CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

1.1 PROBLEM STATEMENT

UHT (ultra-high temperature) milk processing plants in South Africa sporadically experience contamination with *Bacillus sporothermodurans.* Often this contamination is believed to be due to the reprocessing of UHT milk. It may occur as a result of UHT milk from packages used for quality control, UHT milk from defective packaging, or inadequately processed UHT milk caused by other problems that may occur during processing. It should be noted that the main reason for reprocessing is to avoid economic losses.

Internationally in 2008, there was an overall shift towards the consumption of UHT milk (Tetra Pak Dairy Index, 2009). Between 2000 and 2003 there was an increase in the consumption of UHT treated milk compared to pasteurised milk in some European countries like Spain (4%), UK (3%) and Portugal (1%) (Rysstad & Kolstad, 2006). In South Africa UHT milk consumption stood at 32% of all liquid milk and liquid milk products consumed towards the end of 2009 while 60% of the dairy products consumed within this period were in the form of liquid milk and liquid milk products (Coetzee & Maree, 2009).

B. sporothermodurans, first detected in UHT milk in Germany in 1990, affects the stability and the shelf life of commercial UHT milk. This is due to the unusual thermal kinetics of *B. sporothermodurans* spores that enable them to survive high temperatures, namely, 130 ℃ for 4s of UHT treatment (Huemer, Klijn, Vogelsang & Langeveld, 1998). These spores germinate during storage in UHT products causing instability due to their proteolytic activities thereby reducing the shelf life and affecting consumer acceptability.

Increasing the temperature or the holding time in an attempt to inactivate *B. sporothermodurans* spores affects the organoleptic and nutritional qualities of UHT milk (Claeys, Ludikhuyze & Hendrickx, 2001). Severe heating will lead to protein

denaturation, Maillard reactions and lactose isomerisation. Protein denaturation and sugar modification are responsible for the 'cooked' taste while the Maillard reactions induce a decrease of the protein nutritional value by irreversible alteration of the lysine residues (Claeys *et al.*, 2001). It is currently not clear exactly how the structural and chemical components of *B. sporothermodurans* spores influence their extreme wet heat resistance during heating at UHT temperatures (Klijn, Herman, Langeveld, Vaerewijck, Wagendorp, Huemer & Weerkamp, 1997).

B. sporothermodurans spores have been found to be more resistant than other heat resistant spores at temperatures above 130 °C with D_{140} ranging from 3.4–7.9s and Z-values ranging from 13.1–14.2 °C (Huemer *et al.*, 1998). D_{140} is the time required when heating at 140 ^{o}C to inactivate *B. sporothermodurans* spores by one log cycle. Similarly, the Z-value is the temperature required to reduce the D-value by one log cycle (Juneja, Snyder & Marmer, 1997).

When unopened packages of UHT treated milk are incubated at 30 °C for 15 days, *B. sporothermodurans* counts may reach a maximum of 10^5 cfu/ml. Even though *B. sporothermodurans* is not a risk to the consumer, dairy operators are required to address quality problems in order to meet legal demands and avoid trade restrictions (Hammer, Lembke, Suhren & Heeschen, 1995).

B. sporothermodurans has not been isolated from raw milk because of its inability to grow and compete with other bacteria in raw fresh milk. On the other hand, it encounters no competition from other bacteria in UHT treated milk in which it grows without restraint (Huemer *et al.*, 1998). Different authors have postulated possible routes of contamination by *B. sporothermodurans*. Cited are contamination from soil, fodder, digestive tract, dung, udder, teat, milking utensils, raw milk and feed (Vaerewijck, De Vos, Lebbe, Scheldeman, Hoste & Heyndrickx, 2001). Reprocessing of contaminated lots of UHT products and contaminated milk powder has been identified as another possible route of contamination with *B. sporothermodurans* spores during processing (Hammer *et al.*, 1995). However, the precise route of contamination by *B. sporothermodurans* has not yet been established

and this makes it difficult to control contamination (Guillaume–Gentile, Scheldeman, Marugg, Herman, Joosten & Heyndrickx, 2002).

CHAPTER 2: LITERATURE REVIEW

2.1 CURRENT TRENDS IN UHT MILK PRODUCTION AND CONSUMPTION

Between 1997 and 2007, milk production in the world expanded by 122 million tonnes giving an average annual growth of 1.9%. The annual growth rates of milk in 2006 and 2007 were 2.5% and 1.3% respectively with the growth rate expected to increase in 2008 and 2009. Globally there is shift towards the consumption of UHT milk. Consumption of UHT milk increased 18.7% in 2004 to 23% in 2008. An annual growth rate of 5.2% was predicted before 2012 (Tetra Pak Dairy Index, 2009).

In South Africa the total dairy consumption showed positive growth in the five years prior to 2008, however, there was a downward trend in the first quarter of 2008. Pasteurised liquid milk and UHT milk are the major liquid products as shown in Figure 2.1 (Coetzee & Maree, 2009). The world milk consumption and the consumption of other liquid dairy products is expected to rise by an annual growth rate of 2.2% over the next three years, according to a research report released by Tetra Pak which is one of the biggest stakeholders in world food processing and packaging (Tetra Pak Dairy Index, 2009).

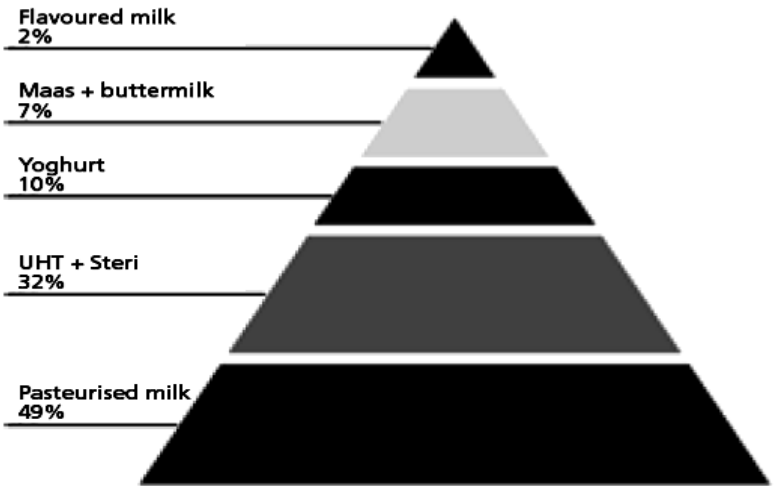

Figure 2.1 Percentage composition of the South African liquid market (Coetzee & Maree, 2009)

2.2 QUALITY AND SAFETY OF RAW MILK

Raw milk and products which have not been pasteurised pose a health risk to consumers, as do pasteurised milk products but to a lesser extent, considering that they have also been implicated in outbreaks (Leedom, 2006). The consumption of raw milk and raw milk products should be avoided especially by high-risk consumer groups because the risk outweighs the sensory good taste benefits associated with it (Leedom, 2006). The shelf life of pasteurised fluid milk is determined by the quality of the raw milk that was processed. Similarly, the bacterial and somatic cell counts of the raw milk determine the amount of heat stable enzymes, plasmin and lipase in the heat-treated milk (Barbano & Santos, 2006). For many years raw milk has been recognised as a vehicle for the transmission of food pathogens. Outbreaks associated with the consumption of raw milk occur routinely every year in different parts of the world. Examples of outbreaks include campylobacteriosis, in USA in May 1983, *Listeria monocytogenes* infection in Canada 1998 and *Yersinia enterocolitica* infection in the USA in 1992 and 1997 (Jayarao, Donaldson, Straley, Sawant, Hegde & Brown, 2006).

