

Scientific Evaluation of Pasteurisation for Pathogen Reduction in Milk and Milk Products

**Dr Harley Juffs and
Associate Professor Hilton Deeth**



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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Disclaimer

This report, '*Scientific Evaluation of Pasteurisation for Pathogen Reduction in Milk and Milk Products*', was prepared for Food Standards Australia New Zealand, Canberra, at their request.

The report is based on refereed scientific papers and reviews from the published literature, industry reports and information provided to the consultant by a representative sample of the commercial milk processors and dairy product manufacturers throughout Australia. The authors have assumed that:

- the scientific papers we have used as sources of information accurately represent the findings of the research carried out under the conditions described in the papers;
- the scientific reviews and industry reports we have used as sources of information accurately reflect the state of knowledge at the date of their publication; and
- data provided by milk processors and dairy product manufacturers on pasteurisation conditions and related matters accurately reflect their commercial practices and technical knowledge.

The report has been prepared with due care and attention to accuracy. The authors accept no liability if, for any reason, the information contained in the report is inaccurate, incomplete or out of date. Any errors in the reporting or analysis of facts are unintended.

The authors will not be responsible for the consequences of any actions taken or decisions made on the basis of any of the information, conclusions or recommendations contained in this document.

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Executive Summary

Introduction

A study titled ‘*Scientific Evaluation of Pasteurisation for Pathogen Reduction in Milk and Milk Products*’ was commissioned by Food Standards Australia New Zealand in February 2005.

The objectives of the study were:

- Define the effect of pasteurisation on levels of pathogenic microorganisms in milk; and
- Determine how current industry pasteurisation practices compare with regulatory requirements.

The results of this study are documented in this report.

Methodology for the evaluation

Three separate but complementary activities were undertaken during the study:

- (a) A desk-top review of the available scientific literature and epidemiological data, from Australian and overseas sources, on the effect of milk pasteurisation and thermisation on the levels of pathogenic microorganisms in milk intended for either human consumption as a liquid milk product or further processing into other dairy products; and
- (b) A survey of the commercial dairy industry in Australia, with the objective of determining current industry practices for the pasteurisation of milk including the methods employed and time/temperature combinations and their relationship to minimum regulatory requirements; and

History of pasteurisation

Pasteurisation of milk as an effective public health measure is now taken for granted. However an understanding of the history of milk pasteurisation, which is long and highlighted by periods of considerable controversy, is an essential prerequisite to a scientific evaluation of the process.

Recommendations on the heating of milk in the home before it was fed to infants were recorded as early as 1824, 40 years before Pasteur’s first experiments. In 1911, the National Milk Standards Committee in the United States was the first professional body to recommend a minimum time-temperature combination for the pasteurisation of milk: 62.8°C (145°F) for 30 minutes (now known as the batch or holder method). This heat treatment was slightly above what many people at the time considered to be adequate exposure for the destruction of *Mycobacterium tuberculosis*, one of the main milk-borne pathogens of concern in that era.

However, it was not until after further research and investigation of the capabilities of the available commercial equipment, that the ‘holding method’ of milk pasteurisation was first officially and legally recognised as an adequate method of pasteurisation in the United States where, in 1924, the first Pasteurised Milk Ordinance was published. In the Ordinance, pasteurisation was defined as ‘*a heating process of not less than 142°F (61.1°C) for 30 minutes in approved equipment*’. However, it is noteworthy that a temperature 3°F lower than that which had been recommend earlier, in 1911, was officially adopted.

Following further studies on the thermal destruction of *M. tuberculosis* and other pathogens, a High Temperature Short Time (HTST) pasteurisation standard - 161°F (71.7°C) for 15 seconds - was included in the 1933 edition of the U.S. Public Health Service Milk Ordinance and Code. The effect of HTST treatment on the creaming ability of milk was also taken into account in setting this standard.

In the late 1930s, it became apparent that *Coxiella burnetii*, the causal agent of Q Fever, was more heat resistant than *M. tuberculosis* / *M. bovis*. Studies reported in 1956 showed that if *C. burnetii* cells were present in raw milk in large numbers, some might survive 143°F (61.7°C) for 30 minutes. These studies resulted in a recommendation by the U.S. Public Health Service to increase the standard for the 'holding method' of pasteurisation to 145°F (62.8°C) for 30 minutes. It was also suggested that at least an additional 5°F (2.8°C) be added to the holding temperature for products with a fat content higher than whole milk or with added sugar.

Apart from some rounding of numbers to take account of Fahrenheit-Celsius conversions, the above standards for pasteurisation have remained unchanged to the present day. According to the International Dairy Federation, the minimum time-temperature combinations now recognised world-wide are 63°C for 30 minutes or 72°C for 15 seconds.

The phosphatase test has been widely used in quality control and food safety programs as an indicator of the efficiency of the milk pasteurisation process. Alkaline phosphatase is an enzyme that is naturally present in raw milk and which, by coincidence, is inactivated when heated at 71.7°C for 15 seconds.

Thermisation is a loosely-defined sub-pasteurisation heat treatment applied to raw milk, typically in the range 62-65°C for 10-20 seconds, first introduced in the late 1950s. There are two schools of thought on its application:

- (a) To extend the storage life of the raw milk before normal pasteurisation, by controlling the psychrotrophic bacteria at an early stage. In this case, the milk is cooled to refrigerated storage temperatures immediately following the thermisation treatment, pending pasteurisation at a later date, i.e. it is not intended to be a replacement for pasteurisation.
- (b) To allow '*cheesemaking to proceed with the positive bacteriological effect of pasteurisation, but without its disadvantages for cheese ripening and whey protein degradation*'. In this case, the milk is not subjected to later pasteurisation and would usually be cooled directly to the cheesemaking temperature only. Some have argued that the application of a sub-pasteurisation heat treatment for this purpose is not 'thermisation'.

As clearly demonstrated by this evaluation, thermisation cannot be relied upon to destroy any pathogenic bacteria that might be present in the raw milk.

Requirements for pasteurisation - Australia New Zealand Food Standards Code

Standard 1.6.2 of the Australia New Zealand Food Standards Code specifies that, for the pasteurisation of milk in Australia, the minimum heat treatment is no less than 72°C for no less than 15 seconds, or any other time and temperature combination of equal or greater lethal

effect. Batch pasteurisation of milk is covered by the latter provision. Sub-pasteurisation heat treatment of milk for the manufacture of certain types of cheese is permitted under specified conditions.

Methods for determination of heat resistance and interpretation of the data

Many different techniques and types of equipment have been used to measure heat resistance of milk-borne pathogens, ranging from the very simple to the very sophisticated and from micro scale to commercial scale. However, there is ample evidence to indicate that the method used to determine heat resistance is a major factor in determining (i) the reliability of the heat resistance data generated, and (ii) its relevance to commercial pasteurisation practice. Hence methodology should always be considered when assessing the veracity of any conclusions about the ability of an organism to survive/not survive commercial heat treatments.

From a commercial perspective, it is the overall impact of the integrated heating profile, plus any other relevant system inputs, on the survival/destruction of any pathogens that may be present in the raw milk on any given day is what really counts. Other system inputs during commercial processing include turbulent flow and, in some cases, homogenization. Thus greatest weight should be given to the results of heat resistance studies carried out using actual HTST pasteurisation equipment, be it either pilot plant- or commercial-scale. Such equipment should, however, comply with recognised design and operational standards.

Ability of the nominated bacterial pathogens to survive pasteurisation

Heat resistance studies conducted using either pilot plant- and/or or commercial-scale HTST pasteurisation equipment, together with additional data from studies using various laboratory techniques, have confirmed that the vegetative forms of 11 of the 18 pathogenic species nominated for review are destroyed by both batch (63°C for 30 minutes) and HTST (72°C for 15 seconds) pasteurisation, with a reasonable margin of safety. These species are:

- *Brucella abortus*;
- *Campylobacter jejuni*;
- *Campylobacter coli*;
- *Coxiella burnetii*;
- Pathogenic *Escherichia coli* (0157:H7);
- *Listeria monocytogenes*;
- *Mycobacterium tuberculosis*;
- *Mycobacterium bovis*;
- *Salmonella enterica* serotypes;
- *Streptococcus pyogenes*; and
- *Yersinia enterocolitica*.

The situation with respect to each of the remaining seven organisms nominated for study is as follows:

- ***Mycobacterium paratuberculosis***. The heat resistance of this organism has been subject to extensive study during the past decade using various laboratory techniques and pilot scale HTST equipment. For a number of reasons, obtaining definitive heat resistance data for this organism has proved to be difficult. While there appears to be ample evidence that this organism is destroyed by batch pasteurisation, studies on the ability of *M. paratuberculosis* to survive heating at 72°C for 15 seconds, even with pilot scale HTST equipment, have given conflicting results. However, the more recent, well-

controlled studies have shown that a minimum 4-log₁₀ reduction (or 4D reduction) is obtained during HTST pasteurisation. In view of the numbers of *M. paratuberculosis* likely to be present in the raw milk, this level of kill in fact provides a reasonable margin of safety for the consumer. More generally, however, population reductions in the order of 6-7D have been reported. The fact that it is necessary for operational reasons to operate HTST equipment at temperatures slightly higher than 72°C - apart from any decision to use higher temperatures for other reasons - provides an additional margin of safety.

A fundamental question with respect to *M. paratuberculosis*, which as yet remains unanswered, is whether the organism is in fact a human pathogen, or whether its postulated association with Crohn's disease is just serendipitous, rather than causal. If studies eventually establish that there is no causal connection between *M. paratuberculosis* and Crohn's disease, any concerns that this organism might be able to survive HTST pasteurisation will prove to have been unfounded. Consideration of this issue was beyond the scope of the present review.

- ***Bacillus cereus***. Although there is limited data available specifically on the heat resistance of the vegetative form of this organism, and none using commercial HTST equipment, it is generally accepted that the vegetative cells are readily destroyed by both batch and HTST pasteurisation. However, this is to some extent academic, as there is more than ample evidence to indicate that the spores of *Bacillus cereus* are very heat resistant and readily survive any heat treatments in the normal pasteurisation range. The pasteurisation heat treatment is sufficient to heat activate the fast-germinating spores of *B. cereus*, but not the slow-germinating spores. Similarly, pasteurisation inactivates diarrhoeagenic toxins produced by *B. cereus*, but not the emetic toxin.
- ***Brucella melitensis***. No definitive data on the heat resistance of the organism (which is not endemic in Australia) were located. However general statements from authoritative sources indicate that the organism is destroyed by pasteurisation.
- ***Enterobacter sakazakii***. Although the data is somewhat variable, and data using commercial HTST equipment is lacking, the consensus view is that the heat resistance of this organism falls within the safety margins of commercial pasteurisation. Its presence in pasteurised milk products has been found to be due to re-contamination of the pasteurised product after the pasteurisation step.
- ***Staphylococcus aureus***. Although this organism has relatively high heat resistance for a mesophilic non-sporing bacterium, and despite the fact that data using commercial HTST equipment is lacking, there is ample evidence from laboratory studies that it is destroyed by both batch and HTST pasteurisation heat treatments with a wide margin of safety. However, the thermal stability of the enterotoxins produced by *S. aureus* greatly exceeds that of its vegetative cells, and readily survives pasteurisation by a wide margin.
- ***Streptococcus agalactiae***. Only one report on the heat resistance of *S. agalactiae* was located. This indicated - under relatively crude experimental conditions - that the organism was inactivated at unspecified population levels in cream by batch pasteurisation. That this is the extent of the data on the heat resistance of this organism is quite remarkable, given that it is a common cause of bovine mastitis and can be transmitted to humans, especially women, who drink raw milk.
- ***Streptococcus zooepidemicus***. Not a single report on the heat resistance of *S. zooepidemicus* was located. This is also remarkable, in view of the fact that human infection with this organism can usually be traced to an animal source, including

ingestion of unpasteurised milk and cheese. Consumption of raw milk was shown to be the source of a severe human infection with this organism in South-East Queensland about 12 years ago.

Approximately 95% of the 265 studies on heat resistance reviewed during this study, either directly or via composite data compiled by other reviewers, were conducted using cows' milk as the heating medium. Limited numbers of studies comparing heat resistance in milk from the different animal species or in different formulations of cows' milk have been reported. In most of these cases, the measured heat resistance has reflected the protective effect of fat and/or total solids content of the milk, i.e. the higher the fat and/or total solids content, the higher the heat resistance. However, the effects generally have not been dramatic and there were some exceptions.

Of the 91 papers directly reviewed during this study, only about 10% reported confidence limits for thermal death time curves and/or D values. These limits add considerable rigour to a data set, particularly when calculating margins of safety for a heat treatment.

Ability of the nominated bacterial pathogens to survive thermisation

Thermisation at 62°C for 15 seconds is generally insufficient to destroy any of the bacterial pathogens likely to be present in raw milk with a reasonable margin of safety. For 8 of the 18 species reviewed, thermisation would have no or little impact on the number of viable organisms. For 7 of the 18 species reviewed, thermisation might give a partial kill, depending upon a range of influencing factors, such as the heat resistance and numbers of the particular strains present in the milk (and, for predictive purposes, the particular D values chosen from the literature for calculation of expected kill). For the remaining three species, no data were available on which to base an assessment of impact of thermisation on them.

Identified gaps in the data on heat resistance of the nominated pathogens

The main gaps in data and knowledge - with respect to traditional thermal pasteurisation - identified during this study include:

- Definitive evidence on whether or not it is valid to classify *M. paratuberculosis* as a human pathogen; and
- Quantitative heat resistance data for *Brucella melitensis*, *Streptococcus agalactiae* and *Streptococcus zooepidemicus* in milk.

In addition, it must be noted that:

- Heat resistance data obtained using commercial HTST pasteurisation equipment appears to be lacking for the vegetative cells of several of the pathogenic species covered in this review, e.g. *Bacillus cereus*, *Enterobacter sakazakii* and *Staphylococcus aureus*;
- The available data for *Streptococcus pyogenes* is of particularly poor quality; and
- The most recent heat resistance data obtained by any method that is available for some of the pathogens is quite dated, e.g. *Mycobacterium tuberculosis* (1927), *Coxiella burnetii* (1956 & 1961), *Mycobacterium bovis* (1960), *Streptococcus agalactiae* (1974) and *Streptococcus pyogenes* (1976).

While the available data are not necessarily in dispute, it would nevertheless be reassuring to have more current data for every pathogen of concern; especially data obtained using modern commercial HTST equipment that complies with current design and operational standards.

Standardised protocols and methodologies for the determination of heat resistance appear to be lacking. Sadly, studies using methodologies known to give unreliable results, e.g. open tubes, are still being reported in the literature. Realistically, however, it would probably be almost impossible to achieve general adherence to such a protocol, particularly on an international basis, even if one did indeed exist. The next best option for an organisation like FSANZ is to establish and publicise its minimum requirements for the type of data that is acceptable for use in submissions on risk assessment studies. For example: milk to be used as the heating medium; confidence limits to be provided for kinetic data; preference to be given to data generated using commercial pasteurisation equipment where possible; and heat resistance data to be based on strains of test organisms known to occur in raw milk.

Epidemiological data on foodborne disease attributed to pasteurised milk

There is a substantial body of mainly anecdotal evidence of the public health benefits of milk pasteurisation particularly during the first half of the last century, which largely drove the eventual acceptance of the process as a mandated public health measure in many countries.

OzFoodNet, the national database for foodborne disease in Australia, has no documented reports of any outbreaks of enteric infection in Australia between 2000 and 2004 due to the consumption of pasteurised milk. However, there have been several outbreaks of enteric infection in Australia in recent years due to the consumption of unpasteurised milk. This has also been the pattern in other Western countries, such as the USA and the UK.

The detection of pathogenic microorganisms in packages of commercially-pasteurised milk is alone insufficient evidence that the organisms are resistant to the pasteurisation heat treatment. There are well-documented cases in which it has been shown that the presence of pathogens in pasteurised milk has been due to inadequate pasteurisation (eg faulty equipment or poor process control) or to re-contamination of the milk after the pasteurisation step in the processing line (eg ineffective sanitising of the equipment). Further investigation of the circumstances surrounding such incidents is always necessary.

Times and temperatures used for the pasteurisation of milk in Australia

From the Australia-wide industry survey conducted during this study and additional data from a survey of the Victorian dairy industry by Dairy Food Safety Victoria in 2004, it was clear that batch pasteurisation is widely used in Australia. However, as the batch method is mainly used by the smaller processors, it would account for only a very small percentage of all milk pasteurised in Australia. Temperatures and times of heat treatment for batch pasteurisation covered a wide range, from 62 to 90°C and from 15 seconds to 30 minutes. Type of product being manufactured was a major influence on the temperature-time combination used.

Several processors reported using what is essentially a HTST treatment, e.g. 72°C for 15 seconds or similar, under batch conditions.

All of the temperatures and times for the pasteurisation of milk by the HTST method reported during the industry surveys showed that the minimum heat treatment for HTST pasteurisation as specified in the Australia New Zealand Food Standards Code, i.e. 72°C for 15 seconds, was being achieved by all respondents to the surveys. Beyond that, however, temperatures ranged from 72-86°C and times from 15-50 seconds, with many different combinations within those ranges. As with batch pasteurisation, type of product was again a major influence on the

heating regime used, with the time and temperatures reported generally being within the expected range for the type of product.

Of particular note is that HTST treatment of milk for liquid milk products, at least by most of the large processors and some of the smaller ones, was mostly in the range 74-78°C for 15-30 seconds. This reflects a recommendation by the peak Australian dairy industry organisation in 2000 that the times and temperatures for HTST pasteurisation of milk for the liquid milk trade be increased as a precaution against the presence in the raw milk of any *Mycobacterium paratuberculosis* organisms that might be resistant to the minimum pasteurisation treatment of 72°C for 15 seconds. Whether use of this enhanced heat treatment is still warranted in the light of more recent studies on the heat resistance of this organism that have been conducted using commercial HTST equipment (refer Section 3.3.8 of this report), particularly in areas of Australia where Johne's disease in cattle is reported to be not endemic, is a matter for conjecture.

Only one processor reported that they were using the 62°C for 15 seconds heat treatment option for cheese milk permitted in the Australia New Zealand Food Standards Code.

Some processors, particularly those in the small and medium size categories, reported that design of their pasteurisers and operational considerations largely dictated the limits on the times and temperatures of heating that they could use in practice.

Overall conclusions with respect to traditional pasteurisation treatments

It is concluded that consumers of pasteurised milk and dairy products in Australia can be assured that pasteurisation continues to be a very effective public health measure. Three complementary observations allow this conclusion to be drawn:

- (a) Ample heat resistance data to indicate that the vegetative cells of the most significant milk-borne pathogens are destroyed by pasteurisation, with a reasonable margin of safety [though it is recognised that there are still some gaps in the data for some organisms and that there are other forms (eg spores) or products (eg toxins) of some species that can withstand pasteurisation];
- (b) With a small number of exceptions (which are related more to process control issues or the interpretation of what constitutes an equivalent treatment, rather than significant deficiencies in the actual times and temperatures used), pasteurisation of milk and cream in Australia meets the minimum time and temperature standards prescribed in the Australia New Zealand Food Standards Code, or recognised equivalents; in many cases, the product is heated to a temperature and/or a time often well in excess of the prescribed minimums; and
- (c) Lack of epidemiological data indicating that pasteurised milk products have been implicated in any outbreaks of foodborne gastrointestinal illness in Australia in recent years whereas, in contrast, such outbreaks continue to be associated with consumption of raw milk, both in Australia and in other countries.

1. Introduction

Food Standards Australia New Zealand (FSANZ) is developing a Primary Production and Processing (PPP) Standard for Dairy.

FSANZ envisages that the Dairy PPP Standard will establish a nationally consistent standards framework, which will ensure that all milk and milk products manufactured throughout Australia are produced to the same rigorous level of safety.

The scope of the Dairy PPP Standard will encompass not only the on-farm production of raw milk and its transport to the primary processing facility, but also its processing into a wide variety of value added dairy products for sale in Australia and overseas.

With few exceptions, pasteurisation of milk is an integral step in its processing. Despite milk pasteurisation's long history as a successful public health measure, the effectiveness of the traditional thermal pasteurisation process in terms of food safety outcomes remains a central issue for FSANZ, particularly as 'new pathogens' emerge or as 'old pathogens' are reassessed.

To assist it with its task of confirming that traditional thermal pasteurisation continues to be an effective food safety measure, FSANZ commissioned a scientific evaluation of pasteurisation for pathogen reduction in milk and milk products.

Objectives and scope of the scientific evaluation

The objectives of the scientific evaluation as outlined by FSANZ were as follows:

- Define the effect of pasteurisation on levels of pathogenic microorganisms in milk; and
- Determine how current industry pasteurisation practices compare to regulatory requirements.

More specifically, the scientific evaluation was to conduct of a review of the current state of knowledge on milk pasteurisation, as defined in Standard 1.6.2 of the *Australia New Zealand Food Standards Code*, with particular reference to:

- (a) its effect on levels of pathogenic microorganisms; and
- (b) current industry practice in Australia (including pasteurisation methods employed and time/temperature combinations and their relationship to minimum regulatory standards).

The review of the scientific literature and available data conducted in fulfillment of activity (a) as stated above, was to cover the effect of thermal pasteurisation methods on levels of pathogenic microorganisms in milk. In particular, thermal death times for the pathogens in milk at the temperatures commonly employed in pasteurisation were to be described.

The scope of the evaluation was further defined by FSANZ, or by agreement with them, in the following terms:

- (a) The microorganisms nominated by FSANZ as being of concern to them were: *Bacillus* spp., *Brucella* spp., *Campylobacter jejuni/coli*, *Coxiella burnetii*, *Enterobacter sakazakii*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium* spp., pathogenic *Streptococcus* spp., *Staphylococcus aureus*, *Salmonella* spp. and *Yersinia enterocolitica*.

- (b) The evaluation was to apply to milk derived from all of the commercial animal species currently utilised in Australia, i.e. cattle, goat, sheep and buffalo, and possibly camel.
- (c) The main focus of the study was to be on the application of ‘traditional’ pasteurisation methods to liquid milk and liquid milk products intended either for direct human consumption or for use in the production of other dairy products such as yoghurt, dried, evaporated and condensed milks, ice cream and cheese. Other processes for the heat treatment of milk and other dairy products such as extended shelf-life (ESL)/ultra high temperature (UHT) treatment of milk and vacreation of cream for butter making were excluded from the scope of the evaluation.

Methodology

The methodology for the conduct of the scientific evaluation was broadly as follows:

- (a) Two separate desk-top reviews of the available scientific literature and epidemiological data, from Australian and overseas sources, with the objective of determining:
 - (i) The effect of milk pasteurisation, as currently defined in Standard 1.6.2 of the Australia New Zealand Food Standards Code, and thermisation on the levels of pathogenic microorganisms in milk intended for human consumption or further processing into dairy products; and
 - (ii) The current state of knowledge on the application of alternative processes for the destruction of pathogenic organisms to foods generally, their effect on levels of pathogenic microorganisms and their potential application to the processing of milk.
- (b) A survey of the commercial dairy industry in Australia, with the objective of determining:
 - (i) Current industry practices for the pasteurisation of milk including the methods employed and time/temperature combinations and their relationship to minimum regulatory requirements; and
 - (ii) Industry interest in and concerns about alternative processes and methods for the treatment of milk that potentially deliver food safety outcomes that are equivalent to those obtained with the thermal methods currently used for the pasteurisation of milk on a commercial basis.

Organisation of the report

This report contains the literature review and the results of the industry survey.

2. Pasteurisation of Milk by Traditional Thermal Processes

2.1 Review of the history of milk pasteurisation and thermisation

Pasteurisation of milk

The pasteurisation of milk has a long and interesting history. Interestingly, destruction of pathogenic microorganisms was not the driving force behind the early commercial versions of the process; the impact of the heating of raw milk on public health was only to become apparent in later years, essentially as a ‘spin-off’ benefit.

The history of heating milk to destroy microorganism was outlined by Westhoff in 1978, largely from a United States’ perspective. The main points from Westhoff’s outline, which covers successive 25-year time periods, are summarised below. Additional information from other sources is inserted as indicated.

Before 1900. William Dewees recommended heating of milk as early as 1824, 40 years before Pasteur’s first experiments. He suggested that milk be heat treated in the home before infant feeding, by bringing it just to the point of boiling and cooling it quickly. According to Dewees, the advantages of this treatment were to increase its shelf life, stating ‘*the tendency to decompose is diminished in the milk*’. Also before Pasteur’s experiments was the contribution of Gail Borden, who obtained a patent in 1853 for the preservation of milk by a process involving heating, condensing and the addition of sugar. However, the fact that heating of milk resulted in microbial destruction was not appreciated until Pasteur’s work on the spoilage of wine in 1864-65. Pasteur demonstrated that heating of wine to 50-60°C prevented abnormal fermentation by destroying the undesirable microorganisms that were causing the spoilage. He made similar studies in beer in 1871-72 and also demonstrated that the souring of milk was due to the growth of undesirable microorganisms (Staal, 1986).

In-bottle pasteurisation of milk at low temperatures for infants came into use in the late 1800s. For example the method of Gerber, which involved subjecting the bottled milk to a temperature of 65°C for one hour, was reported to be in use in 1888.

The first continuously-operating apparatus for the preservation of milk by heating was developed in Germany in 1882. It heated milk to 74-77°C for an unspecified time. In 1884 a Danish dairy scientist, Fjord, received credit for a similar apparatus that heated milk to 70°C. Pasteurisation of milk on a commercial scale using continuous heaters was a common practice in Denmark and Sweden during the mid-1880s. However, heaters of greater heating capacity were quickly developed, as it was required in Denmark that milk be heated to 85°C to prevent the spread of tuberculosis.

An apparatus, known as the Danish pasteuriser, that momentarily heated milk to 85°C was first imported into the United States in 1895. Prior to this, in 1893, some philanthropists established a network of milk depots throughout New York City, where milk was pasteurised in-bottle at 75°C for 20 minutes with a 10 minute come up time and rapid cooling under running water. The benefits of this program were widely promoted and ‘the message of pasteurisation’ was spread throughout the United States and Europe.

Despite lack of compulsory pasteurisation requirements in the United States, heat treatment of milk on a large scale was becoming increasingly popular in the 1890s, since it was recognised by dairy microbiologists and dairy processors as a way of increasing the shelf life of milk. However, objections to the process were raised and commercial pasteurisation of milk was not generally accepted at that time. Some milk companies were forced to adopt the process secretly.

Several milk-borne diseases were recognised before 1900 including typhoid, diphtheria, scarlet fever, tuberculosis and anthrax (and also foot and mouth disease of cattle and some other animals). However, information on the destruction of pathogens by heat before 1900 was limited and variable.

Pasteur's work explained the role of microorganisms in causing undesirable changes in foods. Many of the workers following Pasteur applied heat treatment of milk to kill microorganisms, some recognizing it as a way to prevent milk-borne disease and others as a way to increase the shelf life of milk.

1900-1925. In 1902, 5% of New York's milk supply was pasteurised; by 1914, this figure had increased to 88%. At the turn of the century, low temperature heat treatments (71°C or less) were accepted by many investigators as being effective for destruction of pathogenic microorganisms. Medical authorities, however, opposed commercial pasteurisation of milk, saying it should be done in the home to avoid further post-treatment contamination. Despite growing evidence of the public health benefits of pasteurisation, e.g. a demonstrated reduction in the mortality and incidence of infant diarrhea, criticism continued to be leveled at some forms of pasteurisation, particularly the so-called 'flash' methods. These methods usually employed a temperature of 71°C for 30-60 seconds, but were unreliable. A low-temperature holding method of 60°C for 20 minutes was much more acceptable to the medical profession. In response, a large scale 'holding method' pasteuriser that heated milk at 60-66°C for 30-45 minutes was installed in New York in 1907. This idea spread rapidly and many of the 'flash pasteurisers' were converted to pre-heaters for the holding method (batch) pasteurisers. In many of these units, milk was held at 65°C for 20-30 minutes.

