10^4 cfu.ml^{-1}) and the high inoculum level of 10^6 cfu.ml^{-1}. As was expected it was noticed that the higher the concentration of the inoculum, the longer it took for the cells to die.

The thermal survival/death curves obtained using the HTST pasteurisation treatments (72°C and 90°C for 10 min) for both inoculum levels of 10^4 and 10^6 cfu.ml^{-1} showed again that only the *B. cereus* (S4) strain survived this pasteurisation method. From these results, it was concluded that under the conditions used in this study only the *B. cereus* (S4) strain is resistant to pasteurisation with both LTLT and HTST methods. From these studies, it was also concluded that only the endospores of *B. cereus* are capable of surviving the pasteurisation treatments. Since the endospores survive the heat treatment, they can germinate after the heat process and, multiply even when the milk is stored at temperatures of <6°C. Therefore, it is difficult to determine if these types of endospore formers are from a recontamination source in the process line or if they were already present in the raw milk (Eneroth *et al*., 2001).

Based on the data obtained with the LTLT and HTST methods using the selected microbial strains that were originally isolated by Cronjé (2003), the conclusion was reached that, with the exception of the *B. cereus* (S4) strain, these pasteurisation methods are efficient enough to kill these microbes within the

specified temperature/times. When these LTLT and HTST data, and the results of the studies of bacterial survivals are considered, one can only reach the conclusion that the method of pasteurisation employed played an important role on the survival of these strains in the previous study by Cronjé (2003). To test this hypothesis the survival of the same bacterial strains had to be determined with a "pot" pasteuriser.

It was thus essential that the survival potential of all the selected bacterial strains be evaluated using the "pot" pasteuriser to check the pasteurising status of the "pot" pasteuriser. This is important since the bacterial contaminants were found by Cronjé (2003) to survive in milk that had been pasteurised using the "pot" method. The data obtained in this study on the thermal survival/death profiles when the "pot" pasteuriser was set at 80°C for 30 min showed that, with the exception of the *Can. lipolytica* (G1) yeast strain, the *E. coli* (58), *A. baumannii* (C3), *B. cereus* (S4), *Chr. meningosepticum* (S5) and *P. putida* (S6) strains all survived at least this method of pasteurisation for at least 30 min. These results suggest that the method of pasteurisation employed plays an important role in the survival of these selected bacterial strains. The survival of these strains in the "pot" pasteuriser differ from those obtained with the LTLT and the HTST pasteurisation methods. This can probably be ascribed to the fact that with the "pot" pasteuriser the heat can only be applied from one direction (the bottom of the "pot"). The necessary heat must first heat the liquid at the bottom of the "pot" and then diffuse through the rest of the liquid causing the total liquid of the "pot" to have different temperatures with higher temperatures at the bottom of the "pot". The lid of the "pot" pasteuriser is not heated and thus this does not transfer any heat to the upper liquid. In comparison, the submerged heating of the containers used in the LTLT and HTST pasteurisation methods caused the required total liquid temperature to be reached fast because the heat was applied from all directions.

The data from this study clearly showed that the selected bacterial isolates did not survive the LTLT and HTST pasteurisation but did survive the "pot" pasteurisation. In the South African context the "pot" pasteuriser is ideal for use by new South African entrepreneurs, but in order for it to apply efficient heating resulting in good quality products, it is recommended that the manufacturers of the "pot" pasteuriser improve the heating quality and pasteuriser isolation. If they can design a "pot" pasteuriser that can be tightly closed, with heating from all the sides of the "pot" and have an internal thermometer that facilitates reading the temperature of the

inside liquid outside the "pot", the user can be sure of the inside temperature. By applying these recommendations, the function of the "pot" pasteuriser would be fulfilled.

As it was found that only the *B. cereus* (S4) strain survived all the different types of pasteurisation methods, future research needs to be done to identify at what temperature this heat resistant bacterial strain can be destroyed. This is important because there is a need to destroy all the spoilage organisms that can lead to the deterioration of the food products.

References

Anonymous. (2002). *Foodstuffs, Cosmetics and Disinfectant Act and Regulations. Act no.54 of 1972, G.N.R. 1555/1997*. Johannesburg, South Africa: Lex Patria Publishers.

Cronjé, M.C. (2003). Production of kepi grains using pure cultures as starters. *M.Sc. Thesis,* University of Stellenbosch, Stellenbosch, South Africa.

Eneroth, A., Svensson, B., Molin, G. & Christiansson, A. (2001). Contamination of pasteurised milk by *Bacillus cereus* in the filling machine. *Journal of Dairy Research,* **66**, 189-196.

Grant, I.R., Ball, H.J., Neill, S.D. & Rowe, M.T. (1996). Inactivation of *Mycobacterium paratuberculosis* in cow's milk at pasteurisation temperatures*. Applied and Environmental Microbiology*, **62**, 631-636.

Stauffer, J.E. (1993). Quality assuranceand dairy processing. In*: Dairy Science and Technology Handbook* (edited by Y.H. Hui), Volume 2. Pp. 12-25. New York: VCH Publishers, Inc.