2.3 BACTERIAL COMPOSITION OF RAW MILK

The availability of carbohydrates, proteins and fat, together with the neutral pH makes milk a perfect medium for bacterial microbial growth. Different sorts of bacteria can be found in raw milk. These may include psychrotrophs, coliforms and other Gram-negative bacteria. Different psychrotrophic bacteria belonging to different genera have been isolated from milk. They include: *Pseudomonas*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Aeromonas*, *Acinetobacter*, *Alcaligenes* and *Achromobacter* spp. Some genera isolated from milk are both psychrotrophic and thermoduric. They include: *Bacillus*, *Clostridium*, *Microbacterium*, *Micrococcus* and *Corynebacterium* spp. (Hayes & Boor, 2001). Some thermoduric bacteria that have been isolated from milk are *Microbacterium* (Kazwala, Daborn, Kusiluka, Jiwa, Sharp & Kambarage, 1998), *Micrococcus* (García, Rodríguez, Bernardo, Tornadijo & Carballo, 2002), *Bacillus* spores, *Clostridium* spores (Te Giffel, Wagendorp, Herrewegh & Driehuis, 2002), *Alcaligenes* (Samaras, Kehagias, Arkoudelos & Bocaris, 2003), and *Microbacterium* (Kazwala *et al*., 1998). Similarly, some psychrotrophic bacteria isolated from milk are *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Aerobacter* (Sørhaug & Stepaniak, 1997), *Alcaligenes* (Samaras *et al*., 2003), and *Bacillus* (Te Giffel *et al*., 2002).

The incidence of toxigenic *Bacillus cereus* has been found to be high in milk and cream in particular as toxin production has been linked with aeration (agitation) during growth at 8 °C (Christiansson, Naidu, Nilsson, Wadstrom & Pettersson, 1989). However, the very low incidence of milk-borne food poisoning by *B. cereus* has been attributed to its inability to produce toxin under normal storage conditions of fresh milk (Christiansson *et al*., 1989). *B. licheniformis*, *B. pumilus*, *B. subtilis*, *B. sphaericus*, *B. thuringiensis* and *B. brevis* have been linked to food-borne illness, while *B. anthracis* and *B. cereus* are the only bacilli able to infect humans (Pirttijärvi, Andersson & Salkinoja-Salonen, 2000). Examples of bacterial pathogens associated with milk and the diseases they cause can be seen in Table 2.1.

The contamination of raw milk with bacteria can be due to one or many of the following sources of contamination: interior of the udder, exterior of the udder and milking instruments. Seasonal incidence of spores of *B. cereus* in raw milk has been linked to the pasturing of cattle, considering that housing them in a shelter while they are being fed has been found to reduce the levels of milk contamination (Slaghuis, Te Giffel, Beumer & André, 1997). Automated milking and restricted pasturing have been found to reduce the levels of *B. cereus* spores in raw milk. Examples of thermoduric and psychrotrophic bacteria found in milk can be seen in Table 2.1.

The production of raw milk with a standard plate count (SPC) consistency of less than 10 000 cfu/ml is a reflection of good hygienic practices while an SPC more than 10 000 cfu/ml is a reflection of poor hygienic practices during raw milk production (Cousin & Bramley, 1981). According to the regulations relating to milk and dairy products of the South African Government Notice No. R1555, an SPC of less than 200 000 cfu/ml is recommended for raw milk intended for use or consumption (Regulations relating to milk and dairy products, 1997).

Table 2.1 Food borne pathogens associated with milk and milk products

Organism	Illness
Enterobacteriaceae	
Pathogenic *E. coli* (Buyser *et al.*, 2001)	Gastroenteritis, hemolytic uremic syndrome
Salmonella enterica (Jayarao & Henning, 2001)	Gastroenteritis, typhoid fever
Yersinia enterocolitica (Jayarao & Henning, 2001)	Gastroenteritis
Other Gram-negative bacteria	
Aeromonas hydrophila (Lafarge *et al*., 2004)	Gastroenteritis
Brucella melitensis (Hamdy *et al*., 2002)	Brucellosis (Bang's disease)
Campylobacter jejuni (Jayarao & Henning, 2001) *Escherichia coli* (Griffin *et al*., 1991)	Gastroenteritis
Gram-positive spore formers	
Bacillus cereus (Christiansson *et al*., 1989)	Gastroenteritis (emesis and diarrhoea)
Clostridium botulinum (Franciosa *et al., 1*999)	Botulism
Gram-positive cocci	
Staphylococcus aureus (Buyser *et al*, 2001)	Emetic intoxication
Streptococcus agalactiae (Gillespie *et al*., 1997)	Mastitis
Streptococcus zooepidemicus (Francis *et al*., 1993)	Pharyngitis, nephritic sequelae
Miscellaneous Gram-positive bacteria	
Corynebacterium ulcerans (Barrett, 1986)	Diphtheria
Listeria monocytogenes (Hayes *et al*., 1986)	Listeriosis
Mycobacterium bovis (Kazwala *et al*., 1998)	Tuberculosis
Mycobacterium tuberculosis (Kazwala *et al*., 1998)	Tuberculosis
Mycobacterium avium subsp. Paratuberculosis (Ayele *et al*., 2005)	Johne's disease (ruminants)
Rickettsia	
Coxiella burnetii (Kim *et al*., 2005)	Q fever
Viruses	
FMD virus (Tomasula *et al*., 2007)	Foot-and-mouth disease
Fungi	
Molds (Wouters *et al*., 2002)	Mycotoxins
Protozoa	
Entamoeba histolytica (Rai *et al*., 2008)	Amoebiasis

2.4 QUALITY AND AGE GELATION OF UHT MILK

Proteolytic activities in UHT milk during storage can give rise to a bitter flavour, gelation and sedimentation which can lead to the reduction of the quality of stored products. Proteolysis in UHT milk is largely due to the presence of bacterial proteinase and the natural milk alkaline serine proteinase, known as plasmin (Topçu, Numanoğlu & Saldamli, 2006). The proteolysis deficiencies can be alleviated by conducting UHT processing at a higher temperature (above 150 °C), but this will also lead to the production of a 'cooked' taste in the UHT milk (Topçu *et al.*, 2006). Gelation in UHT milk takes place when whey proteins, especially β-lactoglobulin, interact with casein, mainly κ-casein of the casein micelle to form a three-dimensional protein complex (β-lactoglobulin- κ-casein complex) (Datta & Deeth, 2001).

The process of age gelation can be divided into two steps. In the first step, the *βk*-complexes detach from the casein micelles because of the breakdown of various attachment sites on *k*-casein. In the second step, these complexes aggregate into a three-dimensional matrix. Upon reaching a critical volume, the *βk*-complex forms a gel of custard-like consistency (Datta & Deeth, 2001). Factors that affect the age gelation of UHT milk are the nature of the heat treatment, proteolysis during storage, milk composition and quality, seasonal milk production factors and storage temperature (Datta & Deeth, 2001).

2.5 RAPID METHODS TO DETERMINE THE BACTERIAL QUALITY OF MILK

2.5.1 Bacterial stains

Gram-positive and Gram-negative bacteria in milk can be analysed using a staining technique. In such a technique, the milk sample often pre-treated with EDTA can be filtered through a polysulfone membrane to concentrate the bacteria in the sample. The bacteria on the membrane are stained with toluidine blue and then treated with ethonal-acetic acid which decolourises Gram-negative bacteria while the Gram-

positive bacteria retain the blue colour of toluidine (Yazdankhah, Sørum, Larsen & Gogstad, 2001). The detection limit of this method is at 5×10^6 cfu/ml for *Staphylococcus aureus* and 1×10^6 cfu/ml for *Escherichia coli* for milk samples (Yazdankhah *et al.*, 2001). Resazurin (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide) is the bluish dye used as an oxidation-reduction indicator to test for the presence of bacteria. It has been found to be more sensitive than methylene blue or turbidity alone as an indicator of microbial growth (Takeno, Ohnishi, Komatsu, Masaki, Sen & Ikeda, 2007). The methylene blue dye reduction test (MBRT) has been used to successively count up to 800 life cells within a time frame of 200s (Bapat, Nandy, Wangikar & Venkatesh, 2006). Colony-forming units (cfu) equivalent to 800 live cells have been successfully quantified using MBRT (Bapat *et al.*, 2006). Bacteria can also be enumerated by measuring the amount of adenosine triphosphate (ATP) using the luciferase bioluminescent reaction, however, it is difficult to differentiate between bacterial and somatic ATP (Siragusa & Cutter, 1995).