Public health authorities were in a dilemma, caught between opposition to heat treatment of milk on the one hand and a barrage of reports on the thermal destruction of milk-borne pathogenic bacteria on the other. For example, between 1883 and 1906, no less than 26 reports appeared in the literature on the thermal death times of *Mycobacterium tuberculosis*, using a wide range of times and temperatures. Between 1890 and 1927, at least 31 different time and temperature combinations for adequate pasteurisation were recommended.

In 1911, the National Milk Standards Committee in the United States, a credible body, recommended a time-temperature combination of 62.8°C (145°F) for 30 minutes for the pasteurisation of milk. This was slightly above what many considered to be adequate exposure for the destruction of *M. tuberculosis*, viz. 60°C for 20 minutes. However, an expert committee reported that none of the 4,200 pasteurisation plants in the United States at that time afforded full protection against ineffective pasteurisation, and also recommended 62.8°C (145°F) for 30 minutes. Subsequent extensive engineering and microbiological studies under the direction of Dr Charles E North initiated in 1921 highlighted a range of deficiencies in the equipment in use at the time.

That the 'holding method' represented an adequate method of pasteurisation was first officially and legally recognised in the United States with the publication of the first pasteurised milk ordinance in 1924. This defined pasteurisation as a '*heating process of not less than 142°F (61.1°C) for 30 minutes in approved equipment*'.

1925-1950. The numerous and often conflicting reports on the thermal destruction of *M. tuberculosis* were finally clarified by the work of North and Park (1927), who reviewed the major papers that had been published on the thermal destruction of *M. tuberculosis* to that date. Their data confirmed the work of several earlier investigators and supported the recommendation of 142°F (61.1°C) for 30 minutes as providing an ample margin for the destruction of *M. tuberculosis*.

Although the 'holding method' of pasteurisation was still widely used during the 1920s and early 1930s, work was underway on the effects of higher temperatures and shorter times on the destruction of *M. tuberculosis*. Plate heat exchangers were being developed and used for high temperature-short time methods, which evolved into the current HTST method of pasteurisation. The technology developed rapidly. Following further studies on the thermal destruction of *M. tuberculosis* and other pathogens, a HTST standard - 161°F (71.7°C) for 15 seconds - was included in the 1933 edition of the U.S. Public Health Service Milk Ordinance and Code. The effect of HTST treatment on the creaming ability of milk was also taken into account in setting the standard.

The rickettsia responsible for Q-fever, *Coxiella burnetii*, was first described by Derrick in 1937, in Australia. Derrick also reported that it could be isolated from raw milk. Subsequent studies of Q-fever in California revealed that this organism was more heat resistant than *M. tuberculosis*, and that it could be isolated from pasteurised milk that had been processed according to the then recommended standards.

1950-1975. Work by Enright *et al.* (1956) showed that if *C. burnetii* was present in raw milk in large numbers, some might survive 143°F (61.7°C) for 30 minutes. This study resulted in a recommendation by the U.S. Public Health Service to increase the standard for the 'holding method' of pasteurisation to 145°F (62.8°C) for 30 minutes. It was also suggested that at least an additional 5°F (2.8°C) be added to the holding temperature for products with a fat content higher than whole milk or with added sugar, i.e. 150°F (65.6°C).

The thermal death time curve for *M. tuberculosis* var. *bovis* was later updated by Kells and Lear (1960). Though their data differed from earlier data of other workers for *M. tuberculosis* (see Section 2.3.8), they concluded that heat treatment at 61.7°C for 30 minutes provides a margin of safety of approximately 28.5 minutes for *M. tuberculosis* var. *bovis* (ie organism killed in about 1.5 minutes at 61.7°C), and that a heat treatment at 71.7°C for 15 seconds provides a margin of safety of approximately 14 seconds (ie organism killed in about 1 second at 71.7°C).

The minimum time-temperature combinations recommended by the International Dairy Federation are 63°C for 30 minutes or 72°C for 15 seconds (Burton, 1986). The Federation's definition of pasteurised milk also includes requirements that the product is cooled without delay after heat treatment, is packaged with minimum delay to minimise contamination and gives a negative phosphatase test result immediately after the heat treatment process (Cerf, 1986).

The phosphatase test has been a key element of quality control and food safety programs in the dairy industry. Its methodology, application and interpretation were the subject of much investigation in earlier years (see box for more information on the test).

Phosphatase Test

According to the International Dairy Federation (Anon., 1991), the alkaline phosphatase test developed by Kay and Graham in 1935 is still the most suitable method for control of adequate pasteurisation at 71.7°C (72°C). Alkaline phosphatase is one of the enzymes naturally present in raw milk. By coincidence, it is inactivated when heated at normal High Temperature Short Time (HTST) conditions (71.7°C for 15 seconds).

A simple test for residual alkaline phosphatase is widely used internationally as the confirmatory test to demonstrate that milk has been correctly pasteurised and that, once pasteurised, the milk has not been recontaminated with raw milk. The requirement that pasteurised milk and pasteurised liquid milk products give a negative phosphatase test result was written into most if not all State dairy legislation in Australia, at least prior to 1987 when the original Food Standards Code (now known as Volume 1) developed by the Australia New Zealand Food Authority was gazetted. Volume 1 of the Food Standards Code stated, in Clause 6 of Standard H1, Milk and Milk Products, that '*Pasteurised milk and pasteurised liquid milk products shall not exhibit a phosphatase activity in excess of that required to give a reading of 10µg/mL of p-nitrophenol when tested by the current standard method in AS 2300, Methods of Chemical and Physical Testing for the Dairying Industry*'. However, the current Australia New Zealand Food Standards Code (Volume 2), which was gazetted in December 2000 and now administered by Food Standards Australia New Zealand, does not contain any reference to the phosphatase test or any similar test.

Kay and Graham developed the original test in 1935, based on release of free phenol from phenylphosphate over a 24 hour incubation period. In 1949, Aschaffenburg and Mullen developed a more rapid and simpler test, using p-nitrophenylphosphate as the substrate. Any residual alkaline phosphatase released p-nitrophenol, a yellow substance. The amount of this substance in the milk can be determined semi-quantitatively with a simple colour comparator.

Typical cow's milk shows a phosphatase activity equivalent to 3,500 µg phenol per mL and goats' milk about 600 µg phenol per mL. A residual alkaline phosphatase level of less than 10µg P-nitrophenol equivalent per mL is taken to indicate that milk has been correctly pasteurised and has not been contaminated with raw milk. This is equivalent to 0.3-0.5% of raw milk.

Modifications of the method are necessary for goats' milk. Cream can be tested if diluted. Some coloured milks, e.g. chocolate, mask the yellow colour and alternative though more complex methods are available for such products. Reactivation of the alkaline phosphatase can occur in some products, especially cream, if they are exposed to temperatures above 20°C during storage and magnesium ions are present.

The relationship between the minimum time-temperature combinations recommended by the International Dairy Federation and a summation of conditions for the inactivation of *M. tuberculosis* prepared by Dahlberg (1932) is illustrated in Figure 2.1. This relationship has stood the test of time and is still valid today for this organism.

It is of interest that the impact of heating milk on its creaming capacity ('cream line' – refer box) was a major concern during the evolution of the milk pasteurisation process. This was of course in a period when milk was not homogenized and it was packaged in clear glass bottles. The thickness of the cream layer in the neck of the bottle after standing for a period was promoted as a measure of its 'quality' by the dairy companies. Hence any diminution of the thickness of the cream layer as a result of heating was of serious concern to them. To demonstrate that heat treatments for pasteurisation could be selected that would ensure destruction of *M. tuberculosis* without loss of cream layer, Dahlberg (1932) constructed a chart showing the relationship between the thermal death time curve for *M. tuberculosis*,

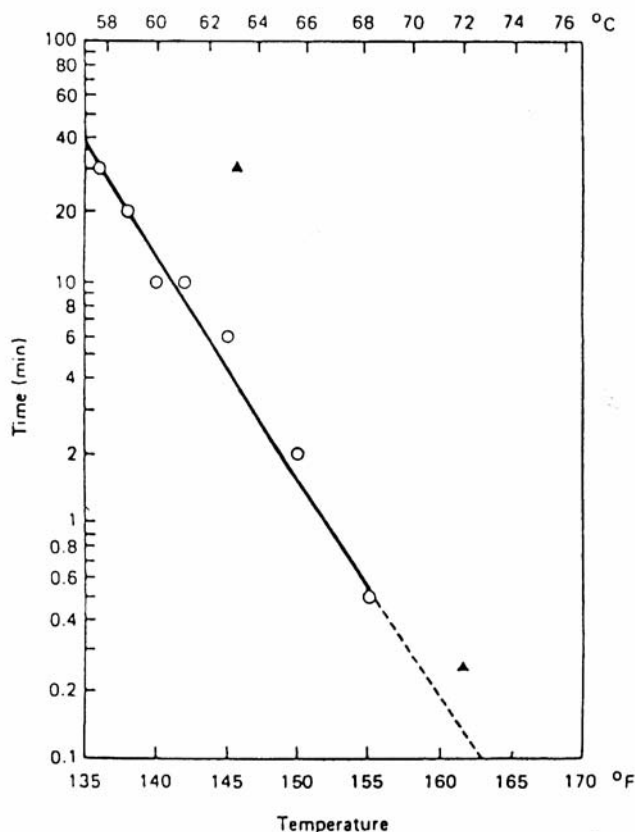


Figure 2.1. Processing times and temperatures required to inactivate *Mycobacterium tuberculosis*, based on the data of Dahlberg (1932) (○) and the minimum heat treatments for pasteurisation recommended by the International Dairy Federation (▲) [after Burton (1986)].

the points below which cream layer was not reduced and the pasteurisation standards as recommended at that time. Conveniently, the latter line fitted neatly between the other two (refer Figure 2.2).

Cream Line

According to Wilson (1942a), the pasteurisation of milk (whole and non-homogenised) affects the size of the fat globules in a way that leads to a diminution in the proportion of the globules that rise to the surface on standing. The average reduction in the cream line of milk heated to 62.8-63.3°C for 30 minutes is 8%, and in milk heated to 64.4°C for the same period, 31%. However, cooling to low temperatures after pasteurisation partially preserves the cream line, e.g. cream line diminution was found in fact to be very small in 'modern' batch pasteurisation plants in which milk was heated to 62.8-63.3°C for 30 minutes then cooled rapidly to 4.4°C. Fortunately, later studies showed that, at temperatures in the range 60-76°C, the commencement of reduction in creaming capacity and the inactivation of phosphatase coincided very closely. For example, it was shown that heating milk at 71.1°C for 15 seconds destroyed the phosphatase without impairing the creaming capacity (Wilson, 1942c).

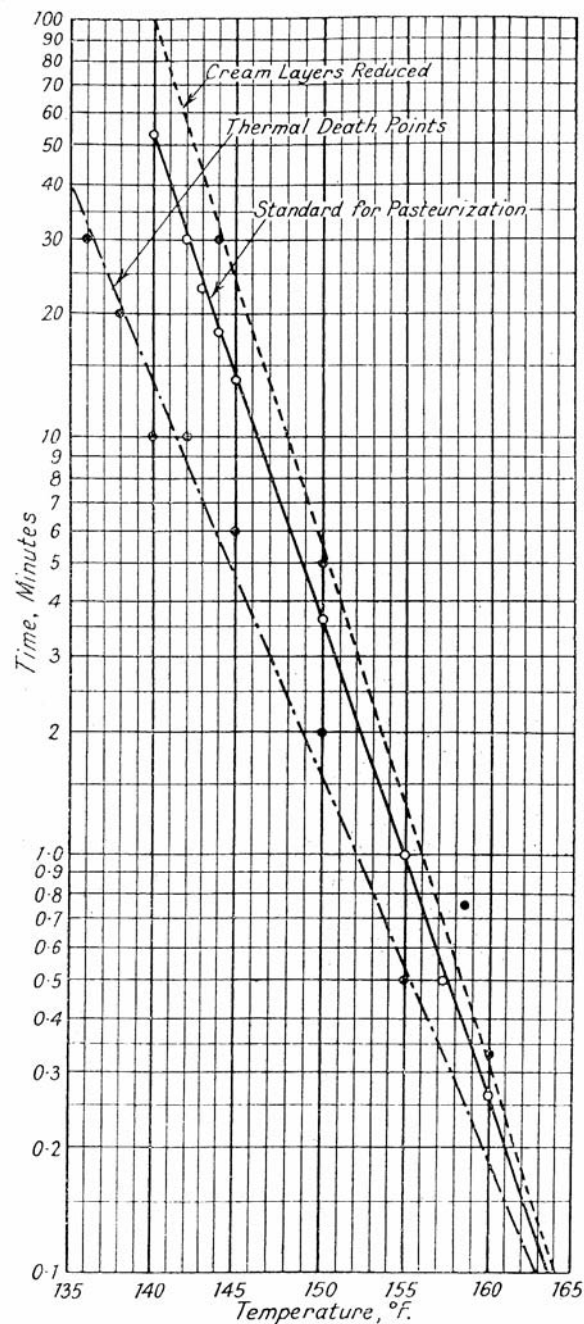


Figure 2.2. Relationship between the thermal death curve for *Mycobacterium tuberculosis*, points below which cream layer was not reduced and the pasteurisation standards as recommended in 1932, according to Dahlberg (1932) [after Wilson (1942b)].

The current Australian requirements for milk pasteurisation are set out in Standard 1.6.2 of the Australia New Zealand Food Standards Code (refer Extract at Attachment 1). The minimum heat treatment specified is no less than 72°C for no less than 15 seconds, or any other time and temperature combination of equal or greater lethal effect.

During the latter half of the last century, i.e. 1950-2000, the focus of research on the pasteurisation of milk moved away from the destruction of pathogens to the maintenance of organoleptic quality and the extension of shelf life. The safety of pasteurised milk - in the context of milk-borne pathogens - was largely taken for granted. For example, Burton (1986) observed that there were no

recorded cases of food poisoning from microorganisms surviving the pasteurisation process, provided it had been properly carried out in accordance with the recommendations of the International Dairy Federation. To support his observation, Burton stated that in England and Wales between 1951 and 1982, there were 213 recorded outbreaks of food poisoning arising from the consumption of raw milk, compared with just 6 from consumption of pasteurised milk, with the latter product accounting for 90-95 % or more of the milk consumption on an annual basis. Four of the six outbreaks associated with pasteurised milk were found to be due to inadequate pasteurisation, contamination of pasteurised milk with raw milk or contamination during packaging. The other two outbreaks were of unknown origin.

It has only been in more recent years, with the emergence of relatively heat-resistant microorganisms of known or possible public health significance, such as *Listeria monocytogenes* and *Mycobacterium avium* subsp. *paratuberculosis*, that the focus has switched back to the adequacy of the pasteurisation heat treatment.

There is however, no doubt that pasteurisation of milk has made an enormous contribution to the public health of populations around the world. During the first half of the last century (and presumably for many centuries prior to that), consumption of raw milk was clearly a dangerous practice (Pearce, 2002). To support his assertion, Pearce quoted data indicating that in the decades between 1900 and 1929, consumption of raw milk in the United States accounted for 68-78% of all cases of typhoid fever, 14-18% of streptococcal infections and 2-8% of diphtheria, plus an unknown number of cases of tuberculosis due to the presence of *M. tuberculosis* and *M. bovis* in raw milk.

Similarly, Wilson in his treatise '*The Pasteurization of Milk*', published in 1942, demonstrated that there had been a direct correlation between a dramatic reduction in the incidence of pulmonary tuberculosis in children in England due to *M. bovis* and the availability of pasteurised milk during the period 1911-1937 (Wilson, 1942c).

Thermisation of milk

According to Van den Berg (1984), the first mention of the term 'thermisation' in the literature was by Casalis in 1958. Casalis was reported to have been in search of a heat treatment for cheese milk with the positive bacteriological effect of pasteurisation, but without its disadvantages for cheese ripening and whey protein degradation. The process of thermisation was defined by Casalis in the following terms:

'Thermisation is a mild heat treatment to destroy the normal bacterial flora and to give a suitable environment for the multiplication of selected starter cultures. A limiting heat treatment of 57-68°C might be accepted.'

However, Van den Berg reported that by 1984, heat treatment of cheese milk was common and that this kind of treatment was not usually referred to as 'thermisation'. Rather, the process of thermisation had instead come to mean a pre-treatment of raw milk that allowed it to be stored for longer periods before processing. In this context, Stadhouders *et al.* (1962), cited by Van den Berg, referred to thermisation as the inflow treatment of raw milk at 63-65°C for 15-20 seconds.

According to Van den Berg, the thermisation process referred to by Stadhouders was not intended to replace pasteurisation. Its purpose was to extend the storage life of the milk before pasteurisation, by controlling the psychrotrophic bacteria at an early stage. Before the 'thermised' milk was used for cheesemaking, it was pasteurised normally. Van den Berg reported that pre-treatment of milk for cheesemaking was a common practice in the Netherlands, as it allowed

cheesemaking to proceed at a constant rate during the week. Van den Berg concluded that thermisation is an in-flow treatment of raw milk at 62-65°C for 10-20 seconds, immediately followed by cooling to refrigerated storage temperatures. Van den Berg further concluded that thermisation does not fulfill the requirements of pasteurisation in terms of minimising health hazards by inactivating pathogenic bacteria. An upper limit of 65°C for 20 seconds should leave sufficient residual alkaline phosphatase in the thermised milk to ensure that a phosphatase test on it will be positive, indicating that the milk is unpasteurised.

Pearce (2003, 2004) confirmed that thermisation in the range 63-65°C for 10-15 seconds is not sufficient to significantly reduce the population of the more heat resistant vegetative bacterial pathogens. This is illustrated by the only published data that Pearce could locate on the heat resistance of bacterial pathogens in milk at thermisation temperatures under turbulent flow conditions. His summary of this data is reproduced in Table 2.1. (Also refer to data on individual pathogens in Section 2.3 of the report.)

Table 2.1. Pathogen kill at thermisation temperatures using a turbulent-flow pasteuriser with a holding time of 16.2 seconds [after Pearce (2003, 2004)*].

Pathogen	No. of strains used	Log-kill at specified temperatures			
		60°C	63°C	64.5°C	66°C
<i>Listeria monocytogenes</i>	10	No change	1-2	2-3	4-5
<i>E. coli</i> 0157:H7	15	2	1-3	>6	na
<i>Yersinia enterocolitica</i>	15	4-5	>5	na	na
<i>Campylobacter</i> spp.	15	4-5	>5	na	na
<i>Salmonella</i> spp.	7	3-5	>5	na	na
<i>Salmonella</i> Seftenberg	2	2-3	4	4	5

* Sources of original data cited by Pearce: D'Aoust *et al.* (1987, 1988); Farber *et al.* (1988).

Pasteurisation of cream

According to Bogh-Sorensen (1992), cream pasteurisation can be defined as heat treatment process applied to cream with the aim of minimizing the public health hazards arising from pathogenic microorganisms. Additional objectives of the heat treatment are to minimise any chemical, physical and organoleptic changes in the cream and to extend the product's shelf life by reducing the number of microorganisms and deactivating enzymes harmful to the cream.

Bogh-Sorensen defined cream as a milk product with an increased milk fat content, ranging from 10-48%, depending on the product. Typical minimum time-temperature combinations needed to give a negative phosphatase test are as follows:

- Holding (batch) method: 65°C for 30 minutes;
- HTST method for cream with 10-20% fat: 75°C for 15 seconds; and
- HTST method for cream with >20% fat: 80°C for 15 seconds.

The Australia New Zealand Food Standards Code does not specify a minimum time and temperature combination for the pasteurisation of cream. However, Standard 1.6.2 does specify that milk and liquid milk products used in the production of cream and cream products must be heated using a combination of time and temperature of equal or greater lethal effect on the bacteria in liquid milk than would be achieved by pasteurisation by heating to a temperature of no less than 72°C for no less than 15 seconds or otherwise produced and processed in accordance with any applicable law of a State or Territory.

2.2 Review of the issues impacting on the determination and interpretation of heat resistance data for pathogenic micro-organisms in milk at temperatures commonly employed in pasteurisation

Terminology and definitions used to define heat resistance of microorganisms

Olson and Nottingham (1980), as contributors to a text published under the auspices of the International Commission on Microbiological Specifications for Food, summarised the basic concepts and kinetic principles involved in the destruction of microorganisms by heat. The following paragraphs are based on that summary unless indicated otherwise.

As the temperature increases above that at which growth of microorganisms ceases, injury and, inevitably, death occurs. Sub-lethal or mild exposures to heat induce stress that may cause injury. Injured cells may remain viable but are unable to reproduce until the damage is repaired¹. At higher temperatures, a homogeneous population of a pure culture will begin to decrease in an orderly manner with time, due to death of individual cells. While exceptions have been noted (see below), it is well established that the order of death is essentially logarithmic. This makes it possible - at least from a theoretical point of view - to develop heat processes with defined time and temperature parameters that will assure with precision the destructive effect against microorganisms.

A typical bacterial 'survivor curve' showing the logarithmic (or exponential) rate of destruction is illustrated in Figure 2.3. When the logarithm of the number of survivors is plotted against time of exposure at a given temperature, the 'curve' will in fact be a straight line, provided of course the population exhibits ideal behaviour and the methodology used to generate the data is error-free. Under defined conditions, the rate of death is constant at any given temperature and independent of the initial number of cells in the population. From such a straight line 'curve', the decimal reduction time or D value - which is the time in minutes required to destroy 90% of the residual population - can be determined.

D values should state the temperature (°C) in a subscript. For example, assuming the hypothetical data set represented in Figure 2.3 was obtained at 65°C, the D value would be expressed as $D_{65\text{C}} = 5$ minutes (ie when heated at 65°C, it would take 5 minutes for the residual viable population to decline from 10^7 per mL to 10^6 per mL, another 5 minutes to decline from 10^6 per mL to 10^5 per mL, and so on). Theoretically, the straight line survivor curve extends beyond the baseline shown in Figure 2.3 and eventually into the area of negative logarithms, e.g. $\log_{10}^{-2} = 1$ survivor per 100 mL, $\log_{10}^{-3} = 1$ survivor per L, and so on. Thus, in theory, the bacterial population might never be totally eliminated.

It is possible, therefore, to predetermine a heat process that will result in a specified level of destruction. For example, a product containing an organism with a heat resistance given as $D_{65\text{C}} = 5$ minutes would have to be held at 65°C for 30 minutes to reduce the population of that organism in the product from an initial level of 10^6 per mL to a final level of 10^0 per mL. In this example, the figure computed is effectively the thermal death time, which has been defined by Brock *et al.* (1994a) as the time in which all cells in a bacterial suspension are killed at a given temperature [assuming of course that in this case the limit of detection is in fact 10^0 (ie <1) per mL].

¹ There is an extensive literature on the injury and repair of damaged cells. However this aspect of heat treatment is beyond the scope of the present study and the topic will not be further addressed in this report.

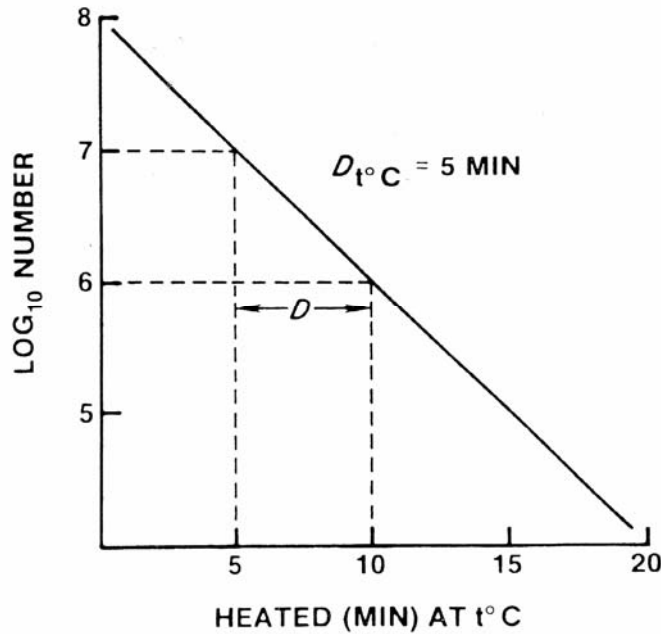


Figure 2.3. Graphical illustration of the decimal reduction time, D , for a hypothetical bacterial population exhibiting a logarithmic rate of destruction over time when heated at a given temperature, i.e. a straight line survivor curve [after Olson and Nottingham (1980)].

Thermal death times are dependent on the initial size of the test population, since the larger the population, the longer the time required to kill all of the cells in it. Thermal death times are only comparable when the numbers of cells in the test populations have been standardised (Brock *et al.*, 1994a). Similarly, for D values to be useful in commercial operations, the upper limit of the population in the product before heating at the temperature applicable to that value must be known or assumed and the target level to be achieved after heating also must be specified.

While the D value is a measure of heat resistance of bacteria at a given temperature, points on a thermal death time curve indicate the relative resistance at different temperatures. Relative heat resistance at different temperatures can be calculated from such a curve. Thermal death time curves can be constructed in several ways. A convenient method, illustrated in Figure 2.4, is to plot the logarithms of the D values against exposure temperatures. The inverse of the slope of the resulting curve is expressed by the term, z , which is the number of $^{\circ}\text{C}$ required for the thermal death time curve to traverse one log cycle of time, i.e. the change in temperature required to increase or decrease the length of the heat treatment (in practice, the holding time) by a factor of 10.

In the example illustrated in Figure 2.4, where $z = 15^{\circ}\text{C}$, increasing the temperature of heating from 80°C by 15°C to 95°C would reduce the holding time required to achieve the same lethal effect from 10 minutes to 1 minute.

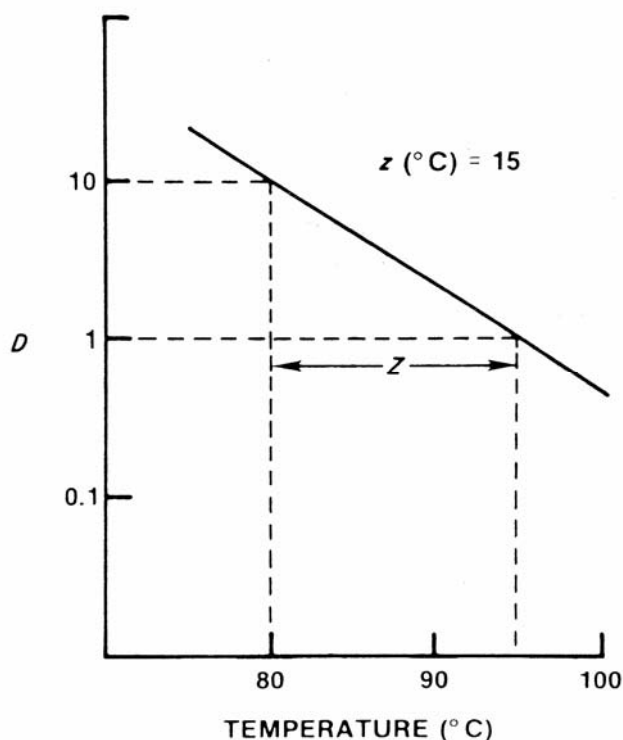


Figure 2.4 Graphical illustration of a thermal death time curve for a hypothetical bacterial population. D = decimal reduction time. Z = the number of $^{\circ}\text{C}$ required for the thermal death time curve to traverse one log cycle of time [after Olson and Nottingham (1980)].

The total integrated lethal effect of a heating process can be expressed as the F value (Zall, 1990). The concept is used mainly in relation to the design of canning or similar processes, where the protocol is to apply sufficient heat to destroy an assumed population of 10^{12} *Clostridium botulinum* spores per g of product. In this case, F_0 is defined as the lethal effect on the spores equivalent to that obtained by heating at 121°C for three minutes (Szabo and Gibson, 2003). F values can be used in other contexts, but the method of calculating the values must be defined in each case. For example, Holsinger *et al.* (1992) calculated F values for *L. monocytogenes* in various ice cream mixes heated at 60°C ranging from 18.8 to 37.3 minutes, depending upon the mix. In this case, Holsinger *et al.* defined the F value as ' $F = 7D + \text{lag period}$ '. However, as will be evident throughout this review, F values have generally not been used in relation to milk pasteurisation.

Another concept, no longer in general use, is the thermal death point. This referred to the lowest temperature at which a suspension of bacteria is killed in 10 minutes. Its use has largely ceased because it was population-dependent and the term 'point' gave the impression that death occurred at a particular point in time, whereas it in fact occurs over a period of time, as outlined above (Pelczar and Reid, 1965).

Factors affecting heat resistance of microorganisms

Olson and Nottingham (1980) also reviewed the factors affecting heat resistance of microorganisms. They found, along with others, that because of the many variables involved in heat resistance studies, e.g. type of organism studied and the technique used, sound interpretation of previously reported data is often difficult. They concluded that the factors affecting heat resistance are of three general types:

1. Inherent resistance, e.g. differences between species, between strains within the same species and between spores and vegetative cells;

2. Environmental influences during growth of cells or formation of spores, e.g. age of cells or culture, growth temperature and growth medium; and
3. Environmental influences during the heating process to determine heat resistance of the cells or spores, e.g. pH and composition of the suspension menstroom or food (fats, salts, etc).

Evidence of death is also a consideration. Post-heating handling and cultural conditions can influence results, the latter being an apparent issue, for example, with the determination of the heat resistance of *Mycobacterium avium* subsp. *paratuberculosis* in milk, (see Section 2.3.8. of the report).

Form of the organism. While bacterial spores are very resistant to heat, some highly so, the vegetative cells of the spore formers are generally no more resistant to heat than the vegetative cells of non-spore formers.

Number of cells. As outlined earlier, the higher the initial population, the longer the time required to reduce the population to a pre-determined number. In practice, commercial heat processes are designed to cope with an anticipated maximum number of bacteria in the product. Thus practices that allow bacterial number to be present in numbers higher than anticipated might result in unsatisfactory outcomes.

Age of cells. The effect of age of cells on their heat resistance does not appear to be consistent. The effect might also be confounded with stage of growth of the culture. However, in some cases, young cells were found to be the most sensitive.

Stage of growth. A number of studies have indicated that cells in the logarithmic phase of growth are more sensitive to heat than at any other stage of the growth cycle.

Growth temperature. The temperature at which bacteria are grown markedly affects their heat resistance. In particular, the cells of non-spore forming bacteria grown at the upper end of their growth temperature range are more heat resistant than those grown at lower temperatures.

Growth medium. Nutrient conditions for cultivation of bacteria for laboratory studies may increase or decrease heat resistance but it is difficult to generalize.

Medium or menstroom in which microorganisms are heated. The physicochemical nature of the heating menstroom profoundly influences the heat resistance of microorganisms. For example, heat resistance of microorganisms is generally greatest between about pH 6.0 and 8.0, and in foods with higher fat contents and lower moisture contents.

Factors affecting the heat resistance of vegetative food-borne pathogens were recently reviewed by Juneja (2004). Of particular relevance to the pasteurisation of milk and milk products is the effect of fat content on heat resistance. In this regard, however, various studies have given conflicting results. For example, one study cited by Juneja showed that D values for *Listeria monocytogenes* in skim milk and whole milk were similar. In another study, it was shown that sheep milk fat added to cow and sheep milk resulted in higher D values than when the same amount of cow milk fat was added; this led to speculation that type of milk fat might also influence D values.

Methods for determination of heat resistance of microorganisms

The most common methods for measuring thermal resistance of bacteria in use prior to 1973 – a period when the protocols for pasteurisation of milk by heating that are in use today were

developed – were broadly classified by Stumbo (1973). Those that have been most widely used in relation to the pasteurisation of milk are described briefly below.

1. **Thermal death time (TDT) – tube method.** In this method, inoculated menstruum, e.g. broth, buffer solution or milk, is distributed in small-diameter (7-12 mm) glass tubes which are subsequently sealed off at the mouth in a flame. The sealed tubes are generally heated by immersion in a thermostatically controlled bath of suitable liquid medium. At predetermined intervals, replicate tubes are removed from the bath, cooled in water, opened aseptically and the contents cultured for survivors.

If tubes are heated for varying periods of time, a survivor curve can be constructed by plotting the logarithm of the number of survivors against the time of heating. The D value can be read directly from the graph. A common variation of this technique was to use just two heating times, and to calculate the D values directly from the number of survivors in each case.

One of the main disadvantages with this method is that heating and cooling lags are considerable and their contribution to the lethal effect is difficult to evaluate. The organism may in fact be killed before the target temperature is even reached. Various procedures for correcting for these lags have been proposed, but it has been established that they cannot give a true correction and are particularly difficult to apply over a range of temperatures.

2. **Flask method.** This method has application for studying the resistance of bacteria to temperatures below the boiling point of water. Generally, a three neck flask with ground glass stoppers (Woulff bottle) is employed as the substrate container. Usually, a thermometer is introduced through one neck and a mechanical stirrer through another. The third neck is used for introduction of inoculum and withdrawing of samples for culturing survivors. When the method is properly employed, heating and cooling lags are negligible. Heating is virtually instantaneous, as the inoculum is introduced directly into the pre-heated menstruum. Cooling of withdrawn samples can also be rapidly achieved.

One of the main disadvantages of the method is that the walls of the flask above the liquid level are easily contaminated with the test organism during addition of inoculum and withdrawing of samples. The under-heated bacteria in the liquid on the walls find their way back into the main body of the menstruum and cause erroneous results, usually evidenced as ‘tailing’ of the survivor curve.

A variation of this method has been to use racks of standard open-ended glass test tubes with some form of cap or plugs. However, this method has the disadvantages outlined for both the tube method outlined in (1) above and the flask method outlined in (2) above.

3. **Thermoresistometer method.** This electro-mechanical device was designed in 1948 and represented advanced technology at the time. Heating was achieved in steam chambers with precise control over heating times and virtually instantaneous heating and cooling of samples. Its use was however limited to temperatures above about 102°C and hence its application was more applicable to bacterial spores than to vegetative cells.
4. **Capillary tube method.** This method is similar to the sealed tube method described in (1) above, except that the inoculated menstruum is sealed in thin-walled, small-diameter glass capillary tubes. This allows for rapid heating and cooling of samples. The procedure is however tedious and there is a risk of sample contamination during opening of the capillary tubes and transfer to a culture medium.

However, with the advent of the continuous flow HTST pasteurisers, questions about the applicability of heat resistance data determined under batch conditions to continuous flow systems was raised. In an attempt to answer these questions, assorted laboratory-scale devices of varying degrees of sophistication were developed over the years. However, most of them lacked one

important feature of the commercial continuous flow systems: turbulent flow. Accurate simulation of the complete heating and cooling profile has also been difficult.

In more recent years, pilot scale versions of commercial HTST pasteurisers have been used for heat resistance studies, though unfortunately to only a rather limited extent. Examples include studies on *Listeria monocytogenes* by Piyasena *et al.* (1998) and on *M. avium* subsp. *paratuberculosis* by Pearce *et al.* (2001) and McDonald *et al.* (2005).

Comparability and reliability of heat resistance data

According to the International Dairy Federation² (Anon., 2004a), heat resistance data are crucial for steering the heat treatment processes used in the dairy industry. The Federation (IDF) went on to state that while many heat resistance data have been accumulated for milk and other foods over the last 50 years, there are good reasons to now revisit the heat resistance properties of microorganisms from the perspectives of different disciplines such as microbiology, technology, standardisation and computational modeling. Reasons given by the IDF for this include:

- The compilation of data (for example, by the International Commission on Microbiological Specifications for Food, 1996a) shows, for the same microbial species, a huge diversity of heat resistance data, ranging from implausible to realistic;
- Many of the heat resistance data for bacteria are very old and their reliability is not always sufficient to meet calculation requirements for modern technologies;
- Observations of unusual or varying heat resistance need an in-depth analysis of the meaning of those results, such as some of those reported for *Mycobacterium avium* subsp. *paratuberculosis* and for *Enterobacter sakazakii*;
- Combined technologies, for example heat and filtration, make it necessary to calculate precisely the efficacy of each component of the process in order to describe the safety of the whole process; and
- Reliable and comparable heat resistance data are deemed necessary to allow the safety of dairy products produced by various technologies to be described in terms that are compatible with modern food safety concepts such as the proposed Food Safety Objectives for food in international trade.

The IDF concluded that while experience tells us that the safety of pasteurised milk is undoubted, the question to be answered is whether the safety to be achieved by pasteurisation can be expressed in comparable and reliable figures.

Pearce (2004) noted that a variety of techniques and methodologies have been used in laboratory studies on heat inactivation. He concluded that most of the methods give reproducible inactivation kinetics and can be useful for comparisons of the heat sensitivities of different strains. However, he found that comparison of results obtained with different methods is difficult, if not impossible, as there are too many variables involved. For example, come-up time with glass capillary tubes is much more rapid than in commercial low temperature holding (batch) pasteurisation which, in turn, has a much slower come-up time than commercial HTST pasteurisation.

To illustrate the fact that laboratory pasteurisation findings do not necessarily correlate with those obtained under commercial conditions, Pearce cited two reports on the heat inactivation of *L.*

² The International Dairy Federation is recognised and supported by the dairy industries world-wide, including Australia. It is often simply referred to as the 'IDF' in English-language countries. The Federation's secretariat is located in Brussels, Belgium.

monocytogenes. Mackey and Bratchell (1989) reviewed numerous laboratory heat inactivation studies that were conducted on this pathogen in the early 1980s and from the data in these studies calculated a mean 5.1-log kill by pasteurisation. In contrast, Piyasena *et al.* (1998) found that *L. monocytogenes* received an 11-log reduction during pasteurisation under commercial conditions with turbulent flow, the first and to date the only such study. Mackey and Bratchell also found a significant difference in D values obtained with different heating methods ($P < 0.05$). Most of the data at $< 65^{\circ}\text{C}$ were obtained using sealed glass tubes and at $> 65^{\circ}\text{C}$ using a slug flow heat exchanger, though with some overlap. (Refer to Section 2.3.7 of this report for more detail on these findings.)

A further example of the conflicting results that can result from the use of different methods for determining heat resistance was reported by Donnelly *et al.* (1987). Thermal resistance of three strains of *L. monocytogenes* as determined by the open test tube and sealed glass tube methods was compared. All *L. monocytogenes* strains, added to sterile whole milk to give an initial population of 5×10^6 per mL, were rapidly inactivated when survival was measured using the sealed tube thermal inactivation method. Calculated $D_{62\text{C}}$ values ranged from 0.1 – 0.4 minutes for the three strains tested, with straight line kinetics. Inactivation at 72°C , 82°C and 92°C was so rapid when measured by the sealed tube method that no surviving cell populations were ever detected. In contrast, extensive tailing of the survivor curves was observed at all temperatures with the open test tube method, with residual populations of 10^{3-4} per mL after 30 minutes at 62°C and 10^{2-3} per mL after 30 minutes at the higher temperatures. The authors concluded that the test tube method for measuring the heat resistance of *L. monocytogenes* was inaccurate and that, as indicated by the sealed tube method, *L. monocytogenes*, dispersed freely in milk, will not survive pasteurisation. It was suggested that the reason for the inaccuracy of the test tube method is simply that some of the bacterial cells can escape heating by accumulating in condensate on the cap of the tube and dripping back into the heating menstrua or by adhering to the wall of the tube above the water level.

Lund *et al.* (2002), in a comprehensive review of the literature on the heat resistance of *M. avium* subsp. *paratuberculosis*, also stated that techniques using partially submerged tubes are not suitable for use in experiments to determine the heat resistance of bacteria. Lund *et al.* selected data on the heat resistance of *L. monocytogenes* published by Donnelly *et al.* (1987) - to illustrate their concerns about the use of partially submerged tubes. The data selected by Lund *et al.* is reproduced in Figure 2.5.

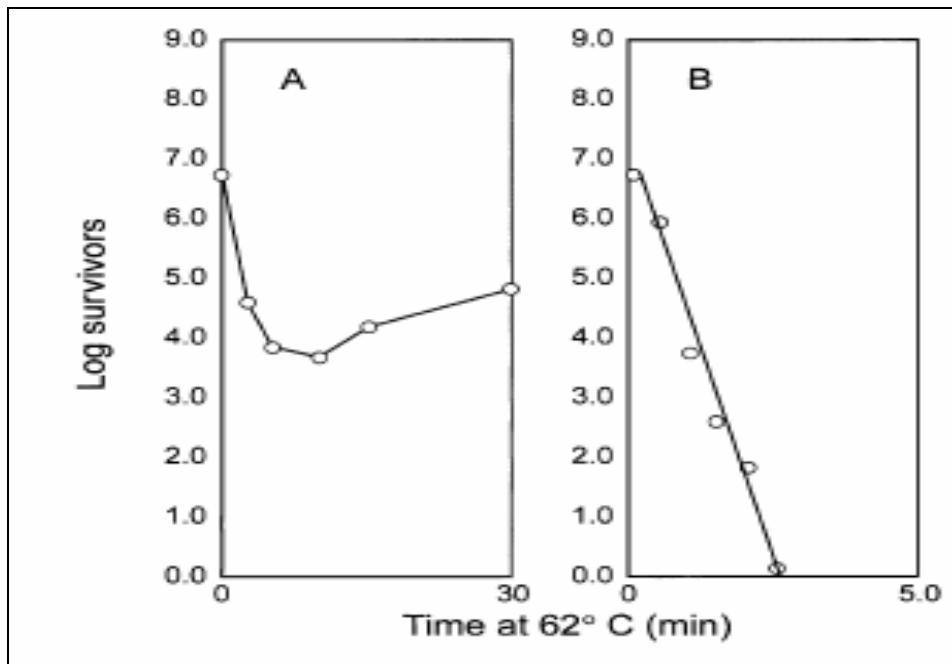


Figure 2.5. Inactivation of *Listeria monocytogenes* strain F5069 in sterile 11% nonfat milk solids at 62°C by (A) the open test tube method or (B) the sealed tube method [after Lund *et al.* (2002) and Donnelly *et al.* (1987)].

Unreliability of thermal inactivation data obtained with partially submerged test tubes was also demonstrated by Sorqvist (1989). For several strains of *Campylobacter jejuni* and *Campylobacter coli*, values of $D_{60\text{C}} = 0.12\text{-}0.14$ minutes were obtained with the capillary tube method. In comparison, values of $D_{60\text{C}} = 2.7\text{-}5.0$ minutes were obtained with the test tube method, a difference of 20-40 fold. The results obtained with the test tube method were considered to be unrealistic.

Thus while it is quite clear that capillary tubes provide more reliable data than partially submerged test tubes or similar, they also have their limitations. According to Fairchild *et al.* (1994), the application of the capillary tube method for determining heat inactivation rates of bacteria is limited to situations where inactivation rates are slow enough to ensure that the time to immerse a sample is relatively insignificant compared to total inactivation time. Also, the capillary method simulates a batch process and is therefore a less than ideal model for continuous flow processes. Using *Listeria innocua* M1 as a test organism in skim milk, they compared heat inactivation data obtained with the capillary tube method with that obtained by a laboratory-scale continuous flow pasteuriser (LSP) that allowed kinetic data to be generated under isothermal continuous flow conditions and also allowed monitoring of thermal activation throughout the length of the holding tube, rather than from single start and end-point analysis of the total heating profile. *L. innocua* M1 was used as the test organism because previous studies had shown it to be non-pathogenic, to have inactivation kinetics similar to *L. monocytogenes* and to be one of the most heat resistant vegetative cells likely to be present in raw milk.

From their studies, Fairchild *et al.* reported that the $D_{65\text{C}}$, $D_{68\text{C}}$ and $D_{70\text{C}}$ values for *L. innocua* M1 were 11.5, 3.5 and 1.6 seconds respectively when determined by the LSP system, and 16.5, 3.9 and 1.5 when determined by the capillary tube method. The D-values of the two methods were predicted to be coincident at 69°C. However, decimal reduction time curves indicated a z value of 4.8°C for the batch system and 5.9°C for the continuous system. Slopes of the curves were significantly different and, as a consequence, predicting the lethality of continuous HTST processes

with respect to *L. innocua* M1 might under-estimate the lethality of the process. The authors concluded that caution should be exercised when applying batch kinetic data to continuous flow systems.

Interpretation of non-logarithmic survivor curves for heat treatments

It is well documented that a plot of log survivors vs. time for bacteria heated at a constant temperature might give a straight line, indicating a logarithmic (exponential) death rate. However, such a plot might also give various types of concave or convex curves, or combinations of different shaped curves in the one line. Deviations from exponential death rates have often been attributed to clumping, either before heating in the case of lags in the death rate or flocculation during heating which would cause apparent death rates to fall more rapidly initially than would be expected from the exponential curve. However, it is sometimes difficult to demonstrate that deviations from straight line kinetics are due to these phenomena alone (Moats *et al.*, 1971).

The occurrence of (what is assumed to be) very heat resistant tails in survivor curves has been reported by a number of workers cited by Moats *et al.* (1977). These tails can be of great practical importance when total destruction of a bacterial population is required. Moats *et al.* were able to demonstrate that sub-cultured populations of the organisms in the heat resistant tails were no more heat resistant than the original populations, suggesting that the occurrence of small numbers of very heat resistant cells might be a normal characteristic of bacterial populations. They also expressed concern that many studies on heat resistance have not been continued through to the end point, thereby failing to detect the presence of a heat resistant tail and leading to the erroneous assumption that the thermal death curve is exponential. Furthermore, the D values calculated from data based on death rates over a narrow population range could give seriously misleading probabilities of kill over a wider population range.

Examples of different shapes of thermal death curves are shown in Figure 2.6. The curves are for five species of *Mycobacterium* in milk at 63.5°C (Grant *et al.*, 1996a). Tailing of the thermal death curve is particularly evident with *Mycobacterium avium* [Chart (c)] and *M. kansasii* [Chart (e)].

Moats *et al.* (1971) argued that the use of thermal death time measurements, i.e. direct measurement of the time required for a given probability of kill at a given temperature, would give valid comparisons without introducing unwarranted assumptions about the exponential nature of the death rate. The reviewer notes, however, that thermal death time measurements used in isolation have their own limitations, being dependent on the initial population of the target organism and accurate determination of the time required to reach the end-point.

Stewart and Cole (2004) raised similar concerns about the interpretation and application of mathematically-derived kinetic data. They observed that since the use of log-linear models to describe microbial death began in the 1920s, it has been common practice for microbiologists to analyse thermal inactivation data using the linear D- and z-value models, even though visual inspection of the plotted data often shows curvature. Process engineers also like the linear models, as they allow for simple, straightforward calculations and comparisons of thermal-process equivalencies to be made.

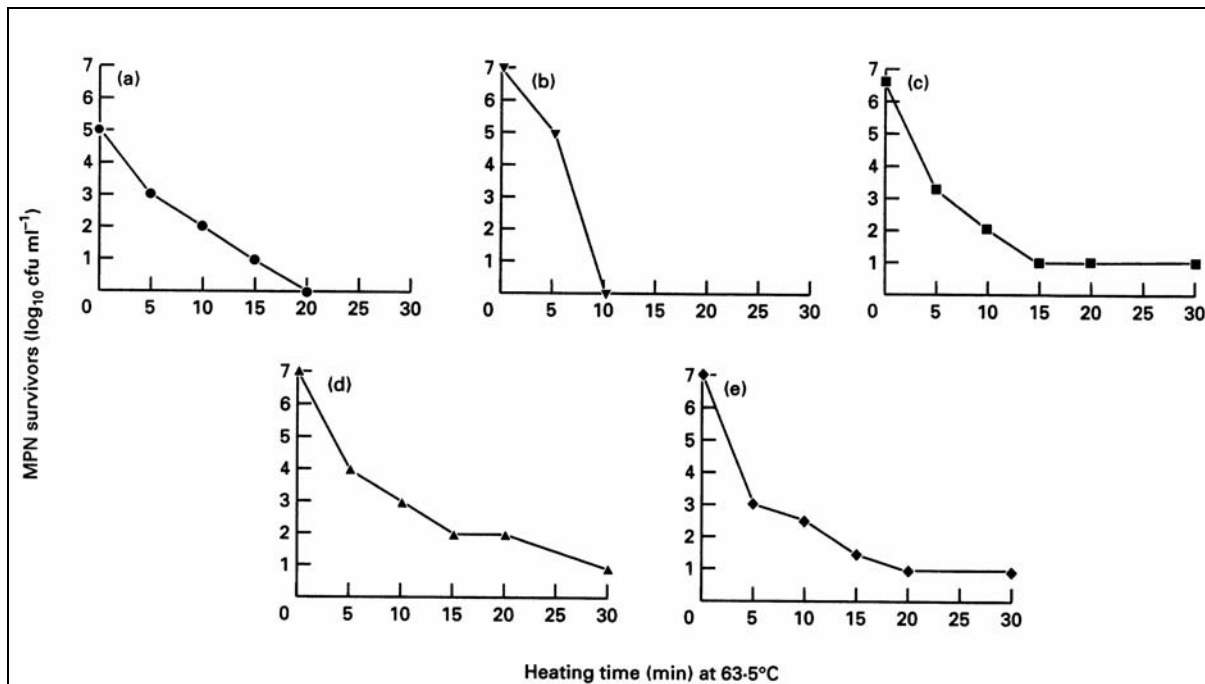


Figure 2.6. Thermal death curves for several *Mycobacterium* spp. in milk at 63.5°C: (a) *M. bovis* T/94/163C; (b) *M. fortuitum* NCTC 10394; (c) *M. avium* NCTC 8552; (d) *M. intracellulare* NCTC 10425; (e) *M. kansasii* NCTC10628 (after Grant *et al.*, 1996a).

Stewart and Cole also noted that, in food microbiology, predictive models are typically empirical, meaning that they relate the microbial growth, survival or death responses to the levels of the controlling factors throughout the experimental design space. As a consequence they caution that empirical models should not be used outside the range of factors used to create them, because there is no underlying principle on which to base extrapolation. Reliance on extrapolation can be problematic in both non-log-linear and log-linear models and can result in under or over processing in terms of food safety outcomes. Interpolation, on the other hand, provides better understanding of the data and hence of the required processing regimes.

2.3 Review of the scientific literature and available data on the effect of pasteurisation and thermisation on levels of pathogenic microorganisms in milk

The literature on the heat resistance of each of the nominated species of pathogenic bacteria is reviewed in separate sub-sections below. The focus of the reviews was on the heat resistance of the organisms in milk when heated at temperatures and for times that equate to commercial batch and HTST pasteurisation and also to thermisation of milk. The aim of the reviews was to demonstrate that the organism is, or is not, killed by each of these heat treatments.

Extensive searches of several commercial databases of the published literature were conducted by the Information Research Centre, Dairy Australia Limited, Melbourne. Databases searched included CAB (Commonwealth Agricultural Bureau), which started in 1972, FSTA (Food Science and Technology Abstracts), which started in 1969 and Foodline (Leatherhead Food Research Association), which also started in 1972. Abstracts of what appeared to be relevant articles were downloaded and reviewed. Full copies of the most relevant papers were then obtained.

As outlined in Section 2.1, studies on the heat resistance of organisms in the context of milk pasteurisation had been carried out for several decades prior to the dates when the electronic database came on line. Identification of papers on relevant work carried in the earlier years was essentially by a 'traceback' system via references in later papers and reviews and in text books. Bulletins and Documents published by the International Dairy Federation were also valuable sources of information.

The library maintained by the Queensland Department of Primary Industries and Fisheries at its Innovative Food Technology Centre at Hamilton in Brisbane has an extensive holding of dairy journals and this was extensively utilised during the review.

The quantity and quality of the published literature on each organism varied widely. For some of the organisms that have been in the spotlight in more recent years because of some doubts about their inactivation by pasteurisation, e.g. *L. monocytogenes* and *M. avium* subsp. *paratuberculosis*, quite a number of studies have been reported. For these, the literature has been reviewed selectively, with emphasis on the more recent studies that - hopefully - have been carried out under more controlled and realistic conditions. At the other end of the spectrum, however, relevant literature was relatively scarce and in some cases relatively old, e.g. pathogenic streptococci.

The method by which heat resistance was determined was an important consideration in the coverage and interpretation of the published literature in the review. In broad terms, the methods fall into a gradation based on relevance and reliability, from the least relevant / reliable to the most relevant / reliable, as follows (though of course there are always exceptions): laboratory studies using partly submerged open tubes or flasks ⇒ laboratory studies using fully submerged sealed capillary tubes ⇒ laboratory studies simulating batch pasteurisation ⇒ laboratory studies simulating continuous pasteurisation ⇒ studies using pilot plant scale continuous pasteurisers ⇒ studies using commercial-scale continuous pasteurisers.

The organisms are listed in alphabetical order in the following sub-sections, by genus. In each case, the material is presented under three sub-headings: Background, Heat resistance and Summary, with additional headings used where appropriate. Generally, the studies reviewed under 'Heat resistance' are covered in approximate chronological order. An overall summary of the data is presented in Section 2.4.

Interspecies differences in the composition of milk

As illustrated in Table 2.2, the gross composition of milk from the five species of animals covered by this review differs widely, particularly with respect to fat content. The composition of human milk is also included in Table 2.2 for comparative purposes. (In some instances, where data on heat resistance in other types of milk was scarce, data on the heat resistance of the nominated organism in human milk has been also included in the following sections of the report.)

Table 2.2. Interspecies differences in the composition of milk [after Wong (1974)].

Species	Composition (g/100g) ¹					
	Fat	Casein	Whey protein	Lactose	Ash	Total solids ²
Cow (<i>Bos taurus</i>)	3.9	2.6	0.6	4.6	0.7	12.7
Goat (<i>Capra hircus</i>)	4.5	2.6	0.6	4.3	0.8	13.3
Sheep (<i>Ovis aries</i>)	7.2	3.9	0.7	4.8	0.9	18.0
Water buffalo (<i>Bubalus bubalis</i>)	7.4	3.2	0.6	4.8	0.8	17.2
Camel ³ (<i>Camelus dromedarius</i>)	4.0	2.7	0.9	5.0	0.8	13.5
Human (<i>Homo sapiens</i>)	4.5	0.4	0.5	7.1	0.2	12.9

¹ Indicative figures only. Actual figures will vary, depending upon factors such as individual animal, breed, animal nutrition, animal health, stage of lactation, calving patterns and the degree of co-mingling.

² Calculated by difference from water content (not included in above table).

³ One-humped (dromedary) camel.

Fifteen reports on the composition of buffalo milk (water buffalo, *Bubalus bubalis*) published prior to 1968 were reviewed by Laximinarayana and Dastur (1968). The average fat content reported in these studies ranged from 5.1 to 9.25%, with most values in the range 6.6-8.0% and a mean value of 7.17%, similar to that reported by Wong (1974) (refer Table 2.2). Average total solids contents ranged from 15.15 to 18.30%, with a mean value of 17.25%, also similar to that reported by Wong.

Data from three studies on the composition of milk from the dromedary (one-humped) camel was summarised by Morton (1984). The figures for fat and total solids contents from the three studies varied, as follows: 5.5, 3.3 and 4.3% fat; 14.3, 13.0 and 13.4% total solids respectively. The third figure in each case was based on pooled data and was similar to the figures reported by Wong (1974) (refer Table 2.2).

Given the differences in the composition of milk between species as outlined above, heat resistance of particular pathogenic microorganisms in each of the milks might vary if, for example, it has been shown that composition of the milk, particularly fat content, can influence heat resistance.

It is noted that the U.S. Public Health Service suggested that the temperature of holding with the batch method of pasteurisation [145°F (62.8°C) for 30 minutes] should be increased by at least 5°F (2.8°C) for products with a fat content higher than whole (cows') milk or with added sugar (Enright *et al.*, 1956).

2.3.1 *Bacillus* spp.

Background

Bacillus cereus was first isolated and described in 1887. However, it was not until the 1950s that its role as a cause of foodborne illness was firmly established. Some forms of foodborne illness now known to be caused by *Bacillus cereus*, a spore-forming organism, were in fact not recognised until the 1970s. Other species of *Bacillus*, including *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*, have also been implicated in foodborne illness, but definitive evidence in some of these cases has been lacking (Jensen and Moir, 2003; International Commission on Microbiological Specifications for Food, 1996f).