2.5.2 Aminopeptidase activity of Gram-negative bacteria

The aminopeptidase test can be use to analyse the microbial quality of milk according to the standard set by the European Union. This method has been designed to measure the aminopeptidase of Gram-negative bacteria. The sensitivity of the assay is at 2×10^4 cfu/ml that is an acceptable limit with respect to the regulations of many countries (Manzano, Ordoez, De La Hoz & Fernandez, 2005). Using this method, the Gram-negative population can be estimated within 2.5h.

2.5.3 BactiFlow™ flow cytometer

The BactiFlow™ flow cytometer can be used to analyse the bacterial population of milk by utilising their esterase activity (Flint, Walker, Waters & Crawford, 2007). The cytometer has the Chemunex system that utilises bacterial esterase activity to label and detect viable cells. The protocol used to analyse viable cells can be modified to analyse only thermophilic bacteria by heat-treating the milk sample. Milk samples that have been diluted with 0.1% peptone are mixed with 0.8%

ethylenediaminetetraacetic acid to minimise the interference from the background (Flint *et al.*, 2007).

2.5.4 Petrifilm™ and SimPlate™ plates

The performance of the Petrifilm™ aerobic count and the SimPlate™ total plate count of pasteurised milk has been found to decline with a decrease in the bacterial quality of the milk (Beloti, Barros, Nero, Pachemshy, de Santana, Bernadette & Franco, 2002). This can be resolved by introducing a preliminary concentration on a filter with the Petrifilm™ technique (Vail, Morgan, Merino, Gonzales, Miller & Ram, 2003). However, the straightforwardness, reliability and low cost of Petrifilm™ plates make them ideal for routine analysis. The counting range of the SimPlate™ is high, with > 1 600 the most probable number (MPN) per single dilution. As a result, SimPlate™ needs fewer dilutions of samples. Some foods, for example, raw liver, wheat flour and nuts contain enzymes that have produced false-positive reactions on SimPlates™. Nevertheless, the SimPlate™ total plate count method is a suitable alternative to conventional SPC for estimating aerobic bacteria in a wide range of foods (Beuchat, Copeland, Curiale, Danisavich, Gangar, King, Lawlis, Likin, Okwusoa, Smith & Townsend, 1998).

2.5.5 Propidium monoazide and real-time PCR

This assay allows for the quantification of only viable bacterial cells present in fermented milk products because propidium monoazide penetrate the dead cells and bind to the DNA that is then cross-linked by photo induction (García-Cayuela, Tabasco, Peláez & Requena, 2009). Propidium monoazide is utilised to discriminate viable and non-viable bacteria in conjunction with real time PCR quantification (Nocker, Cheung & Camper, 2006).

2.6 MILK CONTAMINATION ROUTES

Silage is an important source of contamination of raw milk by bacterial spores and the level of raw milk can be minimised if the spore load in silage is reduced. In addition to silage, factory equipment and packaging materials also contribute to the quality of milk (Te Giffel, Wagendorp, Herrewegh & Driehuis, 2002). A number of milk-contaminating bacteria have been found in the air surrounding the processing machine, in the condensed water on the filling nozzles and in wastewater at the bottom of the filling machine. The majority of these bacteria were identified as *Pseudomonas fluorescens*, *P. putida*, *P. corrugate* and *Janthinobacterium lividum* (Eneroth, Christiansson, Brendehaug& Molin, 1998). Molecular typing methods have been used to reveal genetic diversity of *Bacillus* isolates from dairy farms and dairies from the same location. It has been found that bacteria associated with dairy contamination have not necessarily originated from raw milk, thereby confirming the theory of contamination and propagation during the manufacturing process (Banykó & Vyletělová, 2008). The contamination route can be either external or internal as shown in Figure 2.2 below.

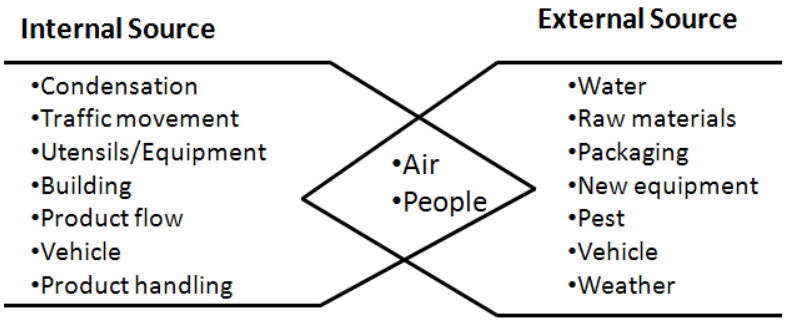

Figure 2.2 Possible sources of cross contamination of pasteurised milk (Burgess, Heggum, Walker & Van Schothorst, 1994)

The high level of Gram-negative psychrotrophic bacteria in refrigerated milk is due to post-pasteurisation contamination at the filling level (Eneroth *et al.*, 1998). In order to extend the shelf life of fluid milk all bacteria that emanate from the raw milk supply

sources have to be eliminated. Different studies conducted have suggested multiple potential entry points for psychrotolerant endospore-forming bacteria into the raw milk at the farm as well as various points of recontamination during the entire production line (Huck, Sonnen & Boor, 2008).

High numbers of *B. cereus* in pasteurised milk can be due to teat contamination especially during grazing (Svensson, Eneroth, Brendehaug, Molin & Christiansson, 2000). Spores of *B. cereus* are very hydrophobic and as such, can adhere to surfaces of steel, glass and rubber but are effectively eliminated during efficient cleaning (Svensson *et al*., 2000).

2.7 BACKGROUND TO UHT MILK CONTAMINATION

In 1985, certain types of *Bacillus* species were detected in UHT treated milk in many European countries (Hammer *et al*., 1995). During that time spoilage encountered in UHT treated milk was thought to have been caused by contamination during filling. Members of the genus *Bacillus*, especially *B. badius*, *B. cereus*, *B. licheniformis*, *B. polymyxa*, *B. subtilis*, and *B. stearothermophilus*, were identified as responsible for the spoilage of UHT milk (Pettersson, Lembke, Hammer, Stackebrandt & Priest, 1996).

Table 2.2 Hazard analysis chart for thermal processing of milk

Process step	Identified hazard	Preventive measures	CCP
Raw milk receiving	Microbiological (M) – Pathogens, *Staphylococcus* toxin	Pasteurisation, Temperature control Antibiotic test	Yes
Filter	Chemical (C)-Animal residues Physical – foreign object.	Passage of foreign object that can be a hazard	No
Raw milk storage	Microbiological (M) – Pathogens, *Staphylococcus* toxin	Pasteurisation, Temperature control	Yes
Clarifier/separator	Microbiological (M) – Pathogens, *Staphylococcus* toxin	Pasteurisation, resident times not enough for *Staphylococcus* toxin production	No
Raw cream storage			
Homogenisation			
Vitamin addition	Microbiological (M) – Pathogens, *Staphylococcus* toxin	Prerequisite programme for ingredient reception, usage record and pump calibration	Yes
Pasteurisation/UHT	Microbiological (M) – Pathogens	Pathogens are eliminated by pasteurisation/UHT	Yes
Pasteurised/UHT storage	Introduction of pathogens, hazard after pasteurisation	Prerequisite programmes are in place to prevent post-contamination	No
Packaging materials	Introduction of pathogens, chemical, or physical hazard after pasteurisation	Pasteurisation/UHT contamination	Yes
Filters			
Cold storage	Properly pasteurised, packaged product contain no hazard	Not applicable	No
Distribution			

Source of table: Byrne and Bishop, 2001

However, it was also found that certain heat resistant spores could survive UHT and autoclaving and subsequently grow in the stored products. These bacteria with highly heat resistant spores were first detected in Southern Europe in 1985. The first detection in UHT milk was in Germany in 1990. Subsequently, the problem was experienced in several other countries (Hammer, Lembke, Suhren & Heeschen, 1995).