The optimum temperature for growth of *B. cereus* is generally within the range 30-37°C, with the maximum temperature for most strains being in the range 45-50°C. *B. cereus* is generally classified as a mesophile, though psychrotolerant strains are not uncommon, particularly in raw and pasteurised milk (Jensen and Moir, 2003).

The psychrotolerant strains can be distinguished from other strains of *B. cereus* by their ability to grow at 4°C, but not at 43°C. In fact, a new species, *Bacillus weihenstephanensis*, has been proposed for this sub-group of *B. cereus* (Jensen and Moir, 2003).

B. cereus has been shown to be a frequent contaminant of raw and pasteurised milk and dairy products in recent decades (International Commission on Microbiological Specifications for Food, 1996f). In a Queensland study conducted about 25 years ago, it was shown that 23% of raw farm milk samples and 31% of commercially-pasteurised milk samples contained psychrotrophic spore-forming bacteria. Of these, *B. cereus* was the most prevalent species. Some of the *B. cereus* isolates showed an ability to germinate and grow slowly in milk at 4°C and even more slowly at 1°C, while others showed an ability to germinate and grow at 4°C but not at 1°C. However, even at 7°C, total growth of the more psychrotrophic isolates of *B. cereus* was very limited, e.g. from an initial plate count of 10³ per mL at day 0, the maximum count reached by one isolate at this temperature in a broth was only 10⁴ per mL, compared with 10¹¹ per mL at 20 and 30°C (Coghill and Juffs, 1979).

The sources of spore-forming *Bacillus* in farm milk supplies in Western Australia were studied by Depiazzi and Bell (1997). Mesophilic spores (ie spores with an ability to survive heating at 80°C for 10 minutes but not 100°C for 30 minutes) were found in 96% of milk samples from the farm vats on 16 irrigated and 26 dryland farms. Mean spore count was 32 per mL and the maximum count was 1,008 per mL. This study did not identify spores to the species level; however 63 of the isolates from this study with the ability to survive 100°C for 30 minutes were subsequently identified at the University of Queensland as *B. cereus* (4), *B. stearothermophilus* (25) and *B. licheniformis* (34) (Intaraphan, 2000). The four *B. cereus* isolates, which accounted for about 6% of this batch of isolates, were classified as mesophiles but their growth at <30°C was not investigated.

A study on the incidence of *Bacillus* spores in Victorian milk supplies was reported relatively recently (Cook and Sandeman, 2000). Milk and environmental samples were taken from 20 farms in each of two major dairying regions of Victoria during six visits to each farm over a two-year period. Mean number of mesophilic spores in the bulk farm milk from one of the regions was 73 CFU³ per mL, range 4-218 per mL, and in the other region, 7 CFU per mL, range 0-78 per mL. However, *B. cereus* accounted for only 3.5% of this category of spores. The spores were widely distributed in the farm environment.

The spores of *B. cereus* survive pasteurisation. However, they are not particularly heat resistant relative to those of other species of spore-forming *Bacillus*. The vegetative cells that result from spore germination after pasteurisation can have an adverse effect on the organoleptic and physical properties of milk products and they can also produce toxins. The vegetative cells themselves are easily inactivated by mild heat treatments (Bergere and Cerf, 1992; ESR Ltd, 2001a).

B. cereus produces a number of extracellular toxins and other harmful metabolites. Of these, the most significant in terms of impact on human health are the diarrhoeagenic enterotoxins and an emetic toxin. These two types of toxins are very different. The diarrhoeagenic toxins are proteins with molecular weights in the range 38,000-46,000, and are antigenic. They are produced by actively growing cells and are inactivated by certain proteolytic enzymes or by heating at 56°C for 30 minutes. Two forms of the diarrhoeagenic toxins are believed to cause food poisoning in humans. In contrast, the emetic toxin is a small peptide (molecular weight <5,000) that is not antigenic. It is extremely resistant to heat, exhibiting thermotolerance at 126°C for 90 minutes. It

³ CFU = colony forming unit.

is also resistant to extremes in pH (stable in range 2-11) and to enzymatic digestion. It is produced during the stationary phase of the growth cycle (International Commission on Microbiological Specifications for Food, 1996f; Granum and Lund, 1997).

The mechanism for of pathogenicity of the diarrhoeagenic form of *B. cereus* illness has not been fully elucidated (International Commission on Microbiological Specifications for Food, 1996f). It had been established that the diarrhoeagenic toxins can be produced in laboratory culture under certain conditions (Christiansson, 1992). However, because these particular toxins are readily inactivated by the proteolytic enzymes present in the human gastrointestinal tract, the presence of these toxins in ingested food might in fact be of little consequence. Rather, it appears that the diarrhoeal toxins that actually cause food poisoning are produced during vegetative growth of *B. cereus* in the small intestine (International Commission on Microbiological Specifications for Food, 1996f; Granum and Lund, 1997). In contrast, there is good evidence that the emetic toxin (cereulide) is produced by *B. cereus* growing in the food before it is consumed (Granum and Lund, 1997).

Some of the documented outbreaks of *B. cereus* food poisoning have been attributed to diarrhoeal toxins and some to the emetic toxins (Christiansson, 1992). Foods most frequently implicated in the diarrhoeal syndrome caused by *B. cereus* have been meat and meat products, soups, vegetables, pudding and sauces and milk and milk products. Foods most frequently implicated in the emetic syndrome have been fried and cooked rice, pasta, pastry and noodles (Granum and Lund, 1997).

B. cereus is not a competitive microorganism, and heating by pasteurisation or cooking actually encourages its growth under suitable conditions, by removing its competitors. Any food containing more than 10^3 *B. cereus* organisms per gram cannot be considered safe for human consumption (Granum and Lund, 1997).

As food poisoning caused by food-borne *B. cereus* is not a reportable disease in any country, reliable statistics on the number of cases are generally not available. Other factors contributing to this situation are that few people seek medical assistance during the active phase of the disease and the quick recovery of the patients (Granum and Lund, 1997).

Many strains of *B. cereus* have the capacity to elaborate the toxins (International Commission on Microbiological Specifications for Food, 1996f). Growth and toxin production by some strains of *B. cereus* have been observed in milk at 6°C, and other studies have shown toxin production in milk after 11-12 days at 7°C and after 24 days at 4°C (Jensen and Moir, 2003). However, the optimum temperature for toxin production in culture is around 32°C; the rate of toxin production is greatly reduced even at 20°C (Christiansson, 1992).

Other factors contributing to the very low incidence of reports of food poisoning due to the presence of this organism in milk products include:

- Little production of at least some types of the *B. cereus* toxins occur under static conditions (as in packaged milk);
- Milk in good condition lacks free glucose and has a low free amino acid content, substances known to promote production of at least some types of the *B. cereus* toxins; and
- Milk has usually undergone detectable organoleptic deterioration before significant toxin production occurs in it (Christiansson, 1992).

Hence, for practical purposes, growth and toxin production by *B. cereus* can be largely prevented by maintaining product at temperatures below 4°C (Jensen and Moir, 2003).

Heat resistance of vegetative cells and spores

The heat resistance of vegetative cells of *B. cereus* in skim milk was studied by Shehata and Collins (1972). The vegetative cells were separated from the spores by a washing and centrifuging technique. Heat resistance was determined using a flask method, with an inoculum level of 10^{5-6} per mL and continuous stirring. No survivors were detected when samples were plated out 5-10 minutes after commencement of heating at 65°C; there were insufficient data to plot survivor curves at this temperature. Examples of D values for the spores of *B. cereus* measured using a similar technique were $D_{85\text{ C}} = 18.5$ minutes and $D_{95\text{ C}} = 1.8$ minutes.

A temperature as low as 45°C has been reported to be harmful to *B. cereus*, the number of viable germinated cells actually decreasing at this temperature. In that study, a D value for the *B. cereus* spores of $D_{45\text{ C}} = 4.6$ hours was calculated (Penna *et al.*, 2002).

No other reports that provided any kinetic data for the heat inactivation of the vegetative cells of *B. cereus* were located. However, there are general statements in the literature that '*the vegetative cells themselves are easily inactivated by mild heat treatments*', for example Bergere and Cerf (1992) and ESR Ltd (2001a).

D values for the spores of *B. cereus* reported in the literature up to 1988 were summarised by Bergere and Cerf (1992). These data show that D values for the spores of *B. cereus* can vary widely. The D values reported from 21 different studies were in the following ranges:

- $D_{90\text{ C}} = 3.6-10.8$ minutes;
- $D_{95\text{ C}} = 0.5-20.2$ minutes;
- $D_{100\text{ C}} = 0.6-27$ minutes;
- $D_{105\text{ C}} = 11.2$ minutes;
- $D_{110\text{ C}} = 11.5$ minutes; and
- $D_{121\text{ C}} = 0.03-2.35$ minutes.

The z values from the same reports - where given - ranged from 6.7 to 13.8°C, with most in the range 8-11°C. In about half of the studies quoted by Bergere and Cerf, the heat inactivation curves were not truly linear; in two cases, the curves had shoulders and in 10 cases, tails. Reasons for the non-linear heat inactivation have been the subject of much study and speculation. According to Bergere and Cerf, factors influencing survival data include the following:

- Sporulation conditions, e.g. inoculum preparation, composition of the sporulation medium and incubation temperature;
- Spore harvesting and storage conditions, e.g. treatments for spore cleaning and inactivation of vegetative cells, storage medium and storage temperature;
- Heating conditions, e.g. composition of heating medium and velocity of heat transfers; and
- Survivor growth conditions, e.g. composition of growth conditions, incubation temperature and time.

Bergere and Cerf also reported that the heat resistance of strains of *B. cereus* can vary widely. Repeated experiments conducted with great precision are therefore necessary to obtain reliable heat inactivation data.

Bergere and Cerf concluded that heat sterilisation, done in an autoclave or by ultrahigh heat treatment, is sufficient to ensure spore populations of *B. cereus* are reduced to safe levels. According to Bergere and Cerf, the presence of *B. cereus* spores in sterilized/longlife products can

be attributed to very high levels of *B. cereus* in the raw product, inadequate heat treatment, e.g. UHT at less than 134°C, or contamination after heat treatment, e.g. a faulty packaging process.

In a more recent study, Wescott *et al.* (1995) established that non-linear survivor curves exhibiting pronounced tailing were observed only when thermal inactivation studies were conducted in a batch system; survivor curves were always linear when a continuous flow system was used. Predicted D values for *B. cereus* spores calculated from decimal reduction (thermal death) time curves, using phosphate buffer as the heating medium, were $D_{94\text{C}} = 81$ seconds, $D_{99\text{C}} = 21$ seconds and $D_{103\text{C}} = 7$ seconds in the batch system, and $D_{99\text{C}} = 13$ seconds, $D_{103\text{C}} = 4$ seconds and $D_{107\text{C}} = 1$ second in the continuous system. The z values obtained by the batch and continuous systems were both 8.5°C; however the continuous flow system was the more lethal.

Post-pasteurisation contamination of pasteurised product found to contain *B. cereus* must always be regarded as a possibility. Spores can germinate, multiply and re-sporulate between processing runs if cleaning has not been fully effective (Van Heddeghem and Vlaemynck, 1992). Spores of *B. cereus* can adhere to surfaces of equipment in the processing line after the pasteurisation step, especially the more hydrophobic surfaces such as gaskets and seals (Jensen and Moir, 2003). *B. cereus* spores attached to stainless steel are more heat-resistant than spores in planktonic form (Simmonds *et al.*, 2003).

Effect of pasteurisation and storage temperature on germination of *B. cereus* spores

In the context of dairy processing technology, spore germination is an important stage in the spore cycle. While spores of *B. cereus* can survive pasteurisation, they then must be able to germinate and the resulting vegetative cells must be able to multiply before product spoilage or toxin production can occur (Bergere, 1992).

Spore germination is a sequential process. Germination is the process by which a dormant spore develops into an actively growing vegetative cell. Often, spores need to be activated before they will germinate, for example by heat (ie heat activation). Triggering of germination involves the interaction of a specific compound with the spore that irreversibly commits the spore to lose its dormant properties. Initiation of germination follows the triggering reactions. Outgrowth is the development of a new vegetative cell from the germinated spore. Each of these stages has its specific requirements (Bergere, 1992).

B. cereus spores are able to germinate without preliminary heat treatment; however the rate of germination and the proportion of germinated spores are higher when the spores have been previously submitted to a sublethal heat treatment, such as 60°C for 60 minutes or 70°C for 30 minutes (Bergere, 1992).

B. cereus spores can be activated by heating them in milk at temperatures in the range 65-95°C for various times. *B. cereus* spores are of two types: slow-germinating and fast-germinating. The slow-germinating spores of *B. cereus* need a more intense heat treatment than the fast-germinating spores. It has been observed that heating of the fast-germinating spores of *B. cereus* in milk at 65°C for two minutes or at 72°C for 10 seconds results in nearly complete germination within 24 hours at 20°C; however, heating at 85-90°C for two minutes is required to achieve the same level of germination with the slow-germinating spores. In other words, batch or HTST pasteurisation is sufficient to trigger germination of the fast-germinating spores of *B. cereus*, but not the slow-germinating spores (Labots and Hup, 1964; Stadhouders *et al.*, 1980; Bergere, 1992).

The main sources of the fast-germinating spores of *B. cereus* are soil, dung and fodder, whereas the slow-germinating spores are mainly found in milking equipment (Bergere, 1992).

One aspect of the rationale for introduction of the thermisation process was the destruction of the fast-germinating spores of *B. cereus* and hence the prevention of the flocculation defect in pasteurised milk known as bittyness. Thermisation, or alternatively an initial pasteurisation, of the milk would trigger germination of the fast-germinating spores during subsequent storage at 5°C for two days, which would then be killed by a subsequent pasteurisation step (Bergere, 1992).

Wilson and Davies (1973) reported that pasteurised milk, as a medium, supported appreciable germination of *B. cereus*, whereas raw milk did not.

The abilities of *B. cereus* strains to germinate and to grow at low temperatures are not necessarily correlated. For example, studies by Anderson Borge *et al.* (2001) on 11 strains of *B. cereus*, inclusive of both mesophilic and psychrotolerant strains, showed that the latter strains exhibited both the highest and the lowest germination rates in milk at 7 and 10°C.

Heat resistance of the toxins

According to one report, the diarrhoeagenic toxin of *B. cereus* is inactivated by heating at 56°C for 30 minutes (International Commission on Microbiological Specifications for Food, 1996f). However, according to other sources, it is inactivated by heating at 56°C for just 5 minutes, but not at 45°C for 30 minutes (Christiansson, 1992).

The emetic toxin is extremely stable to heat, surviving heating at 126°C for 90 minutes (Christiansson, 1992; International Commission on Microbiological Specifications for Food, 1996f).

Thus the diarrhoeagenic toxin is destroyed by both batch and HTST pasteurisation heat treatments, but similar heat treatments would have no impact on the emetic toxin.

Summary

While the vegetative cells of *B. cereus* are reported to be easily destroyed by mild heat treatments, its spores readily survive batch or HTST pasteurisation. Destruction of *B. cereus* spores is a topic for consideration in the context of more severe heat treatments of milk than normal pasteurisation, e.g. UHT, and hence beyond the scope of this study.

B. cereus is widely distributed in the dairy farm environment and hence can often be isolated from farm milk supplies in Australia. However, the numbers of *B. cereus* spores in raw milk can be expected to be relatively small in most cases. For example, a recent study in Victoria showed that the total mesophilic spore count in milk from bulk farm vats was <100 per ml on average and that *B. cereus* accounted for only 3.5% of this category of spores.

Some strains of *B. cereus* are psychrotrophic, but growth at 4°C or lower is slow and limited in terms of the size of the population reached.

Both the batch and HTST pasteurisation heat treatments are sufficient to trigger germination of the fast-germinating spores of *B. cereus*, but not the slow-germinating spores. Pasteurised milk supports the germination of *B. cereus* spores. The spores can germinate in milk at low temperatures, though the rate and level of germination varies with the strain.

B. cereus produces a range of metabolites that are harmful to human health, of which the most significant are two diarrhoeagenic toxins and an emetic toxin. The former are destroyed by both batch and HTST pasteurisation heat treatments, but not the latter.

Cold-stored, packaged pasteurised milk is not an environment that is conducive to significant toxin production by *B. cereus*.

For practical purposes, significant growth and subsequent toxin production by *B. cereus* can be avoided by maintaining pasteurised products at temperatures below 4°C, provided the product is not held for unduly long periods, e.g. 20 days or longer.

2.3.2 *Brucella* spp. including *Brucella abortus* and *Brucella melitensis*

Background

Epidemiological data indicate that foodborne brucellosis (not however the only channel of human infection) is almost completely linked to consumption of milk and milk products (International Commission on Microbiological Specifications for Food, 1996b). The species of relevance to this study are *Brucella abortus* and *Brucella melitensis*.

Brucella abortus causes bovine brucellosis, a highly contagious disease. The dominant feature is late-term abortion and infertility in cattle. The disease is also a serious zoonosis, causing undulant fever in humans, i.e. brucellosis.

The main natural hosts of *Brucella abortus* are cattle, horses and humans. Infection has been found in many other species, although their epidemiological significance is very minor. Infection - with abortion - occurs very uncommonly in pigs, sheep and goats.

Until recently, bovine brucellosis was present throughout the world. However, a number of countries have now succeeded in eradicating this disease. These include Australia, Canada, Israel, Japan, Austria, Switzerland, Denmark, Finland, Norway, Sweden and New Zealand.

Australia has been free of bovine brucellosis since 1989. The disease was probably introduced to Australia in the early years of European settlement. It was prevalent throughout Australia by the 1920s, particularly in dairy herds where it was a source of major economic loss and public health concern. Various regional control schemes were in operation from the 1930s, and a nationally coordinated brucellosis eradication program commenced in 1970 as a component of the Brucellosis and Tuberculosis Eradication Campaign (BTEC). Freedom from bovine brucellosis was achieved progressively - Tasmania in 1975; Western Australia in 1985; the Australian Capital Territory, New South Wales, Victoria and South Australia in 1988; and Queensland and the Northern Territory in July 1989. Australia officially declared its freedom from bovine brucellosis to the Office International des Epizooties (OIE) in August 1989. There have been no recurrences of the disease since 1989 (Animal Health Australia, 2005a).

Brucella melitensis is a major cause of brucellosis in sheep and goats. The disease affects mainly adult female animals, causing abortion and udder infection. It is also a serious zoonosis, causing brucellosis in humans (synonyms according to geographic region: Mediterranean fever, Malta fever, Gibraltar fever, Cyprus fever). *Brucella melitensis* is more pathogenic to man than *Brucella abortus*.

Sheep and goats are the main livestock species affected by the disease. Cattle are occasionally infected by *B. melitensis* in endemic areas but such infections are usually subclinical. Different breeds of sheep vary considerably in their susceptibility to infection, with milking breeds being quite susceptible. The relative susceptibility of Australian breeds of sheep is unknown. Breeds of goats differ little in their susceptibility.

There is a high prevalence of the disease caused by *Brucella melitensis* in countries bordering the Mediterranean Sea and in the Middle East, and it extends further east through Central Asia to China and the southern areas of the former Soviet Union. In Europe, *B. melitensis* infection is absent north of about latitude 45°N. The disease also occurs in some areas of Africa and the Indian subcontinent, and occurs at a high prevalence in Central and South America. North America and South-East Asia appear to be free of the disease.

B. melitensis infection has never been reported in sheep or goats in Australia. However, migrants and tourists occasionally arrive in Australia suffering from *B. melitensis* infection, and they may travel widely in Australia. Since the organism is excreted in the urine of infected humans, infection of sheep and goats from this source is possible, although highly unlikely (Animal Health Australia, 2005b).

Heat resistance

One of the earliest reports on the heat resistance of *Brucella abortus* was by Bryan and Bryan (1944). They added a culture of the organism to cream and pasteurised half of it in the laboratory by a batch process [145°F (62.7°C) for 30 minutes]. The other half was not heat treated. Ripening and salting were additional variables. Butter was made from the cream in a small hand churn and stored at 45°F (7.2°C). *B. abortus* survived in the butter made from unripened cream for four months (both salted and unsalted) and in ripened cream (both salted and unsalted) for three months. Twice weekly examination of the butter made from the pasteurised cream failed to detect any surviving *B. abortus* at any stage, indicating that the organism had been destroyed by the heat treatment under the conditions described.

A more definitive study was carried out by Foster *et al.* (1953). Suspensions of a 96-hour culture of *B. abortus* Strain 2308 were added directly to 1.5 L of pre-heated milk in an elaborate laboratory apparatus that also allowed pre-heating time to be varied and controlled. Initial concentration of *B. abortus* when added to the milk was about 3×10^5 per mL. The test strain of *B. abortus* was killed in 23 minutes at 142.7°F (61.5°C) and in 14 seconds at 161.6°F (72.0°C). Both plate culture and guinea pig assay were used to detect surviving organisms. Pre-heating from a temperature 2°C below the holding temperature over a period of one minute had no measurable effect on the thermal death time at 142.7°F (61.5°C), but reduced it by 2 seconds (to 12 seconds) at 161.6°F (72.0°C). Straight line inactivation kinetics were observed in both cases (see Figure 2.7). The z value for *B. abortus* under these conditions was found to be 9.5°F (5.3°C) in the range 61.5 to 72.0°C. Though lower than some z values reported earlier by other workers (up to 8°C, though with a question mark on their reliability), this was still a relatively high figure, indicating that the lethal values might exceed the pasteurisation curve that applied at the time in the HTST range.

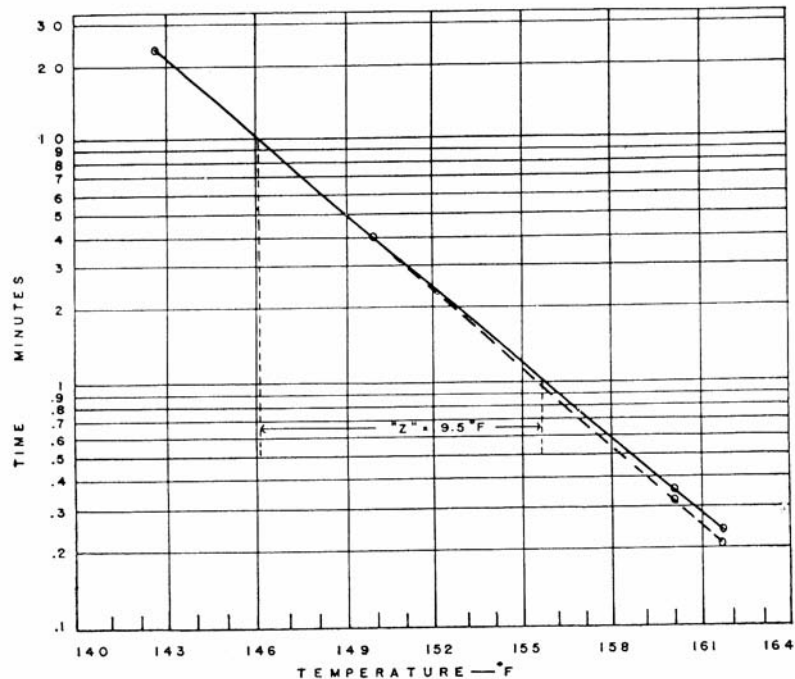


Figure 2.7. Inactivation rate of *Brucella abortus* Strain 2308 in milk, with no pre-heating (-----) and pre-heating for one minute (---) [after Foster *et al.* (1953)].

In view of the high z value for *B. abortus* Strain 2308 reported by Foster *et al.* (1953), further studies on the heat resistance of *B. abortus* in milk at pasteurisation temperatures were carried out by the same group. These were reported by Kronenwett *et al.* (1954). The same apparatus employed by Foster *et al.* (1953) (see above) was used for this study. However, for most experiments, 48-hour cultures, higher inoculation levels ($100\text{--}200 \times 10^6$ per mL) and greater control over other variables, were employed. The experimental range of the z values for the eight strains of *B. abortus* used in this study was $4.3\text{--}4.8^\circ\text{C}$ within the temperature range $61.5\text{--}67.8^\circ\text{C}$. Interestingly, the z values for Strain 2308 - the same strain used by Foster *et al.* - was consistently in the range $4.7\text{--}4.8$ under a range of experimental conditions. One explanation given for the slightly elevated z value results obtained for this strain by Foster *et al.* is that they may have been working with a 'slightly more heat resistant colonial variant' of the strain.

Kronenwett *et al.* concluded that the thermal death times for the *B. abortus* strains studied were considerably below the pasteurisation curve recommended by the U.S. Public Health Service. The then pasteurisation standard for the holding (batch) method of 30 minutes at 143°F (61.7°C) had a margin of safety of approximately 26 minutes. By extrapolating the thermal death curve from 67.8° to 71.7°C , a margin of safety of approximately 12 seconds at 161°F (71.7°C) was indicated (see Figure 2.8).

Davis and Casey (1973) studied the heat resistance of *B. abortus* in milk and skimmed milk using variations of a laboratory test tube method as well as a simple laboratory-scale simulator of a HTST plate pasteuriser that was in fact operated on a batch basis. *B. abortus* was added to the milk to give a level of 10^{6-7} organisms per mL before heating. The organism exhibited classic tailing of the survivor curve when heated in open tubes for at least 60 seconds at $161\text{--}162^\circ\text{F}$ ($71.7\text{--}72.2^\circ\text{C}$). However, survivors could not be recovered from the HTST simulator after heating both artificially ($1\text{--}4 \times 10^6$ viable organisms per mL) and naturally (1.25×10^2 per mL) infected milk at 150.5°F (65.8°C) or above for 15 seconds. Survivors were recovered from milk heated at 149°F (65.0°C) for 5 or 10 but not 15 seconds. At 147.5°F (64.2°C), there was partial survival after 5, 10 and 15

seconds, while at 146°F (63.3°C) there was no evidence of any killing after 5, 10 and 15 seconds. Survival rates in skimmed and whole milk were identical.

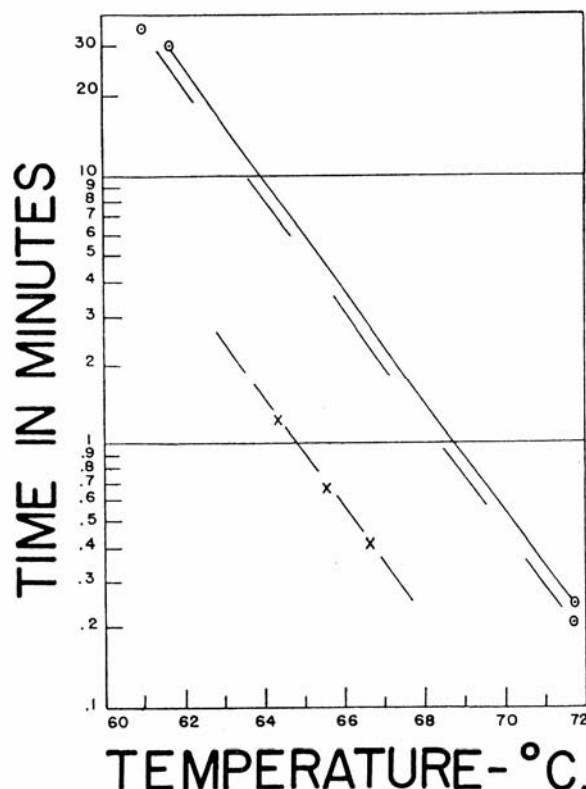


Figure 2.8. Relationship between the thermal death time curve for a 48-hour culture of *Brucella abortus* Strain 2016 in milk (--- x ---) and the pasteurisation (-----) and phosphatase (---) inactivation curves for milk [after Kronenwett *et al.* (1954)]. Viable cell count in the milk prior to heating was 2×10^8 cells/mL.

Stumbo (1973) reported generalised data of $D_{65.6\text{C}} = 0.10\text{-}0.20$ minutes and z values of $4.4\text{-}5.5^\circ\text{C}$ for *Brucella* spp.

In a finding that seems to be rather similar to that reported by Davis and Casey (1973) for the apparent high heat resistance of *B. abortus* in milk when tested by an open tube method (see above), extraordinary heat resistance of *B. abortus* was reported by Swann *et al.* (1981). These workers found that of 40 strains of *B. abortus* isolated from the milk and vaginal swabs of naturally infected cattle in the United States, 95% survived heating at 65°C for 120 minutes while 55% survived 75°C for 120 minutes. However, the methodology used to determine heat resistance in this study involved the heating of an extremely dense cell suspension of each strain (approximately 3×10^9 cells per mL) 'in batches in a water bath' (exact heating mechanism not specified, but assumed to be open tubes). In the opinion of the reviewer, these results lack credibility and should be ignored.

However, the findings reported by Swann *et al.* caused concern in the South African dairy industry, where at the time *B. abortus* had not been eradicated from the cattle population. As a result, a further study on the heat resistance of *B. abortus* was conducted in South Africa by Van den Heever *et al.* (1982). For this study, milk naturally infected with *B. abortus* was pasteurised in a pilot scale batch pasteuriser at 63°C for 30 minutes and in a pilot scale HTST pasteuriser at 72°C for 15 seconds. Numbers of *B. abortus* in the raw milk was not determined but the presence of the organism in the milk was confirmed by both the Brucella Milk Ring Test and guinea pig assay. No

survivors could be detected in the pasteurised milks by guinea pig assay. Van den Heever *et al.* concluded that pasteurisation of raw milk naturally contaminated with *B. abortus* rendered it safe.

Specific reports on the heat inactivation of *Brucella melitensis* were not located during this study. However, Animal Health Australia (2005b) states (without qualification or elaboration) that 'pasteurisation destroys *Brucella melitensis* in milk'.

Summary

Brucella abortus has been eradicated from Australia and *Brucella melitensis* is not endemic in Australia.

Some of the earlier laboratory studies and even some reports published as recently as 1981 gave the impression that *Brucella abortus* might be able to survive pasteurisation. However, some better controlled studies have confirmed that this organism is destroyed by both batch and HTST pasteurisation with wide margins of safety. But thermisation at 65°C for 15 seconds might give only partial destruction, while thermisation at 62°C for 15 seconds might have little or no killing effect.

Quantitative heat inactivation data for *Brucella melitensis* was not located. However statements from authoritative sources indicated that it also was destroyed by pasteurisation.

2.3.3 *Campylobacter jejuni* and *Campylobacter coli*

Background

Since the early 1900s, vibroid-shaped organisms have been well known in veterinary microbiology. They were reported as causing abortion in sheep as early as 1913 and, in 1919, a vibrio isolated from foetal fluids of aborted cattle was named *Vibrio fetus*. In 1913, *Vibrio jejuni* was isolated from cattle with winter scours. Following taxonomic studies of these organisms, the genus name *Campylobacter* was proposed in 1963. *Vibrio fetus* was re-named *Campylobacter fetus* and designated as the type culture for the genus. However, there has been considerable confusion in the naming of the genus, species and subspecies within this group of organisms (Wallace, 2003).

It was not until the 1970s that campylobacters were recognised as a significant cause of human gastroenteritis. Since then, surveillance has shown these organisms to be a common cause of acute human gastroenteritis and, in a survey of hospital laboratories that were cultured for *Campylobacter* species, *Campylobacter* infections were found to be more common than *Salmonella* infections. In the United States, *Campylobacter jejuni* (also designated in some literature as *Campylobacter fetus* subsp. *jejuni*) accounts for 99% of the reported *Campylobacter* spp. from human disease, with *Campylobacter coli* accounting for the majority of the remaining 1% (Wallace, 2003).

In spite of the high contamination rate in slaughtered animals, especially poultry, and the possibility of human infection by contaminated meat, the transmission of *C. jejuni* by consumption of raw milk has been a most important pathway in the epidemiology of human campylobacteriosis. The peculiarity of milk-borne infections is the high number of implicated persons in an outbreak (up to 2500), depending on the method of distribution of the milk. Most of the outbreaks have occurred in countries where pasteurisation of milk is not, or was not, legally prescribed (Hahn, 1994).

Heat resistance

In contrast to some of the other pathogens of interest in this review, reports on the heat resistance of *C. jejuni* only began to appear in the early 1980s, coinciding with the recognition of the species as a foodborne pathogen. Thus it would be reasonable to expect that the methodology used for the heat

inactivation studies should be of a relatively high standard; however, this has not always been the case.

One of the first reports was on the use of a laboratory-scale continuous HTST pasteuriser to determine the heat resistance of some *Campylobacter* spp. in milk (Gill *et al.*, 1981). Pasteurisation at 72°C for 15 seconds destroyed several strains of *C. jejuni* in milk at population levels ranging from 9×10^3 to 2.3×10^6 per mL. In experiments on heat resistance at various time-temperature combinations, it was shown that a human strain of *C. jejuni*, at population levels in the range $1-3 \times 10^6$ per mL, survived for at least four minutes at 50°C, for more than 60 but less than 80 seconds at 60°C, and for more than 10 but less than 20 seconds at 72°C. However - somewhat amazingly - there was some doubt as to the effectiveness of pasteurisation in the latter case, as the phosphatase test on the heated milk was highly positive!

Doyle and Roman (1981) examined the heat resistance of five strains *Campylobacter fetus* subsp. *jejuni* (*C. jejuni*) obtained from human stool specimens. Heat resistance of the organisms in skim milk at temperatures in the range 48-55°C was determined using a flask method. The organisms were added to the milk to give a level of approximately 10^6 per mL. D values at 55°C - the highest temperature used - ranged from 0.74 to 1.0 minute. The D values ranged from 1.56 to 1.95 minutes at 53°C, from 4.4 to 5.4 minutes at 50°C and from 7.2 to 12.8 minutes at 48°C. On this basis of this data, Doyle and Roman concluded that pasteurisation at either 62.7°C for 30 minutes or 71.7°C for 15 seconds should inactivate even unusually large numbers of *C. jejuni* in skim milk.

In a similar study, Waterman (1982) examined the heat resistance of six strains of *C. jejuni*, five from patients suffering from diarrhoea and one from a suspected milk-borne outbreak of enteritis. Cultures were added to milk at a final concentration of 10^8 per mL. Milk in tubes plugged with cotton wool was heated to various temperatures in the range 49.5-56°C by partial submersion in a water bath. (Waterman fortunately acknowledged the short comings of this method.) Death rates were assumed to be linear. Typical D values obtained for different strains at different temperatures at the upper end of the experimental range were: $D_{54C} = 0.7$ minutes, $D_{54.5C} = 0.8$ minutes (strain 17259), $D_{55C} = 1.1$ minutes (strain 16509), $D_{55.5C} = 0.6$ minutes (strain 5388), $D_{56C} = 0.3$ minutes (strain 21033), and $D_{56C} = 0.9$ minutes (strain 17259).

Waterman extrapolated the regression line for pooled data for five of the six strains to 63.5°C, one of the recognised temperatures for batch pasteurisation. The reason for excluding the data for the remaining strain from the pooled data was that this strain was clearly less heat resistant than the other five strains. The z value attached to the regression line for the pooled data was 5.1°C. Based on the extrapolated regression line, Waterman calculated that $D_{63.5C} = 0.6$ seconds (refer Figure 2.9.)

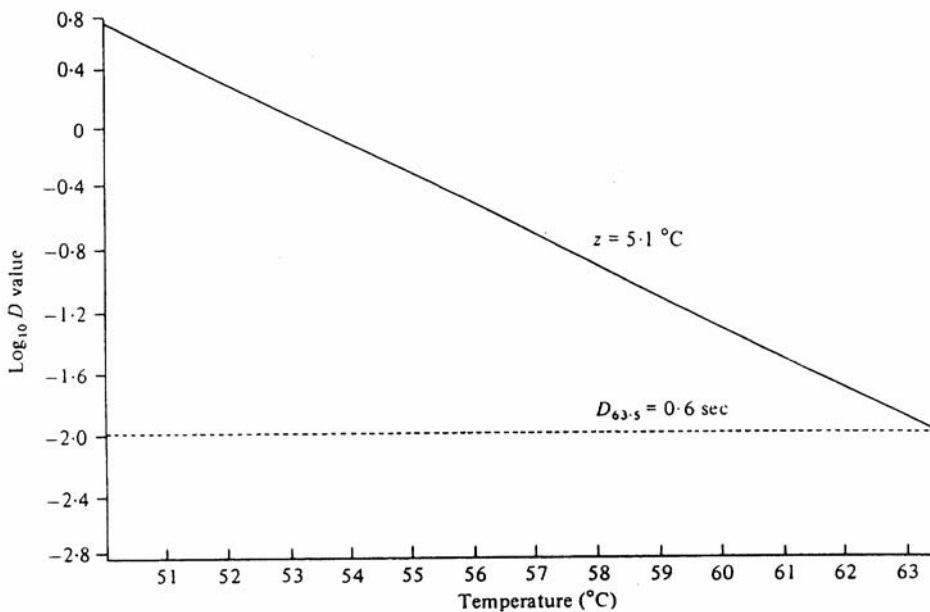


Figure 2.9. Regression line for thermal death times for *Campylobacter jejuni* based on composite data for five strains in the temperature range 49.5-56.0°C, extrapolated to 63.5°C [after Waterman (1982)].

Waterman also added two of the *C. jejuni* strains to milk at a level of 10^8 per mL and laboratory pasteurised it at $63.5 \pm 0.5^\circ\text{C}$ for 30 minutes. No survivors were found in 10 replicate tubes of each strain. Waterman concluded that pasteurisation should give complete protection against the spread of campylobacter enteritis by milk even when large numbers of the organisms are present.

In terms of a thermisation treatment of 62°C for 15 seconds, a value of $D_{62\text{C}} = 0.05$ minutes (3 seconds) can be computed from Waterman's regression line shown in Figure 2.9 above. This translates to a 5D kill for *C. jejuni* in 15 seconds at 62°C .

Considerable variation in the heat resistance of different strains of *C. jejuni* was also reported by Christopher *et al.* (1982). $D_{50\text{C}}$ values of five strains heated in skim milk in sealed glass ampoules fully submerged in a water bath at initial levels of 10^{5-6} per mL ranged from 1.3 to 4.5 minutes.

D'Aoust *et al.* (1988) studied the heat resistance of *C. jejuni* and *C. coli* in whole milk using a regenerative plate HTST pasteuriser operated in the range $60-72^\circ\text{C}$ with a minimum holding time of 16.2 seconds. A 'cocktail' comprised of eight strains of *C. jejuni* and three strains of *C. coli* was added to the milk. Total *Campylobacter* population in the milk before heat treatment was about 10^5 per mL in each of the three trials conducted. Survivors in the range $1.1-2.4 \times 10^1$ per mL were detected after heat treatment at 60°C for 16.2 seconds, i.e. a 4D kill was achieved at this temperature. No survivors were detected at 63 , 66 and 72°C , i.e. a minimum 5D kill was achieved at each of these temperatures. D'Aoust *et al.* noted that while *C. jejuni* and *C. coli* were inactivated by recognised pasteurisation treatments, caution with sub-pasteurisation treatments was required.

Sorqvist (1989) examined the heat resistance of *C. jejuni* and *C. coli* in physiological saline using a glass cup method (shown to give similar results to the submerged capillary tube method). For one strain of *C. coli*, $D_{58\text{C}} = 0.42$ minutes, $D_{60\text{C}} = 0.13$ minutes and $D_{62\text{C}} = 0.07$ minutes, with a z value of 5.07°C . For a mixture of another strain of *C. coli* and one strain of *C. jejuni*, $D_{60\text{C}} = 0.12-0.14$ minutes and the z value was $4.94-5.60^\circ\text{C}$. In terms of a thermisation treatment of 62°C for 15 seconds, the value of $D_{62\text{C}} = 0.07$ minutes for the single strain of *C. coli* translates to only a 3.5D kill.

El Nokrashy *et al.* (1997) isolated *C. jejuni* from 7% of raw milk samples from farms in the Cairo and Giza provinces of Egypt. Heat resistance of two isolates in milk at 50, 55, 60 and 65°C was determined using a capillary tube method. D values for the two strains (average of three replicates) were: $D_{50\text{C}} = 10.5, 6.5$ minutes, $D_{55\text{C}} = 1.25, 1.3$ minutes, $D_{60\text{C}} = 0.175, 0.2$ minutes, $D_{65\text{C}} = 0.075, 0.071$ minutes. The z values for the two strains were calculated to be 7°C and 8°C, much higher than those from other workers quoted above, e.g. 5.1°C (Waterman, 1982) and 5.07°C (Sorqvist, 1989).

Summary

The heat resistance of *C. jejuni* in particular has been studied quite extensively since about 1980. According to laboratory studies on the heat resistance of *C. jejuni* and *C. coli*, both organisms are inactivated by both batch and HTST pasteurisation, with a wide margin of safety. This was confirmed by studies using a regenerative plate HTST pasteuriser, as used commercially.

However, the level of inactivation delivered by a thermisation treatment of 62°C for 15 seconds will depend on the heat resistance of the strains present in the milk, their levels in the milk and the exact time and temperatures of heating. It has been estimated - on the basis of suitable published data - that a heat treatment of 62°C for 15 seconds would result in a 3.5-5D kill of *C. jejuni* and *C. coli*.

2.3.4 *Coxiella burnetii*

Background

Q fever is a zoonosis with a worldwide distribution. The disease is caused by *Coxiella burnetii*, a highly infectious, highly virulent, intracellular, gram-negative rickettsia. Many species of mammals, birds, and arthropods are reservoirs of *C. burnetii* though domestic livestock represent the most significant source of infection for humans. Infection in wild animals is maintained largely by tick vectors, which also provide a source of infection for domestic animals. In Australia, the bandicoot is considered an important reservoir species. Infection in animals is usually subclinical but infected animals can shed large quantities of bacteria into the environment. Infected females can shed very large quantities during parturition and the bacteria can survive harsh environmental conditions.

Although often asymptomatic, Q fever may manifest in humans as an acute disease (mainly as a self-limited febrile illness, pneumonia or hepatitis) or as a chronic disease (mainly endocarditis).

Persons at risk from Q fever include farmers, veterinarians, livestock transport workers, abattoir workers, those in contact with dairy products, laboratory personnel performing *Coxiella burnetii* culture and others working with *C. burnetii*-infected animals.

Q fever is a public health problem in many countries including Australia, United Kingdom, Italy, Spain, Germany, Israel, Greece, and Canada. New Zealand is considered free of Q Fever. Q fever remains primarily an occupational hazard in people in contact with domestic animals such as cattle, sheep and, less frequently, goats. About 600 cases of Q fever are reported in Australia each year, despite the ready availability of a vaccine. Of these, over 200 people are hospitalised as a result of the disease and about three people die as a result of infection. Most infections occur in Queensland and New South Wales and most often affect those who work in the livestock or meat industries, primarily men. The term 'Q fever' (for query fever) was first proposed in 1937 by E H Derrick to describe undiagnosed febrile illnesses in abattoir workers in Brisbane, Queensland.

C. burnetii is excreted in milk, urine, and faeces of infected animals. During parturition the organisms are shed in high numbers within the amniotic fluids and the placenta. Infection with just one organism is thought to be sufficient to cause disease.

Ingestion (mainly though drinking raw milk) has been reported, but is probably a minor factor in the transmission and is now even controversial. Cattle, goats, and sheep are considered the primary reservoirs from which human infection occurs. An effective human vaccine against Q fever for people has been available in Australia since 1989 (Animal Health Australia, 2004).

Heat resistance

During the early 1950s, studies in California showed that *C. burnetii* may be found in the milk of infected dairy cows 'in great numbers' and was more heat resistant than other vegetative pathogens. The latter observation was confirmed with the isolation of the organism from milk pasteurised according to the recommended minimum standards at that time for the low temperature holding (batch or vat) method of pasteurisation (Enright *et al.*, 1956).

Later studies reported by Enright *et al.* (1956) showed that 7.3% of raw farm milk supplies in a particular area of California contained *C. burnetii*. Further studies also confirmed the presence of the organism in raw milk from other parts of California.

Enright *et al.* initially conducted heat resistance studies on *C. burnetii* in whole raw milk containing 100,000 infective doses of the organism per 2 mL in a laboratory heat exchanger that allowed the sample to be fully submerged in a water bath and continuously stirred. The results obtained are set out in Table 2.3.

Table 2.3. Key data from heat inactivation studies carried out on *C. burnetii* in whole milk by Enright *et al.* (1956).

Temperature	Time for 50% destruction (LD ₅₀)	Minimum time for 100% destruction	Minimum time for destruction plus 97.7% (2 σ) confidence interval
143°F (61.7°C)	29.39 min.	33.02 min.	46.03 min.
145°F (62.8°C)	16.29 min.	18.31 min.	25.42 min.
160°F (71.1°C)	11.7 sec.	13.2 sec.	20.4 sec.
161°F (71.7°C)	8.7 sec.	9.8 sec.	15.4 sec.
162°F (72.2°C)	6.5 sec	7.3 sec	11.6 sec

Enright *et al.* concluded that the data as presented in Table 2.3 indicate that heat treatment of raw milk at 143°F (61.7°C) for 30 minutes was totally inadequate to eliminate viable *C. burnetii* from raw milk, while heating at 145°F (62.8°C) for the same time ensures elimination of the organism with a high level of confidence. The regression line derived from the data obtained from the laboratory thermal resistance data is shown in Figure 2.10. The recommended pasteurisation line at the time [based on 143°F (61.7°C) for 30 minutes and 161°F (71.6°C) for 15 seconds] is also shown in Figure 2.10. The former standard was increased to 145°F (62.8°C) for 30 minutes as a result of this work.

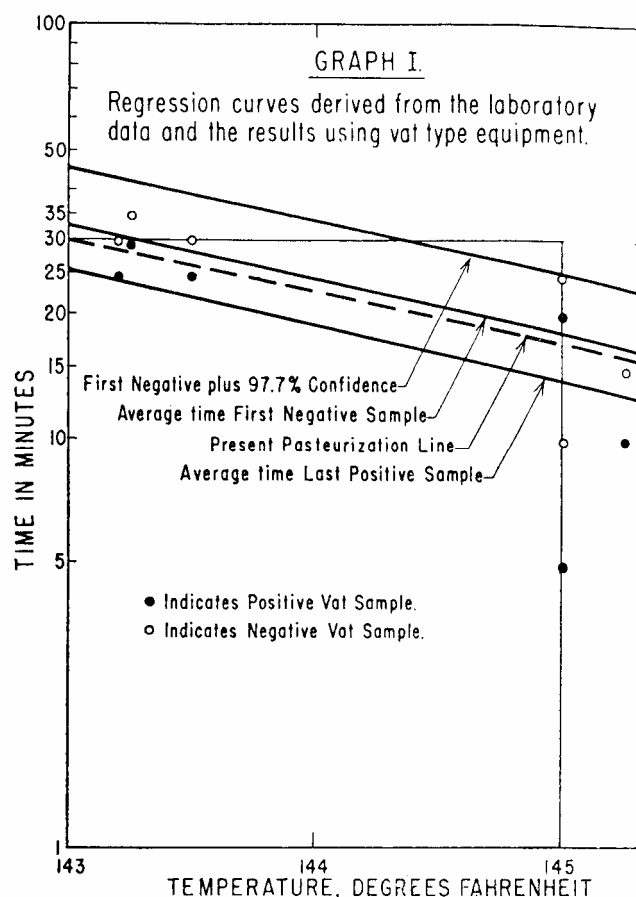


Figure 2.10. Regression line derived from laboratory data on heat inactivation of *C. burnetii* and the results obtained using vat (batch) equipment. Note that the 'present pasteurisation line' on the graph refers to 143°F (61.7°C) for 30 minutes, subsequently increased to 145°F (62.8°C) for 30 minutes [(after Enright *et al.* (1956)].

Further heat inactivation studies were carried out using a commercial HTST pasteuriser, with temperature points in the range 155°F (68.3°C) to 163°F (72.8°C) and 1-10 million infective doses of *C. burnetii* per 2 mL of milk. These studies confirmed the extrapolated regression line derived from the laboratory data and strongly supported the recommended standard for HTST pasteurisation at the time [161°F (71.6°C) for 15 seconds] as adequate to ensure elimination of viable *C. burnetii* from milk (see Figure 2.11). This standard, rounded to 72°C for 15 seconds in Australia and elsewhere, is still in use.

Studies on the inactivation of *C. burnetii* in liquid milk products containing more fat or added sugar or flavouring were reported at a later date by Enright (1961). Heat resistance of *C. burnetii* in the products was determined using a laboratory scale heat exchanger, similar to the one used by Enright *et al.* (1956), as outlined above. Sufficient inoculum of a strain of *C. burnetii* was added to the product to give 100,000 infectious guinea pig doses per mL of product prior to heat treatment. This inoculation rate was stated to be realistic in terms of the levels that can occur in raw milk delivered to a processor. Pasteurisation of cream (up to 40% butterfat) and chocolate milk (4% butterfat and 22.5% total solids) at the recommended standards for milk, 145°F (62.8°C) for 30 minutes and 161°F (71.6°C) for 15 seconds, was shown to be inadequate to eliminate *C. burnetii* from these products. However, increasing the temperature by 5°F, to 150°F (65.6°C) for 30 minutes and to 166°F (74.4°C) for 15 seconds,

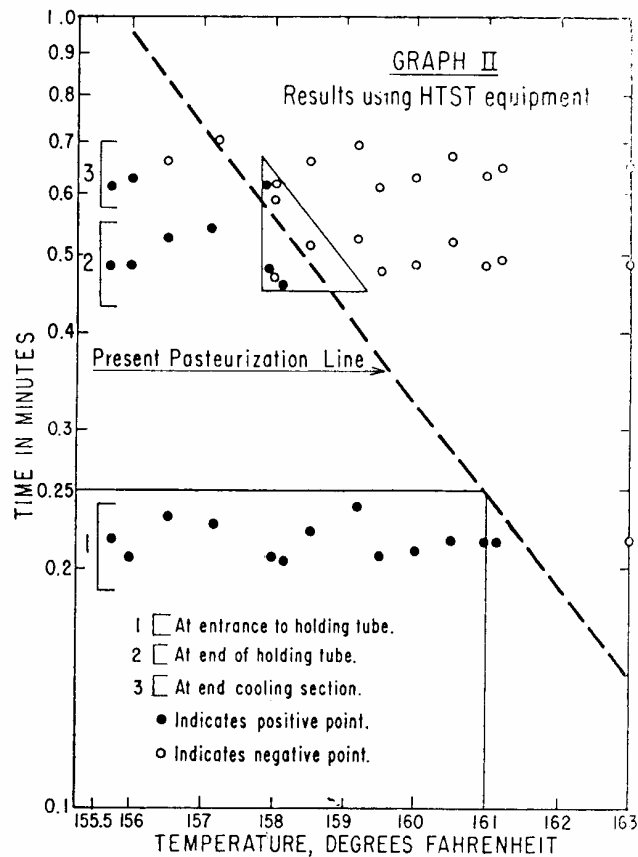


Figure 2.11. Data obtained from a study on heat inactivation of *C. burnetii* in whole milk subjected to HTST pasteurisation in commercial equipment. Note that the 'present pasteurisation line' on the graph is based on 143°F (61.7°C) for 30 minutes [subsequently increased to 145°F (62.8°C) for 30 minutes] and 161°F (71.6°C) for 15 seconds (unchanged) [after Enright *et al.* (1956)].

was adequate. No survivors were found in ice cream mix containing up to 18% butterfat and up to 42.75% total solids that had been pasteurised at 155°F (68.3°C) for 30 minutes and at 175°F (79.4°C) for 25 seconds, the standards as recommend by the United States Public Health Service for this type of product in 1961.

Stumbo (1973) reported generalised data of $D_{65.6\text{C}} = 0.50\text{-}0.60$ minutes and z values of 4.4-5.5°C for *C. burnetii*.

Summary

Pasteurisation of whole milk at 62.8°C (rounded to 63°C) for 30 minutes and 71.6°C (rounded to 72°C) is adequate to inactivate *C. burnetii*. In fact, the pasteurisation standard for batch pasteurisation in the United States was increased from 61.7°C (143°F) for 30 minutes to 62.8°C (145°F) after it had been demonstrated that pasteurisation at 61.7°C (143°F) for 30 minutes did not inactivate the organism.

However, products containing more fat or more solids than whole milk must be pasteurised at higher temperatures and/or for longer times to ensure that *C. burnetii* is destroyed. For example, for cream and chocolate milk, the standard pasteurisation temperature for whole milk must be increased by 2.8°C (5°F), i.e. to 65.6°C (150°F) for 30 minutes and to 74.4°C (166°F) for 15 seconds, to ensure destruction of the organism. Pasteurisation of ice cream mix at 68.3°C (155°F) for 30 minutes or at 79.4°C (175°F) for 25 seconds was shown to be adequate.

Thermisation at 62°C for 15 seconds would not inactivate *C. burnetii*.

2.3.5 *Enterobacter sakazakii*

Background

Enterobacter is one of several genera within the family Enterobacteriaceae that comprise the coliform group of organisms. Other genera within the coliform group include *Escherichia*, *Klebsiella* and *Citrobacter*. *Enterobacter sakazakii* has been implicated in a severe form of neonatal meningitis. Infant formulae containing this organism have been implicated in outbreak and sporadic cases of the disease (Craven *et al.*, 2003).

However, the reason why *E. sakazakii* was present in, and could survive in, infant formulae has been unclear (Breeuwer *et al.*, 2003). Investigations have shown that the organism is one of the most thermotolerant among the Enterobacteriaceae. Nevertheless, the thermal resistance was shown to be insufficient to survive a standard pasteurisation process, suggesting that contamination of the product was occurring during drying or packaging (Nazaro-White and Farber, 1997).

Heat resistance

Nazaro-White and Farber (1997) studied the heat resistance of ten Canadian strains of *E. sakazakii* (5 clinical and 5 food isolates). The test medium was about 50 mL of reconstituted dried infant formula in stainless steel flat-bottomed centrifuge tubes that contained a sterile magnetic stir bar. The tubes were fully submerged and the contents stirred during the heating process. Final inoculum level in the test medium was 10^7 organisms per mL. Heat resistance at 52, 54, 56, 58 and 60°C was determined, with samples withdrawn at intervals to allow survival curves to be constructed.

Pooled D values for the 10 strains used in the above study were as follows:

- 54.8 ± 4.7 minutes at 52°C;
- 23.7 ± 2.5 minutes at 54°C;
- 10.3 ± 0.7 minutes at 56°C;
- 4.2 ± 0.6 minutes at 58°C; and
- 2.5 ± 0.2 minutes at 60°C.

In most cases the D values for the clinical strains were slightly higher than the values for the food strains but the differences were not significant. Thermal inactivation (survival) curves representing pooled data for the five clinical strains and for the five food strains are shown in Figure 2.12.

The calculated overall z value was 5.82°C, confirming that it is one of the most thermotolerant members of the Enterobacteriaceae family that is found in dairy products (refer comparative data compiled by Nazaro-White and Farber in Table 2.4). Nevertheless, it would not survive a pasteurisation process. Using the USDA requirement of a 4-7D (log) kill for various pasteurisation processes as a reference, a 7-log reduction would be achieved in 17.5 minutes at 60°C. From the data it was estimated that the D value for *E. sakazakii* at 72°C was 1.30088 seconds. Thus a minimum HTST pasteurisation treatment (15 seconds at 71.7°C) would give an 11D kill. Nazaro-White and Farber suggested that attention should be focused on process control following pasteurisation to prevent contamination of the pasteurised product with this organism, rather than on the pasteurisation process itself.