In 1985, the European Council (EC) Milk Hygiene Directive 85/397 stipulated a maximum of 10 cfu/0.1 ml for UHT milk after an incubation period of 15 days at 30 ℃ and this led to a change in the quality control protocol for the UHT treated milk. When the German government enforced the EC Milk Hygiene Directive 85/397 in their national legislation through the Hygiene Ordinance of June 1989 (Klijn *et al*., 1997) the quality control for UHT milk was modified to include bacteriological analysis techniques instead of the usual physio-chemical methods like pH, sensory and stability tests, which were not effective enough to detect slow-growing spore forming bacteria (Klijn *et al*., 1997). The bacterium responsible for the non-sterility of UHT milk was identified to be *B. sporothermodurans* (Pettersson *et al.*, 1996). The execution of Hazard Analysis Critical Control Point (HACCP) by the dairy industry is expected to improve consumer confidence in products and decrease the barriers in international trade (Sandrou & Arvanitoyannis, 2000). Figure 2.2 and Table 2.2 above shows the hazard analysis and critical control chart for UHT milk.

2.8 THERMAL PROCESSES AND PROPERTIES OF TREATED MILK

2.8.1 Pasteurisation of milk

According to the International Dairy Federation (IDF), pasteurisation is a process whereby products such as milk are heat-treated in such a way as to minimise possible hazards due to pathogens, without changing the physical, chemical and sensory properties of the product (Lewis, 1986). Raw milk prior to pasteurisation is expected to be of good quality. It should be free of pathogens and should have an acceptable colony count with a minimum of 10^5 cfu/ml.

Regulations relating to milk and dairy products of the South African Government Notice Number R. 1555 of 21 November 1997 state that every particle of milk should be heated at a temperature not less than 63 ℃ and not more than 65.5 ℃ and held at that temperature for at least 30 min. The heating should be followed by cooling within 30 min to a temperature lower than 5 ℃. On the other hand, every particle of the milk can be heated to a temperature of at least 72 ℃ and held at that temperature

for at least 15s with same cooling pattern as mentioned above (Regulations relating to milk and dairy products, 1997).

According to the IDF, pasteurisation should be conducted as follows. For milk use, it is 72 °C for 15s (continuous flow pasteurisation) or 63 °C for 30 min (batch pasteurisation). Other equivalent conditions can be obtained by plotting the line passing through these points on a log of times versus temperature graph. For cream, use 75 °C for 15s (10-20% fat), 80 °C for 15s (above 20% fat) and 65 °C for 30 min (batch pasteurisation) (Codex Committee on Milk and Milk Products, 2000).

2.8.2 UHT processing of milk

UHT treatment of milk is the process within which milk is rendered commercially sterile by heating at temperatures exceeding 135 °C for 1–2s (Kessler, 1981). The processing of milk at high temperatures is aimed at destroying vegetative cells as well as endospores present in raw milk so that it can be stored for prolonged periods, generally several months without refrigeration (Kessler, 1981). According to the IDF, the temperatures for UHT treatment range from 135 to 150 °C used in combination with the appropriate holding times such as 140 °C for 2.3s. The temperatures for sterilisation should be 110 to 125 °C in combination with appropriate holding times such as 121 °C for 3 min or 115 °C for 13 min (Codex Committee on Milk and Milk Products, 2000).

Other equivalent conditions to give an F_0 value of 3 min can be obtained by plotting the line passing through the above temperature/time combinations on a log time versus temperature graph (Codex Committee on Milk and Milk Products, 2000). The F_0 value is any equivalent heat treatment that will cause the same destruction ration of spores similar to that of a reference temperature (Mafart, Couvert, Gaillard & Lequerinel, 2002). The heat process for milk is specifically designed to ensure the safety of products. However, heating might lead to protein denaturation, Maillard reactions and lactose isomerisation (Claeys, Ludikhuyze & Hendrickx, 2001).

Regulations relating to milk and dairy products of the South African Government Notice Number R. 1555 of 21 November 1997 state that UHT treatment comprises heating above 100 °C and aseptic packaging so that the end product, after incubation for not less than 14 days at a temperature of 30 °C, is free from spoilage micro-organisms.

UHT plants operate in a manner to maintain the temperature-time relationship in a given range. There are two types of UHT processes: the direct UHT, which includes a heating and cooling phase, and the indirect UHT. The heating and cooling phases increase with a higher temperature during indirect UHT processes. This implies a longer holding time and, therefore, an increase in the undesired heat-induced changes in milk (Kessler, 1981). For the direct UHT process, which could be either injection or infusion, a high heating temperature can be achieved without the heating and cooling phases. The use of flash evaporation to cool milk rapidly in direct UHT has a problem of heat loss compared to indirect UHT processes. This is because after the flash evaporation the steam form is approximately 80 °C and can be used only for milk preheating (Grijspeerdt, Mortier, Block & Renterghem, 2004).

2.8.3 Important definitions regarding UHT processing

UHT treatment involves a high-temperature/short-time heat treatment aimed at producing a commercially sterile product that can be stored at room temperature. This process aims to destroy all microorganisms. Residual microorganisms are unlikely to cause spoilage under normal storage conditions. UHT-treated milk and cream are packaged aseptically into sterilised, hermetically sealed containers. The total heat treatment is equivalent, in terms of its effectiveness against heat-resistant bacterial spores, to a minimum F_0-value of 3 min. A hermetically sealed container is a container that is designed and intended to be secure against the entry of microorganisms (Codex Committee on Milk and Milk Products, 2000).

Sterilisation involves a high-temperature/long-time heat treatment aimed at producing a commercially sterile product that can be stored at room temperature. The process

aims to destroy all microorganisms. Residual microorganisms are unlikely to cause spoilage under normal storage conditions. Sterilisation is an in-container, batch-wise heating process using minimum temperature-time conditions that achieve an F_0-value of 3 min (Codex Committee on Milk and Milk Products, 2000).

2.8.4 UHT treatment systems

UHT treatment systems can be divided into two types when considering the nature of the equipment used, the direct and the indirect systems (Figure 2.3).