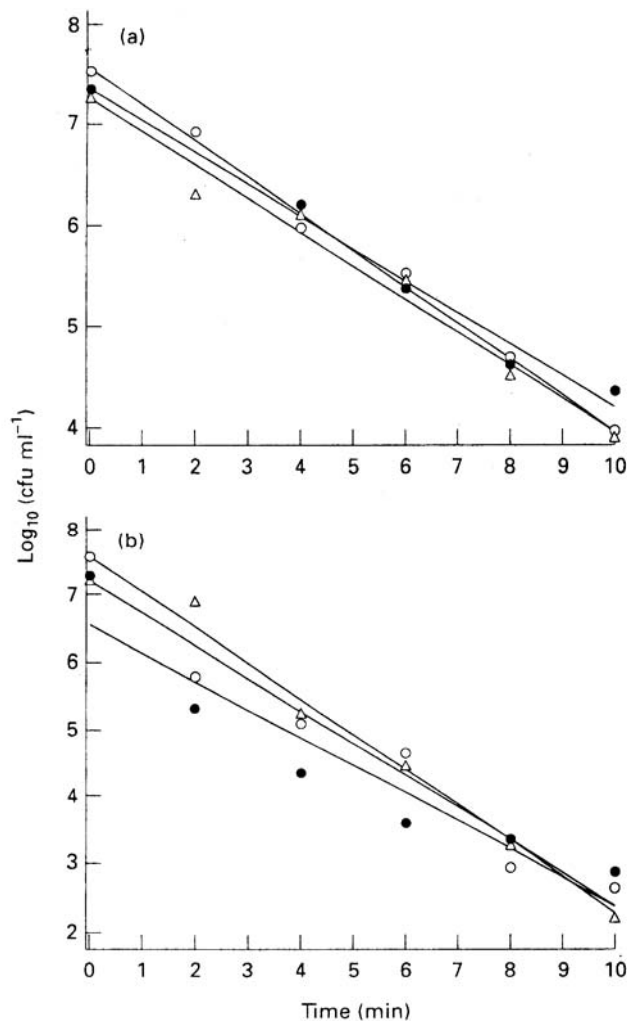


Figure 2.12. Thermal activation of *Enterobacter sakazakii* in reconstituted dried-infant formula at 60°C. Pooled data for five clinical strains (a) and five food strains (b) are shown. In each case, results of triplicate experiments done on three different days are shown (○, ●, ▲) [after Nazaro-White and Farber (1997)].

Breeuwer *et al.* (2003) demonstrated that *E. sakazakii* was not particularly thermotolerant, but remarkably resistant to osmotic stress and drying. Using a capillary tube technique, D values from five independent experiments on one strain in phosphate buffer at 58°C ranged from 0.39 to 0.60 minutes with a mean value of 0.48. This was much lower than the value reported by Nazaro-White and Farber (1997) and more in line with the values of other Enterobacteriaceae (see Table 2.4). D_{58}^C values for five strains in phosphate buffer ranged from 0.27 to 0.50, with similar values heated in reconstituted infant formula. The z values for two of these strains were 3.1 and 3.6°C, relatively low.

However, an average z value of 5.7°C for *E. sakazakii* type strain NCTC 11467 and a capsulated strain across the range 54–62°C in infant formula milk (IFM) and broth was recently reported by Iversen *et al.* (2004). This was similar to the higher value reported by Nazaro-White and Farber (1997), referred to above. Thermotolerance appeared to be determined using tubes or flasks in a water bath. D value of the type strain in IFM at 62°C was 0.3 ± 0.12 minutes and for the capsulated strain, 0.2 ± 0.11 minutes. Iversen *et al.* extrapolated their data to predict $D_{71.2}^C = 0.7$ seconds, equivalent to a 21D kill during HTST pasteurisation with a heating time of 15 seconds.

Table 2.4. Comparison of the heat resistance of various Gram-negative bacteria found in dairy products [after Nazaro-White and Farber (1997)].

Organism	Heating medium	D _{72 C} (seconds)*
<i>Aeromonas hydrophila</i>	Raw milk	0.01476
<i>Campylobacter jejuni</i>	Skim milk	0.07033
<i>Escherichia coli</i>	Whole milk	0.15669
	Human milk	0.01443
<i>Enterobacter sakazakii</i>	Infant formula	1.30088
<i>Klebsiella pneumoniae</i>	Human milk	0.00008
<i>Salmonella</i> Muenster	Whole milk	0.07214
<i>Salmonella</i> Seftenberg	Whole milk	0.08417
<i>Salmonella</i> Typhimurium	Whole milk	0.22000
	Whole milk	0.12125
<i>Shigella dysenteria</i>	Whole milk	0.13045
<i>Yersinia enterocolitica</i>	Whole milk	0.46086
	Whole milk	0.91208

* Data calculated by Nazaro-White and Farber from various publications (references cited by Nazaro-White and Farber). An exception was the value for *Enterobacter sakazakii*, which was calculated from their own data (refer text above).

While the above data indicates that *E. sakazakii* would not survive pasteurisation, the situation with respect to thermisation is not so clear. Extrapolation of the data of Nazaro-White and Farber (1997) to 62°C gives a D value for this temperature of approximately 1 minute, clearly insufficient to ensure destruction of the organism within 15 seconds. The data of Iversen *et al.* indicates that a kill of about 1D would be achieved at 62°C with a heating time of 15 seconds. However, extrapolation of the data of Breeuwer *et al.* (2003) would give a more favourable outcome. For example, the D value at 62°C would be in the order of 3 seconds, resulting in a 5D kill with a holding time of 15 seconds.

Summary

Although somewhat variable, the above data supports the view of the International Dairy Federation (Anon. 2004b) that '*Enterobacter sakazakii* seems not to overcome the current safety margins of commercial pasteurisation. Rather, it is a re-contaminant and not a priority to deal with under the heading of heat resistance'.

However, the destruction of *Enterobacter sakazakii* by thermisation is more problematic, with the predicted achievable kill potentially ranging from very limited kill to complete kill, depending on the circumstances. Three variables will have a significant impact on the outcome: the particular time and temperature combination used for the heat treatment, the particular data set on which the D values used to predict kill are based, and the likely level of the organism in the raw milk.

2.3.6 Pathogenic *Escherichia coli*, primarily *E. coli* O157:H7

Background

Strains of *Escherichia coli* have been considered pathogenic by veterinarians since the early 1900s, when *E. coli* was associated with white scours in calves. However it was not until the 1940s, when outbreaks of infantile diarrhoea were linked to pathogenic *E. coli*, that the concept of *E. coli* as a cause of human diarrhoea was accepted. Since that time, several groups of *E. coli* pathogenic to humans, mostly waterborne, have been identified. However in 1982, after two food-associated outbreaks, foodborne enterohaemorrhagic strains of *E. coli* (EHEC) including *E. coli* O157:H7, have been recognised. Other pathogenic serotypes of *E. coli*, of which there are many, include O26, O111 and O128 (Desmarchelier and Fegan, 2003).

0157:H7 is the best known and most widely studied serotype of *E. coli*. One of its natural habitats is the intestines of cattle, which creates the potential for contamination of milk and dairy products. In spite of this risk, milk and dairy products have only been occasionally implicated in outbreaks of *E. coli* 0157:H7 food poisoning, and even more rarely does an outbreak involve a pasteurised product (Kirk and Rowe, 1999).

There are two important features of *E. coli* 0157:H7 that contribute to its being a particularly dangerous pathogen. Firstly, only very low numbers of the organism - about 100 cells - are required to cause human illness. Secondly, *E. coli* 0157:H7 has been shown to be comparatively acid resistant, which has implications for cheese and yoghurt manufacture (Kirk and Rowe, 1999).

According to the International Commission on Microbiological Specifications for Food (1996d), data on the survival of the pathogenic strains of *E. coli* are scarce.

Heat resistance

In a general statement, the ICMSF (International Commission on Microbiological Specifications for Food, 1996d) noted that thermal inactivation studies have revealed that *E. coli* 0157:H7 is more sensitive to heat than *Salmonella*. Hence, any heat treatments that are sufficient to kill *Salmonella* should also kill *E. coli* 0157:H7.

Singh and Ranganathan (1974, 1980) studied the heat resistance of *Escherichia coli* in cow and buffalo milk. They used three cultures of *E. coli*: 0127:B8 and NP, non-pathogenic strains isolated from pasteurised milk and cream, and 0111:B4, a reference pathogenic culture. Temperatures used for heat treatments were in the range 50-63°C, with times in the range 10-60 minutes. Two mL of skim cows' milk, whole cows' milk or whole buffalo milk containing 1×10^5 *E. coli* per mL were placed in glass ampoules which were sealed and heated by immersion in a water bath.

Singh and Ranganathan found differences in the heat resistance of the three *E. coli* cultures, with the non-pathogenic strains markedly more heat resistant than the pathogenic strain. Also, heat resistance in buffalo milk was slightly higher than that in whole cows' milk, which in turn was slightly higher than that in skim cows' milk, possibly indicating a protective effect of the milk fat or total milk solids. D values were calculated both by the graphic (experimental) method, which involved a manual line of best fit on graph paper, and regression analysis. The z values were also determined by the latter method. If the data was not straight-line, a curvilinear (second degree) line was fitted. The D and z values so obtained are reproduced in Table 2.5.

Singh and Ranganathan concluded from their data, summarised in Table 2.5, that the three strains of *E. coli* tested would be destroyed by batch pasteurisation at 62.8°C for 30 minutes. They also concluded, on the basis of extrapolation, that the non-pathogenic strains (the more heat resistant strains) might survive HTST pasteurisation (71.7°C for 15 seconds) in buffalo milk, but not the pathogenic strain. Effectiveness of thermisation at 62°C for 15 seconds on the pathogenic strain would vary from very effective to partially effective, depending on the type of milk and the *E. coli* numbers present.

Table 2.5. D and z values for three strains of *E. coli* suspended in three types of milk [after Singh and Ranganathan (1980)]. D values are expressed in minutes.

Type of milk	Temperature of heating (°C)	Strain of <i>E. coli</i>					
		0111:B4 (pathogenic)		0127:B8 (non-pathogenic)		NP (non-pathogenic)	
		Graphic method	Calculation method	Graphic method	Calculation method	Graphic method	Calculation method
Skim cow	50	24.00	20.58	47.00	50.37	54.00	51.87
	55	7.00	5.53	30.00	27.32	35.00	34.49
	60	0.00	0.00	7.80	7.79	8.80	8.37
	63	0.00	0.00	2.00	1.78	2.10	1.91
	z value (°C)	-	4.61	-	9.72	-	10.44
Whole cow	50	25.50	24.32	52.50	55.24	59.50	58.52
	55	7.50	6.56	33.75	33.28	39.50	40.13
	60	1.25	0.00	8.25	8.28	9.00	9.40
	63	0.00	0.00	2.00	1.93	2.25	2.05
	z value (°C)	-	5.00	-	10.00	-	10.56
Whole buffalo	50	30.00	30.74	58.00	57.81	65.00	62.22
	55	9.00	8.54	36.50	36.87	41.50	44.71
	60	1.75	1.55	9.25	9.76	10.50	11.12
	63	0.00	0.00	2.20	2.28	2.50	2.67
	z value (°C)	-	5.67	-	10.67	-	11.27

The z values calculated by Singh and Ranganathan for the pathogenic strain are in the range quoted by Lovett *et al.* (1982) expected for vegetative cells (ie $5.56 \pm 1.1^\circ\text{C}$ at heat treatments in the range 54.4 to 71.1°C). The z values for the non-pathogenic strains were, however, much higher.

Morgan *et al.* (1988) studied the heat resistance of pathogenic *E. coli* 2376-81 in human milk using a micro-scale laboratory continuous HTST pasteuriser. A population of $1.1-1.3 \times 10^6$ per mL was completely killed by a heat treatment of 64°C for 33 seconds, but there were $1.1 \times 10^5 - 1.5 \times 10^6$ survivors after a heat treatment of 62°C for 15 seconds. Thus a thermisation treatment would only achieve a kill of 1-2D. D values were as follows (range reflects data obtained with different recovery media):

- 131.9-183.4 seconds at 58°C ;
- 31.5-47.6 seconds at 60°C ;
- 7.7-12.4 seconds at 62°C ; and
- 1.8-3.2 seconds at 64°C .

The corresponding z values were $3.2-3.4^\circ\text{C}$. Thus *E. coli* 2376-81 would be destroyed by both batch and HTST pasteurisation with a wide margin of safety. However kill by thermisation at 62°C for 15 seconds would be limited to a reduction of 1-2D.

D'Aoust *et al.* (1988) studied the heat resistance of *E. coli* 0157:H7 in whole milk using a regenerative plate HTST pasteuriser operated in the range $60-72^\circ\text{C}$ with a minimum holding time of 16.2 seconds. A 'cocktail' comprised of 10 strains of *E. coli* 0157:H7 isolated from human patients and raw ground beef was added to the milk. Total *E. coli* 0157:H7 population in the milk before heat treatment was in the range $1.2-3.1 \times 10^5$ per mL in the three trials conducted. Survivors in the range of $2.3 \times 10^3 - 2.3 \times 10^4$ per mL were detected after heat treatment at 60°C for 16.2 seconds, i.e. a kill of 1-2D was achieved at this temperature. Survivors in the range $4.3 \times 10^{-1} - 9.3 \times 10^3$ per mL were also detected after heat treatment at 63°C for 16.2 seconds, i.e. a kill of 1-6D was achieved at this temperature. No survivors were detected at 64.5 , 66 and 72°C , i.e. a minimum 5D kill was achieved at each of these temperatures. D'Aoust *et al.* noted that while *E. coli* 0157:H7

was inactivated by recognised pasteurisation treatments, caution with sub-pasteurisation treatments was required.

Clementi *et al.* (1995) examined the heat resistance of the pathogenic strain EC10 of *E. coli* in goat milk using capillary tubes and a laboratory slug flow laboratory-scale heat exchanger. Temperature range was 56.5-64.5°C, with residence times in the range 0.055-0.43 minutes. There was good agreement between the two methods in the range 56.5-64.5°C, but the capillary tube method appeared to be less accurate above 60°C. D values obtained with the slug flow heat exchanger were as follows:

- 0.485 minutes at 57.2°C;
- 0.289 minutes at 58.6°C;
- 0.081 minutes at 61.0°C;
- 0.024 minutes at 63.3°C; and
- 0.014 minutes at 64.5°C.

The z value computed from the above data was 4.72°C, close to values reported in the literature for similar organisms. The organism would be destroyed by batch pasteurisation (63°C for 30 minutes) with a wide margin of safety. Extrapolation of the data would indicate that the same would apply to a HTST treatment at 72°C for 15 seconds. It is estimated that a kill of approximately 5D would be achieved by thermisation at 62°C for 15 seconds.

Hassan and Frank (2000) studied the heat resistance of five strains of *E. coli* 0157:H7 in whole milk using a capillary tube method. The unheated milk contained 10^{7-8} of the test organism per mL. Temperatures used for heat treatments were 50, 58, 60 and 82.8°C. D values were calculated from the slopes of the linear regression of mean log survivors vs. time. A regression coefficient was calculated for each D line. For some of the strains, there were insufficient numbers of survivors at some temperatures to allow calculation of D values for them; hence calculation of z values was restricted to the two more heat resistant strains. The D and z values reported by Hassan and Frank are reproduced in Table 2.6.

Table 2.6. Heat resistance data for five strains of *E. coli* 0157:H7 in milk [after Hassan and Frank (2000)].

Strain	Temperature (°C)	D value (minutes)	Regression coefficient (R ²)	z value (°C)
01993	62.8	0.20	0.997	1.8
	60	0.87	0.982	
	58	3.13	0.975	
0019	62.8	0.28	0.997	3.1
	60	1.25	0.990	
	58	4.98	0.995	
009	60	0.45	0.979	Insufficient survival data
	58	1.3	0.995	
923	60	0.43	0.962	Insufficient survival data
	58	0.97	0.999	
933	60	0.35	0.983	Insufficient survival data
	58	0.95	0.980	

The data of Hassan and Frank in Table 2.6 indicates that the D values of different strains of *E. coli* 0157:H7 can vary quite widely at a given temperature, e.g. by a factor of up to 3-4 fold at 60°C.

However, Hassan and Frank concluded that pasteurisation of milk at 62.8°C for 30 minutes would destroy *E. coli* 0157:H7. In contrast however, they also cautioned against sub-pasteurisation heat treatments, e.g. for the five strains tested, heating at 60°C for 15 seconds would achieve a kill of only 0.2-0.7D. The reviewer estimated from the above data that the D value at 62°C for the most heat resistant strain, 0019, is 0.45 minutes, which translates to a kill of 0.55D in 15 seconds.

Sela *et al.* (2003) compared the heat resistance of *E. coli* TG1, a non-pathogenic strain, in camel milk with that in cows' milk. A capillary tube method, with heating in a heat block apparatus, was used. There were small differences in the D values for the two milks in the range 58-65°C, with those in the camel milks the lower of the two. One explanation for these differences is the differences in the gross chemical composition of the milks (see Table 2.7). The higher fat and total solids content of the cows' milk might offer a greater protective effect. However, there other differences in the milks, e.g. the protein and fat components differ in both chemical composition and physical characteristics. The z values in cows' milk were 7.3 (PCA-amp enumeration media) and 8.1°C (VRBA media) *cf.* 7.5 and 10.5 respectively in camel milk.

Table 2.7. Typical chemical composition of camel and bovine milk [after Sela *et al.* (2003)].

Source of milk	Fat	Protein	Lactose	Ash
Camel	2.61±0.58%	2.69±0.36%	4.61±0.32%	0.78±0.06%
Bovine	3.53±0.22%	3.26±0.10%	2.84±0.10%	0.72%

Summary

E. coli 0157:H7 is the most prominent of the foodborne pathogenic strains of *E. coli*, though it has rarely caused problems in pasteurised milk and dairy products. This organism is destroyed by both batch and HTST pasteurisation, with a wide margin of safety.

Estimates of the level of destruction of *E. coli* 0157:H7 and some other pathogenic strains of *E. coli* by a thermisation treatment of 62°C for 15 seconds vary widely, e.g. from <1D kill to a 5D kill. Variables include the strain of *E. coli* present, the type and composition of milk, the numbers of the organisms present and the source of the reference data used to estimate kill.

2.3.7 *Listeria monocytogenes*

Background

The genus *Listeria* contains seven species but only one, *L. monocytogenes*, is regarded as a significant human pathogen. Other species include *L. innocua*, *L. seeligeri*, *L. ivanovii* and *L. welshimeri* (Lund, 1990).

L. monocytogenes was first recognised as a cause of disease in laboratory animals in 1926. Outbreaks of listeriosis in humans were reported in many countries between 1940 and 1980, but in most cases the means of transmission was not established. However, a series of outbreaks in humans from 1980 onwards clearly implicated food as the means of transmission. An outbreak of particular concern to the dairy industry occurred in Massachusetts in 1983. In this outbreak, 49 people were affected by listeriosis, with two deaths from seven perinatal cases and 12 deaths from 42 cases in immunocompromised adults. The pasteuriser had been operated properly and the organism was not isolated from the pasteurised milk. However it was found in the raw milk and in the pasteuriser filters, and cows on farms supplying milk to the processor had previously been diagnosed with listeriosis. The incident was ascribed to accidental downstream contamination of the pasteurised milk with raw milk. Other outbreaks involving milk and dairy products in several countries were subsequently reported (Lund, 1990).

The 1983 outbreak in Massachusetts led to questions about whether *L. monocytogenes* was sufficiently heat resistant to survive pasteurisation. A series of studies on the heat resistance of the organism followed. Unfortunately the findings from these studies differed. Some showed HTST pasteurisation at 71.7°C for 15 seconds was effective, some showed it was not, and some showed it was effective provided the initial population of *L. monocytogenes* did not exceed a certain level, e.g. 10^5 per mL. However the World Health Organization examined the data and concluded that pasteurisation of milk kills *L. monocytogenes*, a view that was generally accepted at that time (Prentice, 1994).

Listeriosis in pregnant women manifests itself as a general bacteraemia, which can lead to transmission of the infection to the foetus via the placenta. Meningitis and meningoencephalitis developed mainly in newborn infants and the elderly. Some patients also develop gastrointestinal symptoms. The average case fatality rate of listeriosis is about 30% (Lund, 1990).

L. monocytogenes occurs widely in the general environment, where it can survive for long periods. A characteristic of particular concern in the dairy industry is its ability to grow at temperatures down to 0.5°C (Lund, 1990).

Heat resistance

A useful starting point in a review of the literature on heat resistance of *L. monocytogenes* is the review of heat resistance data conducted by Mackey and Bratchell (1989). They collated the data from up to 38 papers published on the subject prior to 1989 and presented it as a composite thermal death time plot (refer Figure 2.13). Data sets used by Mackey and Bratchell included those from the papers by Beams *et al.* (1958), Bunning *et al.* (1986) and Bradshaw *et al.* (1987a).

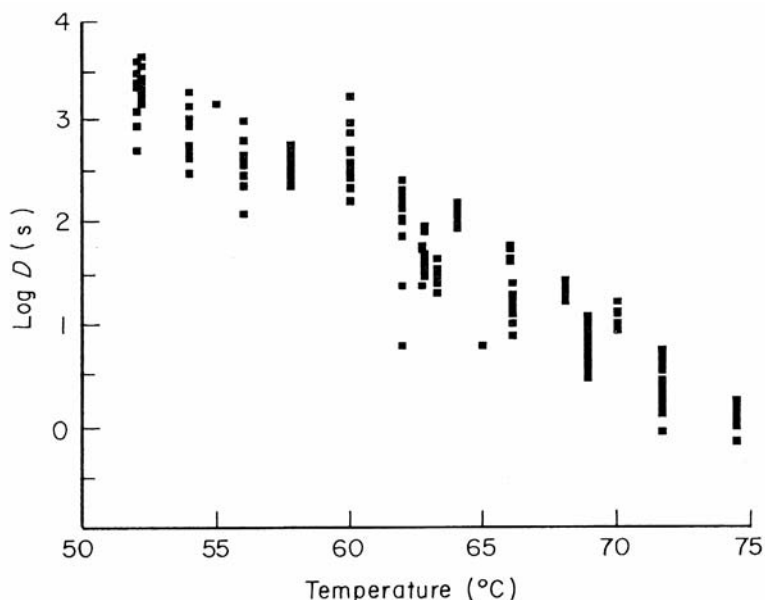


Figure 2.13. Composite heat resistance data for *Listeria monocytogenes* compiled from papers published prior to 1989 [after Mackey and Bratchell (1989)].

The D values included in Mackey and Bratchell's chart reproduced in Figure 2.13 were obtained from studies using raw milk, sterile whole milk, skim milk and reconstituted dried milk, but not cream. One outlier value was excluded. A regression line fitted to the data showed obvious curvature, which was attributed to the method of heating. Up to 65°C, most data were obtained by heating in sealed tubes, whereas at higher temperatures, a slug flow heat exchanger was mostly

used (though with some overlap in methods). Analysis of the data for the two methods in fact revealed a significant difference ($P < 0.05$) in measured heat resistance. The z value of the regression line fitted to the data obtained using sealed tubes was 6.1°C , compared with 7.4°C for the line fitted to the data obtained using the slug flow heat exchanger.

The D values from the two fitted regression lines referred to above are reproduced in Table 2.8. As the slopes of the two lines are different, the estimated D values also differ.

Table 2.8. Estimated D values for the destruction of *Listeria monocytogenes* in milk, based on composite data from reports on studies conducted prior to 1989 [after Mackey and Bratchell (1989)].

Temperature (°C)	D values (seconds)*	
	Sealed tube method	Slug flow heat exchanger
50	5679 (5180-6178)	
55	864 (812-916)	
60	131 (116-136)	110 (82-137)
63	42 (41-44)	43 (35-51)
65	20 (19-21)	23 (20-27)
70	3.0 (2.9-3.2)	4.9 (4.6-5.3)
72		2.7 (2.4-2.9)
75		1.0 (0.9-1.2)

* Values in brackets are the upper and lower 95% confidence limits

According to the sealed tube method, batch (vat) pasteurisation at 62.8°C for 30 minutes would achieve a 39D reduction in numbers while for HTST pasteurisation (71.7°C for 15 seconds), the slug flow model predicts a 5.2D reduction. The latter figure has since been widely quoted, e.g. by Pearce (2004). Increasing the temperature for HTST pasteurisation by just 1.3°C to 73°C would result in a 7.1-8.4D kill, depending on the method used to generate the data. Mackey and Bratchell argued that it was logical to use the data from the sealed tube method below 65°C and data from the slug flow method at higher temperatures.

With respect to the margin of safety with the estimated kill of 5.2D achieved with HTST pasteurisation, Mackey and Bratchell concluded that this was more than adequate. This outcome was in fact confirmed by most of the studies included in their review that had been conducted using actual HTST pasteurisation. Furthermore, they stated that survey work has shown that the *L. monocytogenes* content of raw milk is typically one bacterium per mL.

Mackey and Bratchell also concluded from their analysis that *L. monocytogenes* was more heat resistant than the common *Salmonella* serotypes, but less heat resistant than *S. Seftenberg*. Lund (1990) also briefly reviewed the literature on heat resistance of *L. monocytogenes*. She concluded that the methodology used in some of the studies that had reported high heat resistance for *L. monocytogenes* in fact gave erroneous results. She also observed that some of these studies had used very high inoculum levels. In addition, suggestions that recovered sublethally heat injured bacteria might be multiplying in the pasteurised milk had not been confirmed by studies (refer Crawford *et al.*, 1989). Rather, as noted by some expert groups, the risk of post-pasteurisation contamination of pasteurised milk with *L. monocytogenes* posed a much greater threat than survival of the heat treatment.

Lovett *et al.* (1990) used a commercial HTST pasteuriser to demonstrate that *L. monocytogenes* did not survive pasteurisation at $72-73^{\circ}\text{C}$ for 15-16 seconds in any of five trials. The milk contained $2-3 \times 10^5$ *L. monocytogenes* colony-forming units per mL. The pasteuriser complied with the requirements of the FDA's Pasteurised Milk Ordinance.

Bradshaw *et al.* (1991) also demonstrated that HTST processing was adequate for the pasteurisation of raw milk containing *L. monocytogenes*. Three strains were heated in raw whole milk using sealed glass tubes submerged in a water bath. D values at 71.7°C ranged from 1.5 to 2.2 seconds. A definitive study on the inactivation of *L. monocytogenes* during HTST pasteurisation was reported in 1998 by Piyasena *et al.* They used a pilot scale commercial HTST pasteuriser, allowing the total integrated lethal effect of the process to be taken into account. Whole milk was inoculated with *L. monocytogenes* to a level of 10^8 organisms per mL prior to processing. Using mathematical simulations of the process based on their data, Piyasena *et al.* predicted average log reductions at various temperatures (refer Table 2.9).

Table 2.9. Examples of predicted reductions in the population of *L. monocytogenes* in milk, based on process simulations [after Piyasena *et al.* (1998)].

Temperature (°C)	Average log reduction ^a	95% confidence ^b
67.0	4.17	1.29
69.5	11.6	4.53
72.0	28.1	11.4

^a All simulations were performed using a 16 second holding time.

^b Log reduction achieved using 95% of the 1500 iterations carried out.

The data in Table 2.9 indicate that a conservative estimate of the kill of *L. monocytogenes* at 72°C is 11.4D, more than twice that estimated by Mackey and Bratchell (1989). It also confirmed earlier work by their group, in which it was demonstrated that *L. monocytogenes* could not be detected in milk following HTST processing at >69°C for 16 seconds (Farber *et al.* 1992).

Holsinger *et al.* (1992) reported on a complex study to determine the effect of the various components of ice cream mix on the thermal resistance of *L. monocytogenes*. Nine different formulations of ice cream mix were inoculated with *L. monocytogenes* strain Scott A to a level of 10^9 per mL. The inoculated mix was placed in glass vials which were then heated by submersion in a circulating water bath. The survivor curves were sigmoidal in shape and a lag period was calculated to take account of the shoulder. D values at 60°C were positively correlated with the high fructose corn syrups solids and the stabilizer (carrageen + guar gum). For the nine mixes, the D values at 60°C calculated by the linear regression method ranged from 2.21 to 4.79 minutes; lag periods ranged from 2.04 to 5.24 minutes and F values (where $F = 7D + \text{lag period}$) ranged from 18.84 to 37.28 minutes. Holsinger *et al.* concluded that current guidelines for the pasteurisation of ice cream mix, e.g. 71.1-73.9°C for 30 minutes, were adequate to ensure the inactivation of *L. monocytogenes*.

The thermal inactivation of *L. monocytogenes* in sheep, cow and goat milk was compared by MacDonald and Sutherland (1993). Heating was carried out using both test tubes in a water bath and a modified pilot scale plate heat exchanger (APV Junior Pasteuriser). Skim milk was the basic heating medium, with various levels of milk fat added back. Survival of three strains of *L. monocytogenes* in cow and goat milk in the temperature range 68-74°C was similar. However, they showed greater heat resistance in sheep milk, an effect not solely due to its higher fat content. Despite this finding, MacDonald and Sutherland were able to conclude that *L. monocytogenes* in whole sheep, cow or goat milk at a level of 10^6 per mL could not survive the current HTST plate pasteurisation protocol.

Summary

Despite some early reports indicating that *L. monocytogenes* might survive pasteurisation, later more definitive studies have confirmed that the organism will not survive commercial batch or HTST pasteurisation. However, it would survive a thermisation treatment of 62°C for 15 seconds.

2.3.8 *Mycobacterium* spp.: *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium avium* subsp. *paratuberculosis*

Overview

The genus *Mycobacterium* was officially named as such in 1896 (Buchanan and Gibbons, 1974). It encompasses a diverse group of microorganisms that are widely distributed in the environment. It is comprised of many species, the exact number of which has varied over time, reflecting the dynamic nature of bacterial taxonomy. For example, in 1974, 31 species were officially recognised (Buchanan and Gibbons, 1974). More recently, Sutherland (2003) stated that there were more than 70 species in the genus, of which 15 were reported to be pathogenic to humans. However, just a year later, Katoch (2004) reported that there were more than 95 species in the genus, of which more than 30 were classified as pathogenic.

From a public health viewpoint, the most commonly known species of *Mycobacterium* is *Mycobacterium tuberculosis*, which is associated with human tuberculosis (Sutherland, 2003). *M. tuberculosis* is also the type species for the genus (Buchanan and Gibbons, 1974).

Unpasteurised milk can be a vehicle for transmission of *M. tuberculosis* from an infected human to the consumer of the milk (Burton, 1986).

Another species of mycobacteria, *Mycobacterium bovis*, is pathogenic to both humans and other animals, including cattle. *M. bovis* enters humans via the digestive tract typically from the ingestion of raw milk. After a localised intestinal infection, the organism eventually spreads to the respiratory tract, where it initiates the classic symptoms of tuberculosis (Brock *et al.*, 1994b).

There are many taxonomic similarities between *M. tuberculosis* and *M. bovis*. However, clear differences between the two organisms were recognised as early as 1898. Nevertheless, *M. bovis* continued to be often referred to as a bovine strain of *M. tuberculosis* for many years. It was not in fact until 1970 that the nomenclature '*M. bovis*' was legitimately published [Buchanan and Gibbons (1974); Sinha (1994)]. Thus readers need to be aware that *M. bovis* might be described as *M. tuberculosis* in some of the earlier literature, and *vice versa*. For example, in the review of the history of heating milk published by Westhoff (1978), the definitive data of Kells and Lear (1960) on the heat resistance of *M. tuberculosis* var. *bovis* is also portrayed as data for '*M. tuberculosis*' without any reference to 'var. *bovis*'.

As outlined in Section 2.1, the history of the heat treatment of milk is largely about its application for the inactivation of both spoilage and pathogenic organisms. Historically, the pathogens of initial concern were the tubercle bacilli, *M. tuberculosis* and *M. bovis*, together with *Brucella* species, *Salmonella typhi* and *Salmonella paratyphi* and *Streptococcus pyogenes*. However, it was soon recognised that among this group of pathogenic organisms, the most heat resistant were the mycobacteria, *M. tuberculosis* and *M. bovis* (Hammer, 2004).

Another member of the mycobacteria group, *Mycobacterium avium* subsp. *paratuberculosis* (also known as *M. paratuberculosis* or by the acronyms MAP or Mptb) is the cause of Johne's disease in ruminants (including, of course, cattle, sheep, goats and buffalo) and camelids. *M. paratuberculosis* may be present in the milk of animals infected with the organism asymptotically or symptomatically. While it has been associated with Crohn's disease in humans, there has been insufficient evidence available to prove or disprove whether it has a causal role in at least some cases of this disease [Lund *et al.* (2002); Animal Health Australia (2005d)]. According to Sutherland (2003), the weight of current evidence is that *M. paratuberculosis* should not be treated as a foodborne pathogen.

Despite the fact that the role of *M. paratuberculosis* in Crohn's disease – if any – remains unproven, industry organisations and groups of researchers have considered it prudent to attempt to confirm that the conditions specified for the pasteurisation of milk are adequate to ensure that pasteurised milk is not a vehicle for transmission of *M. paratuberculosis* to humans. Unfortunately, published work on this topic has given widely differing results.

Further attention was focused on *M. paratuberculosis* in milk in 2001 when a research group from Northern Ireland reported that the preliminary results of a survey of raw and commercially pasteurised milk samples obtained from dairy plants throughout the United Kingdom showed that 2.0% of 201 raw milk samples and 2.1% of 476 pasteurised milk contained live cells *M. paratuberculosis*. Where possible, one raw milk sample and several samples of pasteurised milk representing each product type originated from the same batch of milk at each of the dairies (Advisory Committee on the Microbiological Safety of Food, 2000). In the completed survey, the incidence of samples that were culture-positive for *M. paratuberculosis* was 1.8% (Grant, 2004). This finding was interpreted by Grant *et al* (2001) as indicating that commercial HTST pasteurisation is insufficient to inactivate this bacterium; however others have argued that these results alone are insufficient to support that interpretation, and that there might be other explanations for the results, e.g. post-pasteurisation contamination (Lund *et al.*, 2002).

Thus there are three species of *Mycobacterium* of interest with respect to their heat resistance in milk: (a) *M. tuberculosis*, (b) *M. bovis* and (c) *M. paratuberculosis*. The heat resistance of each of these will be addressed separately below.

(a) *Mycobacterium tuberculosis*

Background

The organism currently known as *Mycobacterium tuberculosis* was officially first assigned that name in 1896. It was known as *Bacterium tuberculosis* or *Bacillus tuberculosis* prior to that date and has been officially named *Mycobacterium tuberculosis typus humanis* or *Mycobacterium tuberculosis var. hominis* during certain periods after that date (Buchanan and Gibbons, 1974).

M. tuberculosis was first isolated and described as the causative agent of tuberculosis in humans by Koch in 1882. At one stage in history, tuberculosis was the single most important infectious disease of humans and accounted for one-seventh of all deaths. Despite the success of eradication programs, tuberculosis remains a significant disease world-wide and has re-emerged as a significant infectious disease in many Western countries. Primary infection is by inhalation of droplets or dust particles from an infected individual (Brock *et al.*, 1994b). However, as mentioned above, milk can also be a vehicle for transmission of the infectious agent for the disease (Burton, 1986).

Heat resistance

As outlined in Section 2.1, it was discovered about a century ago – largely accidentally – that heating of milk prevented the spread of tuberculosis. This in turn led to studies on the heat resistance of *M. tuberculosis* which, initially at least, were largely of an empirical nature (Hammer, 2004).

In 1911, the National Milk Standards Committee in the United States, a credible body, recommended a time-temperature combination of 62.8°C (145°F) for 30 minutes for the pasteurisation of milk. This was slightly above what many considered to be adequate exposure for the destruction of *M. tuberculosis*, 60°C for 20 minutes (Westhoff, 1978).

The numerous and often conflicting reports on the thermal destruction of *M. tuberculosis* were finally clarified by the work of North and Park (1927), who reviewed the major papers that had been published on the thermal destruction of *M. tuberculosis* to that date. Their data confirmed the work of several earlier investigators and supported the recommendation of 142°F (61.1°C) for 30 minutes as providing an ample margin for the destruction of *M. tuberculosis* (Westhoff, 1978). The thermal death time data used by North and Park, also presented as a scatter diagram on which they based - using what would now be considered to be a rather unorthodox approach - a linear 'thermal death curve', is reproduced in Figure 2.14 (taken from Hammer, 2004). From this curve, North and Hammer concluded that a heat treatment of 142°F (62.2°C) for 30 minutes would ensure a margin of safety for the destruction of *M. tuberculosis* of 20 minutes or 6°F (3.3°C). Note that initial population levels for the thermal death time data were not provided in the review by Hammer (2004).

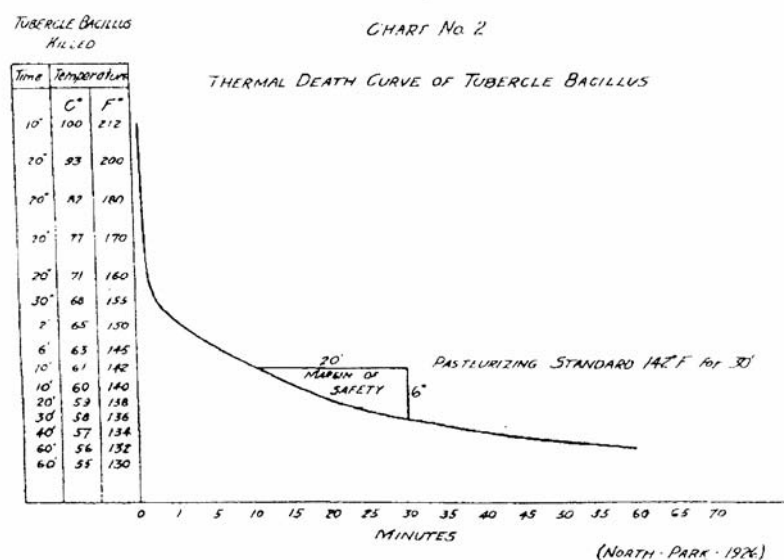


Figure 2.14. Thermal death curve of *M. tuberculosis* prepared by North and Park (1927) [after Hammer (2004)]. *Note.* The first six values in the time column are in seconds; the remainder are in minutes.

Although the 'holding method' method of pasteurisation was still widely used during the 1920s and early 1930s, work was underway on the effects of higher temperatures and shorter times on the destruction of *M. tuberculosis*. Plate heat exchangers were being developed and used for high temperature-short time methods, which evolved into the current HTST method of pasteurisation. The technology developed rapidly (Westhoff, 1978).

A summation of the heating conditions for the inactivation of *M. tuberculosis* prepared by Dahlberg (1932) is illustrated in Figure 2.1. This relationship has stood the test of time and is still valid today for this organism. Following the summation of the studies on the thermal destruction of *M. tuberculosis* and other pathogens by Dahlberg in 1932, a HTST standard - 161°F (71.7°C) for 15 seconds - was included in the 1933 edition of the U.S. Public Health Service Milk Ordinance and Code. The effect of HTST treatment on the creaming ability of milk was also taken into account in setting the standard (see Section 2.1).

The above standards are still in force today, with some minor adjustments. Following the work of Enright *et al.* (1956) showing that some cells of *C. burnetii* might survive 143°F (61.7°C) for 30 minutes if it was present in raw milk in large numbers, the U.S. Public Health Service recommended that the standard for the 'holding method' of pasteurisation be increased 145°F

(62.8°C) for 30 minutes. The only other changes have been as the result of rounding 62.8°C to 63°C and 71.7°C to 72°C in some countries, including Australia.

Stumbo (1973) reported generalised data of $D_{65.6\text{C}} = 0.2\text{-}0.3$ minutes and z values of 4.4-5.6°C for *M. tuberculosis* in pasteurised foods. Hammer (2004) observed that more than 100 scientific reports on the heat resistance of both *M. tuberculosis* and *M. bovis* had been published prior to 1973, though most of these were of an empirical nature.

Summary

There is ample evidence to indicate that *M. tuberculosis* is destroyed by both batch (62.8°C for 30 minutes) and HTST (71.7°C for 15 seconds) pasteurisation. In fact, the standards for pasteurisation of milk were originally based on the heat resistance of *M. tuberculosis*.

It is likely that, in at least some of the reported studies on *M. tuberculosis*, the test organism would have been now classified as *M. bovis*.

Thermisation of milk at 62°C for 15 seconds could not be relied upon to destroy *M. tuberculosis*. Based on the thermal destruction time curve published by Dahlberg (1932), the D value for *M. tuberculosis* at 62°C would be approximately five minutes.

(b) *Mycobacterium bovis*

Background

Tuberculosis (TB) in cattle is caused by *Mycobacterium bovis*. It is a chronic disease that seldom becomes apparent until it has reached an advanced stage. The disease can be spread to many other species, including humans, by drinking raw (unpasteurised) milk or by inhaling bacteria exhaled from an infected animal (Animal Health Australia, 2005c).

Bovine TB has occurred in Australia virtually since European settlement, particularly in the dairy industry. A national eradication campaign (BTEC — the Brucellosis and Tuberculosis Eradication Campaign) commenced in 1968. BTEC was based on the tracing of any cattle found to be infected at slaughter, quarantining of infected herds, intradermal testing of cattle, and slaughter of positive reactors (Animal Health Australia, 2005c).

Impending freedom from tuberculosis in Australia was achieved progressively by area: Tasmania (January 1963); New South Wales, Victoria, South Australia and the Australian Capital Territory (January 1988); Queensland (January 1990); and Western Australia and the Northern Territory (December 1992). National 'impending freedom' from bovine TB was declared on 31 December 1992 (Animal Health Australia, 2005c).

A further five years of monitoring were required before Australia could be declared free of bovine tuberculosis on 31 December 1997. This date marked the end of BTEC and the start of the Tuberculosis Freedom and Assurance Program (TFAP). TFAP is a surveillance program to ensure that any resurgence of TB in Australia is promptly and effectively eliminated. Surveillance is based primarily on the examination of animals at slaughter and submission of any granulomas found for laboratory examination as part of the National Granuloma Submission Program (Animal Health Australia, 2005c).

All known cases of bovine TB have been eradicated from cattle in Australia, although, because of the nature of the disease, an occasional case is detected (and eliminated). Clinical cases of TB in cattle are now rarely seen in Australia (Animal Health Australia (2005c).

As mentioned above, the organism now known as *M. bovis* was only officially assigned that name in 1970. Prior to that date, it was officially known as *Mycobacterium tuberculosis typus bovinus* from 1907 and as *Mycobacterium tuberculosis* var. *bovis* from 1934 (Buchanan and Gibbons, 1974).

Heat resistance

A huge amount of experimental work has been performed all over the world on the heat resistance of *M. bovis* (and *M. tuberculosis*), mainly during the period 1930-1960. Although largely of an empirical nature and carried out using a variety of commercial scale methods and equipment, this work led to the common acceptance of the proposition that holder (batch) and HTST pasteurisation are sufficient to inactivate the classical mycobacteria *M. bovis* and *M. tuberculosis*. From a practical perspective, this situation was supported by the fact that an investigation based on the testing of 1736 samples of pasteurised milk in the UK during a 20-year period from 1945 to 1965 yielded only two positive results, one in 1947 and one in 1948. Nevertheless, there were almost no reports of the elaboration of kinetic data prior to 1960 (Hammer, 2004).

Perhaps in fact the only study ever conducted with the specific aim of generating kinetic data for organisms explicitly identified as *M. bovis* was carried out by Kells and Lear (1960).

Kells and Lear determined the heat resistance of three strains of *M. tuberculosis* var. *M. bovis* (*M. bovis*) in milk using a laboratory apparatus that appeared to simulate batch pasteurisation (ie sample constantly agitated). Only approximate initial counts of *M. bovis* were reported, because of clumping. Also, only a final 'thermal death time' at temperatures in the range 64-69°C were reported; no counts at any intervening time points were included in their report. Clumping was controlled by agitating cell suspensions with glass beads and checking the suspensions microscopically.

Thermal death times and z values reported by Kells and Lear are reproduced in Table 2.10, together with indicative D values calculated from the available data.

Table 2.10. Indicative D values and z values for three strains of *M. bovis* in milk [after Kells and Lear (1960)].

Strain of <i>M. bovis</i>	Approx. initial count of <i>M. bovis</i> (CFUs ¹) per ml of milk	Cell dispersion		Heating temperature (°C)	Indicative D values ² (sec)	Z value (°C)
		Single cells %	Cells per clump of remaining colony forming units			
ATCC 12621	10 ⁶	10-20	2-10	64.0	6.6	4.8°C
				65.0	3.4	
				66.0	2.3	
				67.0	1.4	
				68.0	0.9	
ATCC 11756	10 ⁶	10-20	2-10	64.0	4.8	4.9°C
				65.0	3.3	
				66.0	2.4	
				67.0	1.3	
				68.1	0.8	
	10 ⁴	60-70	2-5	64.0	>8.5	4.8°C
				65.0	5.0	
				66.0	>3.0	
				67.0	1.9	
				68.0	1.4	
USDA 854	10 ⁶	10-20	2-10	64.0	4.8	5.2°C
				65.0	2.3	
				66.1	1.8	
				67.0	1.3	
				68.0	0.9	
				69.0	0.6	

¹ CFU = colony forming unit.

² Calculated manually by the reviewer, by dividing the mean of the thermal death time ranges quoted in the paper by the log₁₀ of the initial population shown in the table.

The time and temperatures required to inactivate *M. bovis* obtained by Kells and Lear (refer Table 2.10) were much lower than those reported by Dahlberg (1932), on which the pasteurisation standards were based. The relationship between the thermal inactivation data of Kells and Lear on *M. bovis* ATCC 11756 and the current pasteurisation standards (63°C for 30 minutes; 72°C for 15 seconds) and the cream line reduction curve developed by Dahlberg (1932) is shown in Figure 2.15 (taken from Pearce, 2004).

The extent of cell clumping, a topic of much study and speculation with respect to the heat resistance of *M. paratuberculosis* and the issue of tailing, appeared to have no effect on the z values obtained by Kells and Lear for strain ATCC 11756. The thermal death time curve for each of the strains of *M. bovis* studied by them was straight line.

Only two more recent studies on the heat resistance of *M. bovis* have been reported (Hammer, 2004). Both reports confirmed the known data for inactivation of *M. bovis*. However, no kinetic data were published in either case. In one of these studies, reported by Grant *et al.* (1996b), milk was inoculated with a strain of *M. bovis* isolated by a veterinary laboratory in Northern Ireland and heated in submerged sealed glass tubes. The milk was inoculated to a level of 10⁷ colony-forming units per mL, a level reported to be much higher than that likely to be encountered in raw milk that was to be commercially pasteurised. In the other study, reported by Pavlas (1990; paper not sighted), both batch and HTST pasteurisation were simulated using submerged glass capillary tubes.

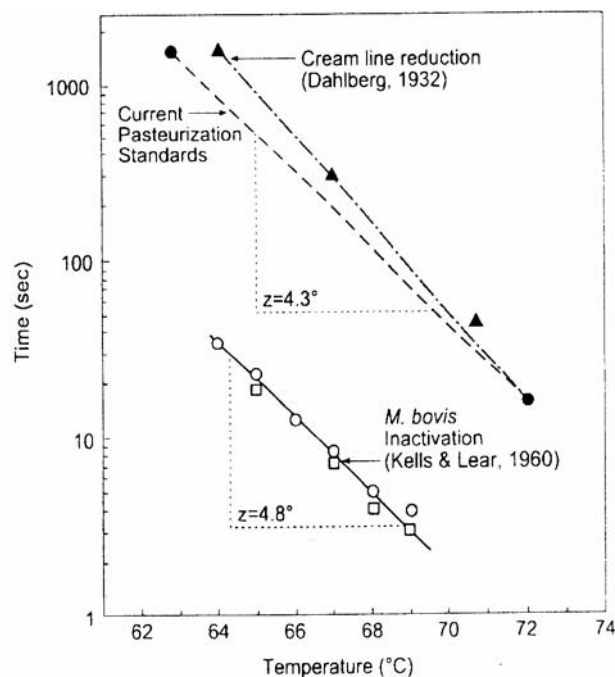


Figure 2.15. The thermal death time curve for *M. bovis* ATCC 11756, based on the inactivation data of Kells and Lear (1960) [(○)= high clumping; (□) = low clumping] relative to the current pasteurisation standards (63°C for 30 minutes; 72°C for 15 seconds) and the cream line reduction curve developed by Dahlberg (1932) [after Pearce (2004)].

Summary

M. bovis is destroyed by both batch and HTST pasteurisation. However, *M. bovis* is not destroyed by thermisation 62°C for 15 seconds. Based on extrapolation of data from Kells and Lear (1960), the D value at 62°C of *M. bovis* is 90 seconds. Kinetic data for this organism is limited.

(c) *Mycobacterium avium subsp. paratuberculosis* (*Mycobacterium paratuberculosis*, MAP, Mptb)

Background

Johne's disease (JD) is a chronic, granulomatous enteritis of ruminants and camelids caused by *Mycobacterium avium subsp. paratuberculosis* (*M. paratuberculosis*). Typically, there is a long incubation period, with clinical disease usually only occurring in older animals. There is no effective treatment and affected animals become emaciated and eventually die or are destroyed (Animal Health Australia, 2005d).

There are two distinct sub-types of *M. paratuberculosis*, one with a host preference for cattle (BJD), and the other for sheep (OJD). Although Johne's disease in cattle and sheep are regarded as separate entities, cross-over between the species has been found to occur sporadically. Goats, deer and camelids may be affected by either sub-type of *M. paratuberculosis* (Animal Health Australia, 2005d).

Johne's disease has a worldwide distribution. BJD was first recorded in Australian cattle over 70 years ago. Most of Australia and the majority of Australian cattle herds are not infected with BJD. However the disease is endemic in the high rainfall zones and irrigated districts of south-east Australia, mainly in dairy herds. It now occurs in the states of Victoria, Tasmania, South Australia and parts of New South Wales. Approximately 1350 cattle herds were known to be infected in

south-east Australia early in 2005. While most common in dairy herds, the disease also occurs in beef cattle, goats, deer and alpacas (Animal Health Australia, 2005d).

Western Australia was declared a BJD-Free Zone in August 1999. Queensland and the Northern Territory have traditionally also been free of the disease and have been declared Protected Zones, along with a large part of New South Wales (Animal Health Australia, 2005d).

National eradication of BJD is not technically feasible or economically justified in the foreseeable future but a national approach to BJD management is being taken to reduce the spread of BJD to other herds and to other parts of the country (Animal Health Australia, 2005d).

The reader is referred to the Overview at the beginning of this section of the report (2.3.8) for the reasons why the dairy industry has an interest in the heat resistance of *M. paratuberculosis*. The organism now officially known as *Mycobacterium avium subsp. paratuberculosis* (or simply as *M. paratuberculosis*, for reasons of convenience) was first officially designated a separate species, initially known as *Mycobacterium paratuberculosis*, in 1923. Since then, it has been successively officially named *Mycobacterium enteritidis* (1927), *Bacterium paratuberculosis* (1929), *Bacillus paratuberculosis* (1941) and *Mycobacterium johnei* (1943), with its current name adopted post-1974 (Buchanan and Gibbons, 1974).

Heat resistance

An extensive literature on the heat resistance of *M. paratuberculosis* has accumulated, with much of it published in the last decade or so. For example, a complete Bulletin of the International Dairy Federation published in 2001 (No. 362) was devoted to *M. paratuberculosis* (International Dairy Federation, 2001), an extensive review of the heat resistance of *M. paratuberculosis* was published by Lund *et al.* in 2002, and a Bulletin of the International Dairy Federation published in 2004 (No. 392) included extensive proceedings of an international workshop on the heat resistance of *M. paratuberculosis* (International Dairy Federation, 2004).

Lund *et al.* (2002) highlighted some of the many problems that have been encountered in the conduct of heat resistance studies on *M. paratuberculosis* and in the interpretation of the results from these studies. These include:

- slow growth rate of *M. paratuberculosis* in laboratory media making it susceptible to being overgrown by other heat resistant bacteria that may be present and by contaminants generally;
- the fastidious nature of *M. paratuberculosis* in relation to its cultural conditions, requiring sophisticated cultural techniques;
- the hydrophobic nature of the *M. paratuberculosis* cells;
- the tendency of *M. paratuberculosis* to form large clumps, which may have a protective effect during the heat treatment and also interferes with quantitative enumeration of survivors;
- different methods of preparing inoculum and different inoculation rates; and
- different methods of carrying out heat treatments.

Results of seven studies on the heat resistance of *M. paratuberculosis* conducted by several different research groups and published between 1993 and 1999 were collated by Lund *et al.* (2002). The collated data are reproduced in Table 2.11. Type of heating procedure is taken from Pearce (2004).

Table 2.11. Comparison of estimates, by several groups of workers, of the lethality of heat treatment of *M. paratuberculosis* in milk [after Lund *et al.* (2002); Pearce (2004)].

Reference	Heat treatment				
	63°C for 30 minutes		72°C for 15 seconds		
	Inoculum, mL ⁻¹	No. of decimal reductions	Heating procedure	Inoculum, mL ⁻¹	No. of decimal reductions
Chiodini and Herman-Taylor (1993)	10 ⁴	<2	Test tubes	10 ⁴	<2
Grant <i>et al.</i> (1996b)	10 ⁶⁻⁷	5-6	Lab scale HTST apparatus	10 ⁶⁻⁷	4.3-6
	10 ³⁻⁴	2-3.7		10 ³⁻⁴	2-3.7
Grant <i>et al.</i> (1999)	-		Lab scale HTST apparatus	10 ⁵⁻⁶	5.6-6
Hope <i>et al.</i> (1996)	-		Pilot plant, laminar flow	<10 ⁵ (a)	~5
Stabel <i>et al.</i> (1997)	-		Test tubes	10 ⁶⁻⁷ (b)	0.5-3
	-		Armfield lab pasteuriser	10 ^{5.5-6} (c)	>4.5-5
Sung and Collins (1998)	10 ⁵⁻⁶ (d)	>6	Capillary tubes	10 ⁵⁻⁶	1-2
Keswani and Frank (1998)	10 ⁶⁻⁷	>10	Capillary tubes	10 ⁵	~4

(a) Because of laminar flow, 25 second heating time was needed so that every particle of milk would be heated at 72°C for at least 15 seconds.

(b) Heated in tubes.

(c) Heated in flow through pasteuriser with laminar flow.

(d) Heated at 62°C.

From Table 2.11 it is evident that the different groups of workers reported contrasting estimates of the effect of heat treatment on *M. paratuberculosis*. In three of the reports, it was estimated that heating at 63°C for 30 minutes gave more than five decimal reductions, while the two other reports that included data for this treatment gave decimal reductions of between 1 and 3.7 from inoculum levels between 10³ and 10⁴ per ml. A survival curve at 63.5°C typical of the 11 strains studied by Grant *et al.* (1996b) showed reduction in viable numbers of approximately 6D at that temperature when the inoculum level was between 10⁶ and 10⁷ per mL (refer Figure 2.16). Tailing was then evident, with low number surviving after 30 minutes. However, other data from Grant *et al.* (1996b) showed that a reduction of only 2-3.7D was achieved when the starting concentration was between 10³ and 10⁴ per mL.

Lund *et al.* suggested that the tailing was most likely due to artifactual reasons related to method, rather than clumping. (There is ample evidence presented elsewhere in this review to indicate that some methods, particularly those that rely on heating in test tubes, give unreliable results.) Lund *et al.* also argued that, when the mathematical basis of heat inactivation was considered, the evidence for clumping as the cause of the tailing was weak. In contrast, Klijn *et al.* (2001) used a sophisticated mathematical modeling technique to demonstrate that while tailing is not due to the presence of a more heat resistant cell fraction, clumping might be a contributing factor.