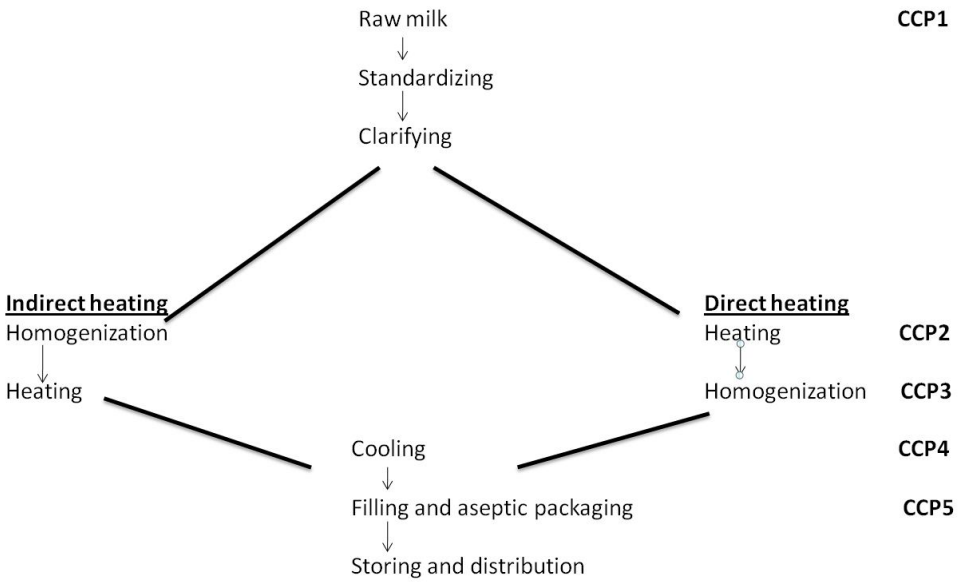

Figure 2.3 Flow diagram for production of UHT milk (Sandrou & Arvanitoyannis, 2000)

2.8.4.1 Direct UHT system

With the direct UHT system, heating is performed by mixing the product and steam at high pressure of about 9 bars. With this system the heat transfer is higher than that of indirect UHT and because the residence time is very short, results in less fouling. This process is difficult to control and therefore not often used (Grijspeerdt *et al.*, 2004). There are two types of the direct heating system: direct heating by injection and direct heating by infusion as shown in Figure 2.4. In the injection system, the

steam injected comes into contact with the product through a specially designed nozzle. With the infusion system, the product is distributed in strings across the centre of a chamber in which steam is distributed.

2.8.4.2 Indirect UHT system

The indirect UHT system consists of a solid barrier that separates the heat transfer medium (often water or steam) and the dairy product. The indirect system can further be subdivided into two types depending on the nature of the heat exchanger incorporated into it. The two types of heat exchanger are the plate and tubular heat exchangers as shown in Figure 2.5 and Figure 2.6 (Grijspeerdt *et al.*, 2004). The quality of UHT milk in packs processed via indirect UHT differed from that produced by direct UHT when they were analysed after storage for 24 weeks. Thermally induced changes in lactulose, furosine and acid-soluble whey proteins revealed that directly heated UHT milks suffer less heat damage than indirectly heated milk (Elliott, Datta, Amenu & Deeth, 2005).

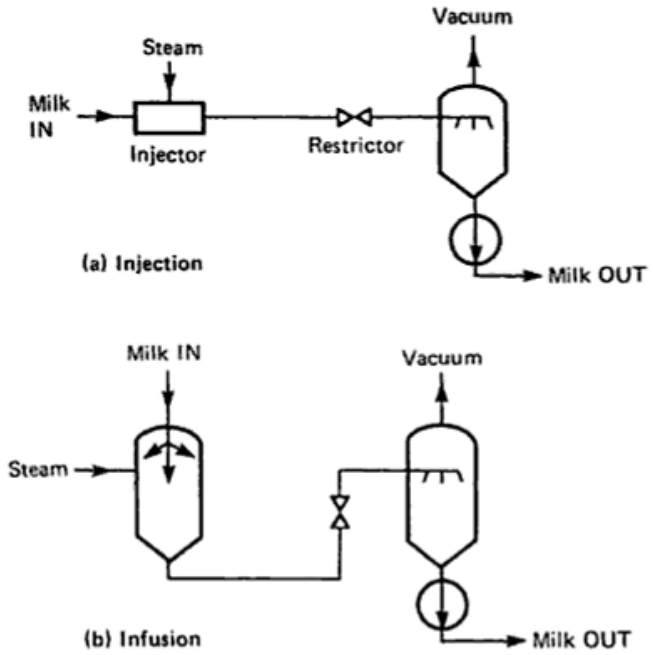

Figure 2.4 Diagram of UHT injection and infusion systems (Lewis & Heppel, 2000)

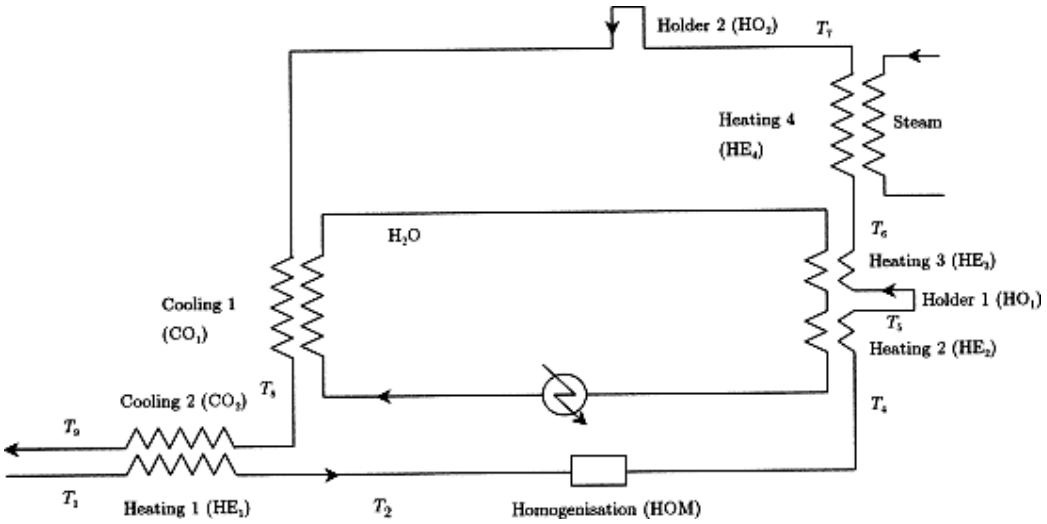

Figure 2.5 Schematic layout of a UHT system using a tubular heat exchanger (Grijspeerdt *et al.*, 2004)

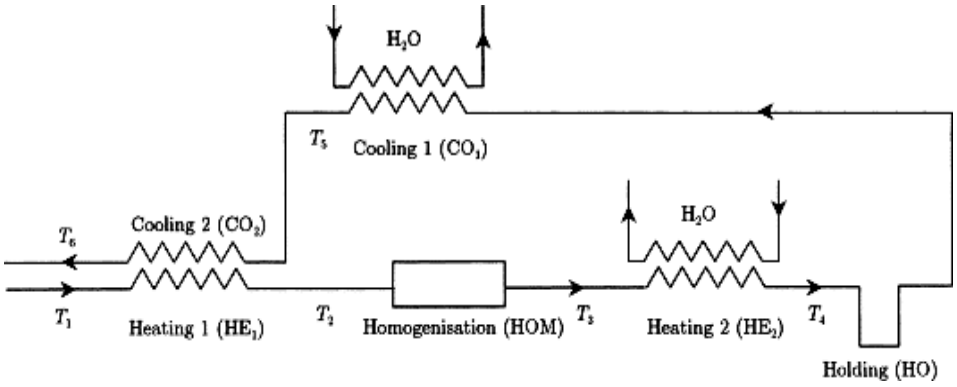

Figure 2.6 Schematic layout of a UHT system using a plate heat exchanger (Grijspeerdt *et al.*, 2004)

2.8.5 Holding time during thermal processing of liquid milk

When liquids flow through a pipe, there is a distribution of holding (residence) time along the pipe. It is possible to measure and analyse this distribution using various methods. One method involves the injection of a pulse of a tracer material in a liquid followed by sampling and analysing the outlet stream at defined intervals (Lewis, 1986). Based on this method, three types of flow have been determined: the plug flow, streamline flow and turbulent flow. The plug flow is the ideal situation where

the velocity profiles within a liquid do not vary. Due to internal friction or the viscosity of the fluid, there is always velocity distribution within the tube. This distribution of velocity and the flow type (streamline or turbulent) can be determined by using a dimensionless constant known as the Reynolds number (Re) (Lewis, 1986).

$$\text{Re} = \frac{VDP}{U} \quad \text{or} \quad \frac{4QP}{\prod UD}$$

V = Average velocity (m/s)

D = Tube diameter (m)

P = Fluid density (kg/m^3)

U = Dynamic Viscosity (Ns/m^2)

Q = Volumetric flow rate (m^3/s)

$\prod = {}^{22}\!/_{7}$

VOT = Volumetric of tube (m^3)

The resident time (t_{av}) is based on the average velocity and the length of the tube.

$$\text{Resident time } (t_{av}) = \frac{L}{V} \text{ or } \frac{VOT}{Q}$$

2.8.6 Tubular heat exchanger fouling by milk during thermal processing

Fouling, which is the accumulation of unwanted deposits on the surfaces of food processing equipment, mostly heat exchanger, causes resistance to heat transfer and as a result reduces the efficiency of thermal processing (Swartzel, 2007). The presence of these deposits represents a resistance to heat or mass transfer and therefore reduces the efficiency of the particular food process. Fouling may be as a result of an accumulation of the following: constituents of the fluid food being processed or products of chemical reactions occurring during the processes or particulate matter suspended in the fluid or microorganisms (Bansal & Chen, 2006). Two different types of fouling are commonly found in the food industry: thermal fouling and membrane fouling. Each manifests distinctly different mechanisms and processes. A

holistic approach is recommended in controlling fouling within the entire plant rather than focusing solely on the heat exchangers as fouling may shift to other parts of the plant (Bansal & Chen, 2006). The fouling of milk commences when milk is heated at 80 °C. At this temperature, milk starts to form deposits that can be monitored by weighing or indirectly by monitoring the pressure drop in the system. In practice the time taken for the pressure to drop varies considerably. When the pressure starts to drop, it does so in an increasingly parabolic pattern until a limiting pressure is reached (Lewis, 1986). It should be noted that the formation of different types of deposits is temperature dependent. Deposits formed between 80 °C and 105 °C, are a white, voluminous precipitate that is mainly protein. Beyond 110 °C the deposits are granular and are of mineral origin. At this stage build-up of deposit is usually within the final heating compartment, hence reducing heat transmission (Lewis, 1986).

The quality of milk also determines the speed of fouling and ageing of milk. The stability of raw milk with regards to deposit formation during processing has been shown to improve when held at 4 °C for 10–24h. This is probably due to the lypolytic activity that must have taken place during ageing. Milk with high levels of β-casein can easily form deposits (Lewis, 1986). Equal volumes of alcohol and milk can be mixed to determine the heat stability of the milk (Lewis, 1986).

2.8.7 Changes in milk on storage as a result of UHT treatment

Plasmin, which is an alkaline proteinase, is the main proteolytic enzyme in bovine milk. This bovine milk plasmin is found in two forms, the active and the inactive forms. This inactive form, otherwise known as plasminogen, turns to the active form upon activation as discussed in the following paragraphs. Plasmin is a trypsin-like serine proteinase that can hydrolyse β-casein and αs_2-casein readily and αs_1-casein slowly (Enright, Bland, Needs & Kelly, 1999).

Plasminogen activators are responsible for activating plasminogen to plasmin. In milk there are two types of plasminogen activators: the tissue-type (tPA) and the urokinase-type (uPA). There are also varied systems of inhibitors in milk that prevent

the activity of plasmin. These include the inhibitors to plasmin activator and inhibitors of plasmin itself. Pasteurisation of milk gives rise to an increase in the proteolytic activity of plasmin in milk. This might be due to the inactivation of inhibitors of plasmin activators, causing an increase in the activation of plasminogen (Enright *et al.*, 1999). An increase in the severity of preheating during UHT treatment, for example, 72 °C for 30s to 80 °C for 30 min, delays gelation of UHT milk during storage. This is due to increased levels of whey protein, mostly *β*-lactoglobulin denaturation (Datta & Deeth, 2001).

2.9 *BACILLUS SPOROTHERMODURANS*

B. sporothermodurans are psychrotolerant, mesophilic aerobic endospore-forming bacteria which produce extreme heat-resistant spores. When incubated for 2 days at 37 °C on a Brain Heart Infusion (BHI) agar plate, they are rod shaped which could become filamentous rods after laboratory cultivation. This filamentous morphology is linked to growth in laboratory media. They react with Gram stain producing a granular appearance and staining is not even (Hammer *et al.*, 1995).

B. sporothermodurans are aerobic spore formers of considerable importance in the food industry considering their ubiquitous nature. Regularly used pasteurisation processes that are adequate in inactivating vegetative cells have failed to inactivate *B. sporothermodurans* spores. The surviving spores may germinate and grow rapidly in products with little or no competition from other growing bacteria (Scheldeman, Herman, Foster & Hendrickx, 2006). *B. sporothermodurans* is not known to be pathogenic but can cause milk spoilage during production, storage and distribution thereby rendering products unsuitable for human consumption which can lead to considerable economic losses despite modern manufacturing techniques (Scheldeman *et al.*, 2006).

In 1995, *B. sporothermodurans* were detected for the first time in raw milk from farms. In 1996, 100 raw milk samples were screened using PCR fingerprints and only three samples from the same geographical area tested positive for *B.*

sporothermodurans. This positive result could not be confirmed in subsequent samplings. Only 2 out of 120 feed samples of corn, grass silage and sugar beets tested positive. These results suggest that contamination of raw milk at the farm through feed samples such as corn silage, grass silage and sugar beets are only incidental (Hammer *et al.*, 1995).

2.10 *B. SPOROTHERMODURANS* CHARACTERISATION TECHNIQUES

BHI agar plates that have been supplemented with Vitamin B_{12} are used for the isolation of *B. sporothermodurans* when incubated at 37 °C. Due to the competitive nature of the background flora, it is difficult to isolate *B. sporothermodurans* from raw milk or other farm sources. However, if samples are heated at 100 °C for 30–40 min and plated on BHI agar, *B. sporothermodurans* can be isolated (Scheldeman *et al.*, 2006). Different molecular methods have been used to identify and characterise *B. sporothermodurans*. Conventional PCR using the primers: BSPO-F2 (forward) (5' ACG GCT CAA CCG TGG AG 3') and BSPO-R2 (reverse) (5' GTA ACC TCG CGG TCT A 3') specific for *B. sporothermodurans* have been used to amplify portions of the 16SrRNA gene. The sizes of amplicons are used to confirm the presence of *B. sporothermodurans* (Scheldeman, Herman, Goris, De Vos & Hendrickx, 2002). Similarly, sequencing of the 16S rRNA gene can be used for the identification of *B. sporothermodurans* because the 16S rRNA genes have regions with high variability (Klijn *et al.*, 1997).

2.10.1 16S rRNA gene sequencing

The 16S rRNA genes of *B. sporothermodurans* can be amplified by PCR using conserved specific primers and the resulting PCR products purified and subsequently sequenced. A combination of the 16S rRNA gene sequencing primers can be used to generate a continuous stretch of the 16S rRNA gene (Scheldeman, Pil, Herman, De Vos & Heyndrickx, 2005).

In order to identify unknown bacteria, the 16S rRNA gene can be sequenced and the identity of the bacteria obtained by matching the sequence to those present on known databases. The 16S rRNA sequence analysis of *B. sporothermodurans* is complicated by the occurrence of many 16S rRNA gene copies in the bacterial genomic DNA but it can be used to determine other species that are closely related to *B. sporothermodurans* (Klijn *et al*., 1997).

2.10.2 Polymerase chain reaction (PCR)

This is a PCR reaction in which primers specific to the 16S rDNA gene of *B. sporothermodurans* are used to amplify segments that can be analysed on agarose gel electrophoresis. Different authors have published primers that are used to detect *B. sporothermodurans* (Scheldeman *et al.*, 2002; Herman *et al*., 1997).

Generally, a form of PCR reaction in which primers, specific to a region containing repetitive sequences in genomic DNA, is amplified. These amplified sequences, which could be of different lengths, can further be separated by denaturing polyacrylamide gel electrophoresis and then stained. At present, REP-PCR has only been used to successfully distinguish *B. sporothermodurans* from other known *Bacillus* species.

2.10.3 Amplified ribosomal DNA restriction analysis (ARDRA)

ARDRA is a PCR-based analysis in which the ribosomal DNA of bacteria is amplified with specific primers and the resulting amplicons are restricted using restriction enzymes. After restriction, fragments are analysed on a gel and robotypes are generated. This technique has been used to discriminate *B. sporothermodurans* from other *Bacillus* spp. isolated from milk **(**Guillaume-Gentile *et al.*, 2002). Of all the typing molecular techniques used to analyse *B. sporothermodurans*, only the REP-PCR with gel separation (Klijn *et al.*, 1997) and ribotyping (Guillaume-Gentile *et al.*, 2002) can present maximum discrimination between strains of *B. sporothermodurans* and other closely related species of *Bacillus*. These two molecular typing methods

have been used to reveal great genetic heterogeneity among *B. sporothermodurans* isolates from dairy farms and hence have been used to distinguish isolates of *B. sporothermodurans* from different origins (Guillaume-Gentile *et al.,* 2002).

2.11 *BACILLUS SPOROTHERMODURANS* SPORES

The analysis of *B. sporothermodurans* spores using Transmission Electron Microscopy (TEM) revealed structural differences between spores of *B. sporothermodurans* isolates emanating from various origins and from those of other species. The spores of the UHT strains either belonging to *B. sporothermodurans* or *Paenibacillus lactis* had cores that were very dense and the surrounding cortex comparatively large. The spores of *B. sporothermodurans* originating from raw milk and of *B. cereus* had cores that were proportionally larger in relation to the cortex size and less compact (Scheldeman *et al.,* 2006). The spores of *B. sporothermodurans* originating from feed concentrate showed intermediate properties. A compact spore core could be the quality of a more complete dehydration, an important aspect in heat resistance.

2.12 ANALYSIS *B. SPOROTHERMODURANS* SPORE COMPONENTS

It is possible to quantify micrograms of protein using the principles of protein-dye binding as described by Bradford (1976). This involves the use of Coomassie Brilliant Blue G-250 dye to bind protein. The binding of the dye to protein causes the dye's maximum absorption to shift from 465 to 595 nm; hence the absorption at 595 nm is used for monitoring the dye protein complex. Potassium and sodium cations, as well as carbohydrate such as sucrose, have little or no interference on this assay. On the other hand, large amounts of detergents such as sodium dodecyl sulphate, Triton X-100, and commercial glassware detergents can interfere on this assay. Small acid soluble proteins (SASP), which are small moleculer weight proteins located in the core of *Bacillus* spores, can be potential biomarkers for the identification of spores of *Bacillus* species by mass spectrometry considering that genetically distinct species

and strains have differentiated and their identity been confirmed by genetic analysis (Hathout, Setlow, Cabrera-Martinez, Fenselau & Setlow, 2003).

Dipicolinic acid (DPA), a component of the spore core, is often used to measure the integrity of the spore after subjection to stress. DPA extractions are often conducted by heating about 0.1 to 2 mg of spores per ml in 0.2 M K phosphate at pH 1.8 for up to 10 min at 100°C. Spore dipicolinic acid can be analysed using the reverse-phase liquid chromatography according to the method described by Warth (1979).

2.13 THERMAL INACTIVATION KINETICS OF *BACILLUS SPOROTHERMODURANS*

Studies based on the heat resistance of spores of three *B. sporothermodurans* strains isolated from non-sterile UHT milk and *B. sporothermodurans* spores conducted within the temperature ranges of 110–145 °C and 130–145 °C revealed that for higher temperatures, the D_{140} values of *B. sporothermodurans* (3.4–7.9s) were higher when compared to that of *B. stearothermophilus* (0.9 s) as shown on Figure 2.7 below. Similarly, the Z-value of *B. sporothermodurans* (13.1–14.2 °C) was higher when compared to that of *B. stearothermophilus* (9.1 °C) (Huemer *et al.*, 1998). Furthermore, spores *B. sporothermodurans* isolated from UHT have been found to possess high heat resistance compared to that isolated from the feed, as seen in Figure 2.8 below.

Major factors that contribute to the wet heat resistance of bacterial spores include (Melly, Genest, Gilmore, Little, Popham, Driks & Setlow, 2002):

- The core water content
- Mineralisation of the spore core due to accumulation of high levels of divalent cations and DPA
- Presence of α/β-type SASP.

An increase in the mineralisation of spores due to high levels of divalent cations and DPA increases the wet heat resistance of bacterial spores (Paidhungat, Setlow, Driks & Setlow, 2000). SASP binds to spore DNA thereby protecting it from wet heat and other treatments like chemicals, radiation, and so forth (Setlow, Atluri, Kitchel, Koziol-Dube, & Setlow 2006).

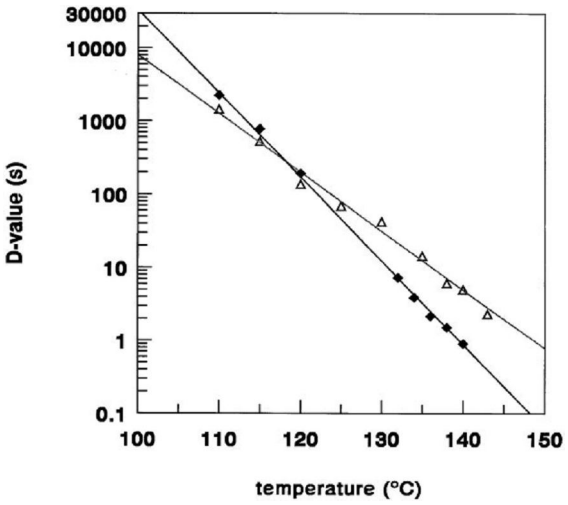

Figure 2.7 Thermal death time curves of *B. stearothermophilus* spores (Δ) and *B. sporothermodurans* spores J16B (▲); best plot lines through experimental data (Huemer *et al.*, 1998)

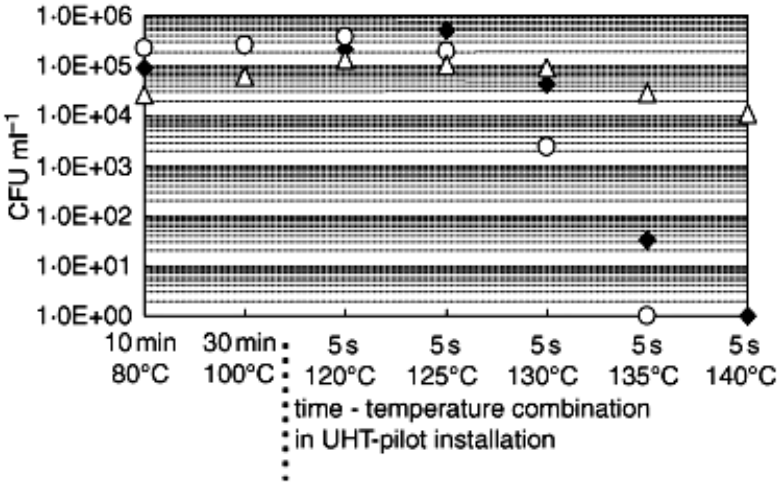

Figure 2.8 Survival of *Bacillus sporothermodurans* spores from different origins in a pilot ultra-high temperature (UHT) installation (direct mode). ◆, Feed concentrate isolate MB 1316; ○ Feed concentrate isolate MB 1317; industrial spores (Scheldeman *et al.*, 2006)

The SASP are of two types (α/ß-type and γ-type) and have an average molecular weight ranging from 5–10 kilodaltons named after the major protein(s). These types in *B. subtilis* spores are synthesised only in developing spores late in sporulation (Hathout *et al.*, 2003). It has been reported that killing spores by wet heat is not through DNA damage but rather by rupturing the spore's inner membrane permeability barrier and the inactivation of core enzymes (Warth, 1980; Setlow, 2000).

The heat resistance of spores has been found to increase about tenfold for sporulation between 30–44 °C while sporulation at 52 °C did not show any additional increase in heat resistance in the 100–120 °C treatment temperature (Condon, Bayarte & Sala, 1992). The condition of a particular food product prior to heat treatment is very crucial considering that the waiting time at warm temperature can lead to non-sterility in the processed food (Leguérinel, Couvert & Mafart, 2007).

2.14 STRESS RESPONSE ADAPTATION OF *BACILLUS* SPECIES

Bacteria stress response adaptation is of interest to the dairy industry because it influences the survival of *Bacillus* spp. during processing. When *Bacillus* spp. adapt to a particular stress they become more resistant to that stress during subsequent application (Van Schaik & Abee, 2005). After being exposed to a certain stress, bacteria may overcome subsequent sub-lethal stresses only to survive in food systems (Hill, Cotter, Sleator & Gahan, 2002). Furthermore, a stress response to one stress can lead to an adaptive response to other stresses (Hill *et al.*, 2002).

Most bacteria possess networks that enable them to adapt to their changing environments and survive under certain stress conditions. Therefore, these networks can affect processing and storage under certain circumstances (Abee & Wouters, 1999). Bacteria adaptive responses are due to certain genetic transformations within the genome that modify the metabolism of bacteria. Most regulatory systems involve the alteration of sigma (σ) factors whose main function is to attach to the core RNA polymerase giving promoter specificity which directs the expression of heat-shock genes involved in heat-shock response (Abee & Wouters, 1999).

Heat-shock spores (activated spores) have been found to be more heat resistant than those that have not been heat-shocked for the same spore type, despite both having the same protoplast density. It appears that spore heat-shock causes the cortical peptidoglycan to expand against the intact spore coats thereby resulting in lower water content in the protoplast and higher water content in the cortex (Beaman, Pankratz & Gerhardt, 1998). Heat-shock has been found to cause complete and partial deactivation of spores causing disruption and relaxation of the outer membrane. These spores also release some of their dipicolinic acid (DPA) and minerals, increasing their ability to germinate as well as acquiring heat-induced resistance (Teofila, Pankratz & Gerhardt, 1998).

2.15 OTHER *BACILLUS* SPECIES RELATED TO THERMALLY PROCESSED MILK

Bacillus species, particularly *B. licheniformis* and *B. cereus,* are the most commonly isolated species of *Bacilli* present in milk at various processing stages. *B. licheniformis* counts were found to be higher in heated milk during winter while *B. cereus* was found to be higher during summer according to studies conducted in the United Kingdom Crielly, Logan & Anderton, 1994). However, this pattern was found to change when milk samples were pre-incubated before plate counts due to the fact that *B. cereus* grows faster at ambient temperature than *B. licheniformis* does. *Bacillus* species that are frequently isolated from milk include: *B. circulans*, *B. firmus*, *B. subtilis*, *B. coagulans*, *B. sphaericus* and *B. mycoides.* However, the identity of some *Bacillus* strains remains undetermined (Crielly *et al.*, 1994).

2.15.1 *Bacillus cereus*

B. cereus are ubiquitous microorganisms which can be found in the soil, air, dust, water as well as in some processed food products consisting of rice, dairy products, meat, spices and egg. They cause food spoilage and with doses as low as 10^3–10^4 bacteria/g, they can cause food poisoning (Andersson, Rönner & Granum, 1995).

Quality problems caused by *B. cereus* include aggregation of the creamy layer of pasteurised milk owing to their lecithinase activity. They also cause sweet curdling of milk in low pasteurised milk that occurs without any rise in pH. Processing techniques such as pasteurisation tend to target spore producing, non-competitive bacteria by eliminating the vegetative cells while the spores survive. The spores became heat activated and proceed to germinate in the stored products (Andersson *et al.*, 1995). *B. cereus* causes two types of food poisoning. The first type is caused by an emetic toxin and results in vomiting, while the second is caused by enterotoxins and results in diarrhoea. However, the two types of symptoms can occur at the same time in situations where both toxin types are produced (Granum & Lund, 1997).

2.15.2 *Bacillus licheniformis*

B. licheniformis has been linked with septicemia, peritonitis, ophthalmitis and food poisoning in humans as well as in bovine toxemia and abortions in cattle. *B. licheniformis* is a regular contaminant of dairy products. However, it is mostly linked to cooked meats and vegetables. Heat-stable toxin-producing *B. licheniformis* and *B. pumilus* have been detected in milk from mastitis cows (Salkinoja-Salonen, Vuorio, Scoging, Kämpfer, Andersson & Honkanen-Buzalski, 1999). An isolate was considered toxin producing when it inhibited motility of boar sperm upon exposure to boiled bacterial suspension (≤ 4 mg wet wt. of bacteria per ml) within a 3-day exposure.

2.15.3 *Bacillus stearothermophilus*

B. stearothermophilus are often related to the contamination of dairy products, especially milk powder. They produce thermophilic spores that can withstand pasteurisation at 73 °C for 15s and can grow at 65 °C. They can cause spoilage in circumstances involving the reconstitution of milk powder. The growth of *B. stearothermophilus* during the manufacture of milk powder is considered to take place as a biofilm. Biofilms are defined as the growth of microorganisms and their extra-cellular polymeric material on a surface (Abraham, Debray, Candau & Piar, 1990**)**. *B. stearothermophilus* have been found to enhance the acidic conditions in milk and lactose solution. Their growth effects protein stability during processing which results in accelerated aggregation of milk proteins (Yoo, Hardin & Chen, 2006).

2.15.4 *Paenibacillus* spp.

Paenibacillus are psychrotolerant endospore-forming bacteria that also can cause spoilage in raw and pasteurised milk. These bacteria produce endospores that can withstand pasteurisation and can survive and reproduce at refrigeration temperatures **(**Huck, Sonnen & Boor, 2008).

Strains of *Paenibacillus* have been isolated from a variety of sources that include the soil, the rhizosphere, water, diseased insect larvae and foods. They produce endospores that can withstand industrial sterilisation and UHT processing of milk. Furthermore, they have been isolated from UHT milk alongside *Bacillus sporothermodurans* (Scheldeman, Goossens, Rodríguez-Diaz, Pil, Goris, Herman, De Vos, Logan & Heyndrickx, 2004). Examples are: *Paenibacillus lautus*, *Paenibacillus azotofixans*, *Paenibacillus polymyxa* and *Paenibacillus macerans.*

2.15.5 *Anoxybacillus spp*.

Strains of *Anoxybacillus flavithermus* alongside other *Bacillus* spp have been found in milk powders irrespective of the origin. These *Bacillus* spp can impart significant economic loss when they exceed limits set aside by regulatory authorities (Rueckert, Ronimus & Morgan, 2005).

2.15.6 Research trends on *B. sporothermodurans*.

Previous research studies on *B. sporothermodurance* have demonstrated the extrme heat resistance of it'spores (Huemer *et al*., 1998). Similarley PCR techniques that can detect *B. sporothermodurans* from both sterilized or UHT treated as well as those from non heated milk sources such as the feed (Scheldeman *et al.*, 2002). Different analyses have been conducted to determine the clonal relationship of *B sporothermodurans* isolated from different sources (Herman *et al.*, 2000). Further research need to conducted to determine the mechanism of destruction of *B. sporothermodurans* spores in order to come up with ways of inactivating them during UHT processing, using less severe heat treatment hence maintaining the sensory properties of treat milk products.