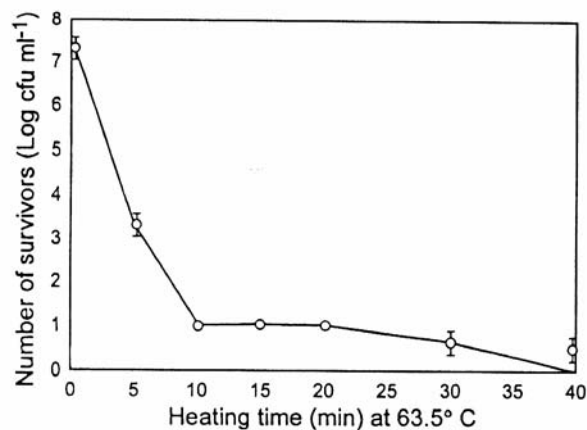


Figure 2.16. A survival curve at 63.5°C typical of the 11 strains of *M. paratuberculosis* studied by Grant *et al.* (1996b) [after Lund *et al.*, 2002]

Five of the nine sets of data listed in Table 2.11 estimated that heating at 72°C for 15 seconds gave more than a 4D reduction. In at least two sets of data, the decimal reduction achieved was limited by the inoculum level. The finding that 4.3-6 decimal reductions occurred from an inoculum level of 10^{6-7} per mL, but only 2-3.7 reductions occurred from an inoculum level of 10^{3-4} , was most likely also due to artifactual reasons, rather than clumping, according to Lund *et al.* (2002).

Analysis by Lund *et al.* of several studies on the effect of clumping failed to show any statistical differences in the heat resistance of ‘clumped’ and ‘declumped’ cells of *M. paratuberculosis*. Lund *et al.* (2002) also noted that ingestion of *M. paratuberculosis* cells by macrophages did not protect them from HTST treatment.

Lund *et al.* also examined the issue of increasing the time and/or temperature of heating on the survival of *M. paratuberculosis*. One of the studies reviewed by them showed, for example, that a greater lethal effect on *M. paratuberculosis* was achieved by increasing the heating time at 72°C from 15 to 25 seconds than by increasing the heating temperature from 72 to 90°C. However, using the principles of kinetic theory, they demonstrated that this finding was almost certainly erroneous. Rather, and as also pointed out by some other workers, much greater lethal effect will always be expected by increasing the temperature of heating over this range than by increasing the time of heating. Lund *et al.* assumed that the D value of *M. paratuberculosis* at 72°C was three seconds (a reasonable assumption from the data in Table 2.11) and the z value is either 4, 6, 8, or 10°C [covering the range for most vegetative bacteria given by Stumbo (1973) and the value of 7.11°C for *M. paratuberculosis* in milk reported by Sung and Collins (1998)] and calculated the number of decimal reductions that would be obtained by increasing time or temperature of heating from the equation for lethal rate. The results of these calculations are given in Table 2.12.

From their review, Lund *et al.* concluded that there was still a need to establish, perhaps with different methodology, that HTST pasteurisation in particular was adequate to ensure destruction of *M. paratuberculosis* in sufficient numbers to prevent infection, with an acceptable margin of safety. They also suggested that there was a need to set a Performance Criterion for pasteurisation of milk in relation to this bacterium. In addition, they suggested that the commonly used phosphatase test might not be adequate to ensure that pasteurised milk was completely free of unpasteurised milk, as it has a detection limit of 0.05-0.1% raw milk.

Table 2.12. Calculated effect on survival of *M. paratuberculosis* in milk from changing the time and temperature of heating [after Lund *et al.* (2002)].

Temperature / time of heating	Number of decimal reductions if z =			
	Z = 4	Z = 6	Z = 8	Z = 10
72°C for 15 sec	5	5	5	5
72°C for 25 sec	8.3	8.3	8.3	8.3
75°C for 15 sec	28	16	12	10
80°C for 15 sec	500	108	50	31
90°C for 15 sec	158,113	5,000	889	315

A report on a New Zealand study of the heat resistance of five strains of *M. paratuberculosis* (ATCC type strain, human isolate and three bovine isolates), using a pilot scale HTST pasteuriser with validated turbulent flow (Reynolds Number, 11,050), was published shortly after the review by Lund *et al.* had been completed [Pearce *et al.* (2001); Pearce *et al.*, (2004); Pearce (2004)]. Heat resistance of the five strains was similar. All strains survived 63° for 15 seconds; mean $D_{63\text{C}}$ for the five strains was 15.0 ± 2.8 seconds. Only one survived 69°C for 15 seconds. No strain survived 72°C for 15 seconds; mean $D_{72\text{C}}$ for the five strains was <2.03 seconds, equivalent to a >7 log kill. The mean z value for the five strains was 8.6°C.

When milk was inoculated with fecal material from a cow that was a high level shedder of *M. paratuberculosis* by Pearce *et al.* (2001), a log kill >4 was obtained with heating at 72°C for 15 seconds.

From their work, Pearce *et al.* concluded that pasteurisation of milk 72°C for 15 seconds using properly maintained and operated commercial equipment should ensure the absence of viable *M. paratuberculosis* organisms in retail milk and other pasteurised dairy products. However, they also noted that the widespread commercial practice of pasteurising 1-2°C above 72°C is an additional safeguard.

However, studies by Hammer *et al.* (2004) on the heat resistance of *M. paratuberculosis* in artificially-infected milk using a pilot scale commercial HTST system produced less definitive results. They found low numbers of survivors in all samples following heat treatment at a range of time and temperatures, including 45 experiments using 18-30 seconds at 72-75°C (3-6 \log_{10} reduction obtained) and 48 experiments using 40-60 seconds at 72-90°C (4-6 \log_{10} reduction obtained). There was no difference in the results with phagocytized and free *M. paratuberculosis* cells.

Stabel and Lambertza (2004) reported on a study designed to evaluate the effectiveness of the recognised heat treatments for both the holder (batch) and HTST methods on the destruction of *M. paratuberculosis*. One hundred eighty experiments were conducted in this study using a two-phase, slug-flow, laboratory-scale heat exchanger (allows precise control over residence time, plus some turbulence) and a laboratory scale HTST pasteurizer unit. Milk that had been previously 'commercially sterilised' by UHT processing was inoculated with three different field strains of *M. paratuberculosis* at two concentrations, 10^5 and 10^8 CFU per mL. Five different time-temperature combinations were evaluated: 62.7°C for 30 minutes (U.S. standard for batch pasteurisation), 65.5°C for 16 seconds (a thermisation treatment), 71.7°C for 15 seconds (U.S. standard for HTST pasteurisation), 71.7°C for 20 seconds and 74.4°C for 15 seconds. Three replicates of each experiment were run for the pasteurizer unit, time-temperature combination, and strain of *M. paratuberculosis*. Treatment of milk as per minimum pasteurisation requirements or higher, regardless of bacterial strain or type of pasteurizer unit, resulted in an average 5.0- and 7.7- \log_{10} kill

for the low and high concentrations of inoculum, respectively. However, milk heat treated for cheese production (65.5°C for 16 seconds) resulted in a much lower and more variable kill.

Stabel and Lambertza concluded that the results of their study indicated that the current U.S. minimum standards for batch and HTST pasteurization of grade A milk significantly reduced the survivability of *M. paratuberculosis*, but some bacteria survived the sub-pasteurization heat treatment of milk used for cheese manufacture. These findings were broadly consistent with those reported by Pearce *et al.* (2001) (see above) and McDonald *et al.* (2005) (see below).

Grant (2004) reported that in three surveys of commercially pasteurised milk samples, two of which were conducted in the UK and one in Ontario, low levels of *M. paratuberculosis* were found in only one of them: there was evidence of viable *M. paratuberculosis* organisms in 1.8% of samples from a UK-wide milk survey (see above under 'Overview' for more information). Grant observed, however, that the methodologies used in the three surveys differed quite markedly.

In addition, Grant (2004) also reported on a study that involved commercial-scale pasteurisation of milk likely to be naturally-infected with *M. paratuberculosis* over a period of 12 weeks, with heat treatment at 73°C for 15 or 25 seconds with and without prior homogenization as variables. A total of 6.9% of the samples of the heat treated milk contained viable *M. paratuberculosis*, with each of the four treatments represented in the 6.9% of positive samples. Grant concluded that these results were evidence that *M. paratuberculosis* in naturally infected milk is capable of occasionally surviving commercial pasteurisation, if they are present in high enough numbers.

An extensive Australian study on the incidence of *M. paratuberculosis* in raw milk supplies and on the heat resistance of *M. paratuberculosis* under commercial pasteurisation conditions was conducted during 1999 and 2000 with the support of the Dairy Research and Development Corporation/Dairy Australia Limited [McDonald *et al.* (2000); McDonald *et al.* (2002); McDonald *et al.* (2005)]. This study identified the effectiveness of pasteurisation and the concentration of *M. paratuberculosis* in raw milk as the most critical factors influencing the potential presence of the organism in pasteurised milk and dairy products. A quantitative assessment of the lethality of pasteurisation was undertaken using an industrial HTST pasteuriser with a validated Reynolds number of 62,112 [satisfying the criterion of >26,000 for turbulent flow in commercial pasteurisers quoted by Grant (2004)] and a flow rate of 3,000 litres per hour. Raw milk was inoculated with a mixture of four field isolates of *M. paratuberculosis* strain C1 or a mixture of five field isolates of strain C3, then homogenised, pasteurised and cultured using a sensitive technique capable of detecting one organism per 10 ml of milk. Twenty batches of milk containing 10^{3-4} organisms per mL prior to homogenisation were processed using combinations of three temperatures, 72, 75 and 78°C, and three holding times, 15, 20 and 25 seconds. Homogenisation increased the count of *M. paratuberculosis* prior to heat treatment by a factor of about 10 (ie 1 log₁₀).

In 17 (85%) of the 20 processing runs carried out by McDonald *et al.* (2005) no viable *M. paratuberculosis* organisms were detected after any of the heat treatments, representing >6-log₁₀ reductions during pasteurisation. However, in three (15%) of the processing runs, where milk was processed at 72°C for 15 seconds, 75°C for 15 seconds and 78°C for 15 seconds, viable organisms were detected but only at very low levels of detection (maximum of 1 CFU of *M. paratuberculosis* per 250 mL of milk in one case; maximum of 1 CFU per 500 mL of milk in the other two cases). In these three cases, 4-6 log₁₀ reductions of *M. paratuberculosis* occurred during pasteurisation. Thus, overall, the absolute minimum log₁₀ reduction in *M. paratuberculosis* organisms that was achieved with pasteurisation was >4.

Based on surveys of cattle and farm milk supplies in Victoria conducted as part of the above study, McDonald *et al.* (2000) concluded that the levels of *M. paratuberculosis* entering raw milk storage

silos at processing factories were likely to be quite low, probably not exceeding 100 (10^2) organisms per mL. On this basis, they further concluded that a $>4\text{-log}_{10}$ reduction of *M. paratuberculosis* during pasteurisation (see above) in fact provides a reasonable margin of safety.

Klijn *et al.* (2001) reviewed the methodologies that had been used to study the heat resistance of *M. paratuberculosis*. They came to the conclusion that it continued to be very difficult to compare the results of different studies, because of differences in methodologies, and made a plea for a more consensual approach. Studies to date had shown that critical parameters in heat inactivation experiments were: strains and culture conditions; application of heat; data sets; and quantification of colony-forming units. Kinetic data continued to be largely limited to that obtained using simulation of holder (batch) pasteurisation. They noted that in studies using continuous flow pasteurisation, generally only end-point measurements were performed, which cannot be used to calculate reliable inactivation kinetics.

Summary

The interest of the dairy industry in various countries including Australia in the heat resistance of *M. paratuberculosis* has been largely driven by three things: (i) a suggestion - so far unproven - that *M. paratuberculosis* infection might be associated with the incidence of Crohn's disease in humans; (ii) data from surveys of commercially pasteurised milk samples conducted in the UK indicating that low levels of *M. paratuberculosis* were found in some samples in some of these surveys (a result that does not necessarily mean that the *M. paratuberculosis* in the samples were heat resistant; there are other possible explanations); and (iii) knowledge that *M. paratuberculosis* can be present in raw milk supplies as a consequence of Johne's disease in cattle (still widespread amongst dairy herds in southeastern Australia).

However, *M. paratuberculosis* has proved to be a difficult organism to work with and some of the laboratory studies on its inactivation by heat treatment, particularly at 72°C for 15 seconds (the temperature and time used in HTST pasteurisation), have given conflicting results. Some studies have shown low levels of survivors, which some reviewers argue are an artifact of the method that was used to study heat resistance.

However, a well-controlled study by Pearce *et al.* (2004) in New Zealand using pilot scale HTST equipment with validated turbulent flow showed that a 7D kill of *M. paratuberculosis* in milk heated to 72°C for 15 seconds was obtained with no survivors. Pearce *et al.* noted that increasing the heating temperature by 1-2°C above 72°C, a common commercial practice, would provide an additional margin of safety. A similar study in Australia by McDonald *et al.* (2005) showed that in most cases, pasteurisation using commercial HTST equipment with a Reynolds number $>62,000$ gave $>6\text{-log}_{10}$ reduction of *M. paratuberculosis*, though in a few cases only a $>4\text{-log}_{10}$ reduction was achieved. However, with studies showing that the levels of *M. paratuberculosis* entering raw milk storage silos at processing factories in Victoria were unlikely to be exceed 10^2 organisms per mL, a $>4\text{-log}_{10}$ reduction of *M. paratuberculosis* during pasteurisation in fact provides a reasonable margin of safety for the consumer.

M. paratuberculosis does not survive batch pasteurisation at 63°C for 30 minutes, but would survive thermisation at 62°C for 15 seconds.

2.3.9 *Salmonella* spp.

Background

The primary reservoir of the bacteria belonging to the genus *Salmonella* is the intestinal tract of vertebrates. They are widely distributed in nature. The type species is listed as *Salmonella*

cholerae, first isolated from pigs in 1885. Salmonellosis is one of the most important public and animal health problems worldwide. It is a communicable disease readily transmissible to man from animal directly or via contaminated products of plant or animal origin. Serovars (serotypes) of *Salmonella* vary widely in their pathogenicity and some are host specific (Jay *et al.*, 2003).

There has been much confusion and controversy over the taxonomy and nomenclature of the *Salmonella* group over the years. Two forms of nomenclature are currently in use. The first follows traditional usage where *Salmonella* is given as the genus and serovars are given species status, e.g. *Salmonella typhimurium*. While this system is now officially outdated, it is still commonly used in the literature. The other form is where the *Salmonella* genus contains only two species: *Salmonella enterica* divided into six subspecies and *Salmonella bongori*. Only subspecies I serovars are given serovar names, which are written like a species name but not in italics, as they no longer have species status. However, proper use of this system can be cumbersome and the names are often abbreviated for general use, e.g. *Salmonella enterica* subsp. *enterica* serovar Typhimurium is conveniently recorded as *Salmonella* Typhimurium or *S.* Typhimurium (Jay *et al.*, 2003).

The discovery of *Salmonella* Newbrunswick in dried milk powder prior to 1968 generated interest in the heat resistance of the *Salmonella*. The aim was to determine whether they were in fact capable of surviving pasteurisation (Read *et al.*, 1968).

Outbreaks of human salmonellosis have underlined the importance of milk and milk products as vehicles for spread of infection. *Salmonella* Heidelberg, Muenster and Typhimurium have all been the cause of outbreaks of illness following consumption of cheese manufactured from raw or improperly pasteurised milk. There was also a large outbreak in Illinois involving 14,000 cases of illness following consumption of raw milk contaminated with *Salmonella*. The observation that *Salmonella* can survive in cheese for periods exceeding the mandatory 60-day refrigerated storage period for cheese manufactured from non-pasteurised milk has also been a concern (D'Aoust *et al.*, 1987).

Certain serotypes of *Salmonella enterica* are notorious for their resistance to thermal treatments, the most prominent being *Salmonella* Seftenberg 775W (Doyle and Mazzotta, 2000). However *Salmonella* Seftenberg is relatively rare (ESR Ltd, 2001b).

Although *Salmonella* Seftenberg 775W is not an important foodborne pathogen, it is often used as a test organism, the implication being that if a particular thermal process destroys it, the process will also be effective against more the common types of salmonellae (Doyle and Mazzotta, 2000). Salmonellae other than *Salmonella* Seftenberg 775W are not particularly resistant to heat (ESR Ltd, 2001b).

Examination of thermal death curves of salmonellae reveals a distinct tailing or biphasic inactivation kinetics under some conditions. This may indicate the presence of two populations of otherwise genetically identical cells, one more heat resistant than the other (Doyle and Mazzotta, 2000). Another explanation is the result of faulty technique as demonstrated elsewhere in this report.

Heat resistance

Studies on the thermal resistance of the salmonellae were extensively reviewed by Doyle and Mazzotta (2000). They reported that heat resistance of salmonellae in dairy products, particularly milk, has been investigated by a number of researchers. However, some of the early reports were

based on survivor curves with extensive tailing, which puts a question mark over the validity of their kinetic data.

Increasing the solids content of milk increases the heat resistance of *Salmonella*. For example, in one study cited by Doyle and Mazzotta, the $D_{55\text{C}}$ value increased from 4.7 minutes at 10% total solids to 18.3 minutes at 42% total solids.

D values for the destruction of salmonellae in raw milk from two of the more recent studies on this topic were collated by Doyle and Mazzotta (2000). These data are reproduced in Table 2.13. Additional information from the two original papers from which these data were drawn is provided below.

The data of Bradshaw *et al.* (1987b) summarised in Table 2.13 indicates that *Salmonella* Typhimurium would be destroyed by batch pasteurisation at 62.8°C with a wide margin of safety (approximately 270D kill). Bradshaw *et al.* calculated D values for this organism at 71.7°C - by extrapolation - of 0.24 seconds for the human isolate and 0.22 seconds for the milk isolate. These values indicate that *Salmonella* Typhimurium would also be destroyed by HTST pasteurisation at 71.7°C for 15 seconds, again with a wide margin of safety (65D kill). Bradshaw *et al.* confirmed the effectiveness of pasteurisation by subjecting the two of their most heat resistant strains in each group to heat treatment in a heat exchanger; D values were equivalent to or less than the extrapolated values. The z value of 5.3°C for *Salmonella* Typhimurium was consistent with the values for most other vegetative bacteria.

Table 2.13. Summary of the more recent available data on the thermal resistance of *Salmonella* in raw milk [after (Doyle and Mazzotta, 2000)].

<i>Salmonella</i> serotype	D value (minutes) at temperature specified (°C)										z value (°C)
	51.8	57.2	60.0	61.5	62.8	63.0	64.5	67.5	68.3	71.7	
Typhimurium, from milk ^a	21.1	1.7			0.11				0.015		5.3
Typhimurium, from human ^a	24.0									0.004	5.3
Human mix ^b			0.058	0.046							
			0.098	0.059							
Non-human mix ^b			0.068	0.054		0.049	0.034				
Seftenberg 775W ^b			0.122	0.107		0.067	0.067	0.046			
Muenster ^b			0.097	0.051		0.038					
			0.058								

^a Data of Bradshaw *et al.* (1987b). Outbreak-associated strains isolated from milk or humans were cultured, added to milk and heated in small glass tubes. See text below for further details.

^b Data of D'Aoust *et al.* (1987). Human mix contained *Salmonella* serotypes: Typhimurium, Infantis, Hadar, Agona, Enteritidis, Heidelberg, Newport, Saint-paul, Thompson and Schwarzengrund. Non-human mix included *Salmonella* serotypes: Muenster, Kentucky, Anatum, Montevideo, Mbandaka, Albany, Brandenburg, Seftenberg, Newington and California. In each case, cultures were grown up and added to a bulk tank containing 1200 L of raw whole milk to a final concentration of 10⁵ per mL. The inoculated milk was then heat treated in a pilot scale continuous flow regenerative HTST plate pasteuriser at temperatures in the range 60-74°C with a minimum residence time in the holding tube of 16.2 seconds and a mean residence time of 17.6 seconds. See text below for further details.

Based on the data of Bradshaw *et al.*, thermisation at 62.8°C for 15 seconds, for example, would achieve only a 2.3D kill, less at 62°C.

The D values from the report of D'Aoust *et al.* (1987) listed in Table 2.13 were calculated using the following formulae, based on first order reaction kinetics:

$$k = [2.303 \log (a/b)] / t] \quad \text{and} \quad D = 2.303/k$$

where a = initial number of cells;
b = number of surviving cells at time t;
t = heating time (17.6 seconds); and
D = time to destroy 90% of cells

D'Aoust *et al.* found that thermal processing at >64.5°C for a minimum holding time of 16.2 seconds was effective in reducing the numbers of all test organisms (see Table 2.13) from an initial population level of $1-5 \times 10^5$ per mL to undetectable levels, with one exception. *Salmonella* Seftenberg 775W survived a heat treatment of 67.5°C, albeit in low numbers, for a minimum of 16.2 seconds, but was undetectable after similar treatment at 68.3°C and higher. D values at 60°C for all strains except Seftenberg 775W were in the range 0.058-0.098 minutes and, at 63°C, 0.037-0.061 minutes, comparable to values reported by other workers. However, $D_{66\text{C}} = 0.050-0.054$ minutes for *Salmonella* Seftenberg 775W was lower than that reported by some other workers (0.56-1.00 minutes at 65.6°C).

Based on the data of D'Aoust *et al.*, pasteurisation by both batch and HTST methods would ensure destruction of all *Salmonella* strains, including Seftenberg 775W, with a very wide margin of safety. However, thermisation at 62°C for 15 seconds would destroy some strains but only have a partial effect on others.

In an earlier study, Read *et al.* (1968) studied the heat resistance of several serotypes of *Salmonella* in whole milk using a thermoresistometer. The serotypes were Anatum, Binza, Cubana, Meleagridis, Newbrunswick and Tennessee (isolated from dried milk), and Seftenberg 775W. Excluding Seftenberg 775W, D values were $D_{62.8\text{C}} = 3.6-5.7$ seconds, $D_{65.6\text{C}} = 1.1-1.8$ seconds and $D_{68.3\text{C}} = 0.28-0.52$ seconds. For Seftenberg 775W, $D_{65.5\text{C}} = 34.0$ seconds, $D_{68.3\text{C}} = 10.0$ seconds, $D_{71.7\text{C}} = 1.2$ seconds and $D_{73.9\text{C}} = 0.55$ seconds. Read *et al.* concluded that pasteurisation of milk in accordance with the standards recommend by the U.S. Public Health Service (see Section 2.1) will inactivate all seven strains of *Salmonella* studied, provided the initial concentration of salmonellae does not exceed 3×10^{12} per mL.

Thomas *et al.* (1966) concluded that *Salmonella* Seftenberg 775W, because of its relatively high heat resistance, provided a good reference strain when determining safe heat treatments in food processing. Any heat treatment necessary to eliminate it are almost certain to kill any other salmonellae and also any staphylococci present.

Thomas *et al.* cautioned against extrapolating thermal death time curves to more than 5.6°C above the highest temperature for which actual test data was available.

Summary

All salmonellae, even the most heat resistant serotype *Salmonella* Seftenberg 775W, are destroyed by both batch and HTST pasteurisation with a wide margin of safety. However, effectiveness of a thermisation treatment at 62°C for 15 seconds would depend on *Salmonella* strains and numbers present in the milk. For practical purposes, it would have to be assumed that thermisation might reduce the numbers of salmonellae present in the raw milk, but would not totally eliminate them.

2.3.10 *Staphylococcus aureus*

Background

Staphylococcus was first named by Ogston in 1882, after it was discovered in pus from human abscesses. It was first grown in pure culture in 1884 and the orange colony-forming coccus was named *Staphylococcus aureus*. The organism was associated with a large outbreak of food poisoning in Michigan in 1884, believed to have been caused by consumption of cheese, but its role was not confirmed (or believed) by investigators despite – in retrospect – clear evidence. It was not until 1914 that staphylococcal food poisoning was clearly demonstrated; in this case the food poisoning was caused by consumption of stored, un-refrigerated raw milk from a cow that had staphylococcal mastitis. Since that time, *S. aureus* has become known as one of the most common causes of foodborne illness (International Commission on Microbiological Specifications for Food, 1996e; Stewart, 2003).

Staphylococcal food poisoning is caused not by ingestion of the organism itself, but by a filterable enterotoxin produced by *S. aureus* growing in the food under suitable conditions prior to consumption. Thus absence or low numbers of *S. aureus* in a heat treated food product does not guarantee its safety; absence of the enterotoxin must also be demonstrated. Species of *Staphylococcus* other than *S. aureus* can produce enterotoxins, but the overwhelming majority of staphylococcal food poisoning outbreaks have been caused by *S. aureus* (Stewart, 2003).

It is generally agreed that the thermal stability of the enterotoxins produced by *S. aureus* greatly exceeds that of its vegetative cells. For example, while the vegetative cells are readily killed at pasteurisation temperatures, the enterotoxins can survive the process used to sterilise low acid canned foods. As a comparison, the D values for vegetative cells at 60°C are reported to be in the range of 0.43-8.0 minutes; however to gain a reduction in toxin activity of similar scale, a heat treatment of 121°C for 3-8 minutes would be required. For this reason, it is advisable that heat-treated foods be examined for the presence of residual toxin in addition to, or instead of, viable cells of *S. aureus* (Stewart, 2003).

S. aureus has high heat resistance for a mesophilic non-sporing bacterium. Its heat resistance is higher in foods with a lower water activity (Stewart, 2003).

The primary source of staphylococci in milk is cows with a mastitic infection (Firstenberg-Eden, *et al.*, 1977). The mean count of coagulase-positive *S. aureus* in raw milk from a group of 36 Queensland farms with a history of high somatic cell counts was 2,200 per mL, with a range from <100 per mL to 90,000 per mL (Juffs *et al.*, 1982). Sheep's milk may contain up to 10^6 *S. aureus* per g (Firstenberg-Eden, *et al.*, 1977). *S. aureus* has been isolated from milk from goats with subclinical mastitis (Mallikeswaran and Padmanaban, 1989).

The following review is confined to the heat resistance of the vegetative cells of *S. aureus*.

Heat resistance

One of the first reports on the heat resistance of *S. aureus* in dairy products was by Bryan and Bryan (1944), who studied heat resistance of the organism in cream. A source of contaminated cream was obtained by injecting the test organism into different quarters of the udder of a dairy cow. Half of the cream obtained from the milk from the cow over a period of a week was pasteurised in the laboratory by a batch process [145°F (62.7°C) for 30 minutes]. The other half was not heat treated. Ripening and salting were additional variables. Butter was made from the cream in a small hand churn and stored at 45°F (7.2°C). *S. aureus* survived in the butter made from unripened cream (both salted and unsalted) and in ripened cream (both salted and unsalted) for at

least six months. Twice weekly examination of the butter made from the pasteurised cream failed to detect any surviving *S. aureus* at any stage, indicating that the organism had been destroyed by the heat treatment under the conditions described. However, it must be noted that Bryan and Bryan did not provide any indication of the population levels of the test organisms in the product prior to heat treatment.

Thomas *et al.* (1966) studied the heat resistance of two strains of *S. aureus* in pasteurised skim milk diluted with an equal quantity of sterile distilled water. An open flask method was used. Heat treatments were in the range 60-68.3°C and initial level of inoculum was 10^{7-8} per mL. Calculated D values for *S. aureus* MS149 were $D_{60\text{C}} = 3.28$ minutes and $D_{65.6\text{C}} = 0.39$ minutes, with a z value of 6.04°C. Calculated D values for *S. aureus* 196E were $D_{60\text{C}} = 3.44$ minutes and $D_{65.6\text{C}} = 0.28$ minutes, with a z value of 5.10°C. Both strains would thus be destroyed by batch pasteurisation at 63°C for 30 minutes, with a wide margin of safety. However, as previously mentioned, Thomas *et al.* cautioned against extrapolating thermal death time curves to more than 5.6°C above the highest temperature for which actual test data was available.

Thomas *et al.* (1966) also suggested that *Salmonella* Seftenberg 775W, because of its relatively high heat resistance, provided a good reference strain when determining safe heat treatments in food processing for *S. aureus*. Any heat treatment necessary to eliminate *Salmonella* Seftenberg 775W is almost certain to kill any staphylococci present.

Walker and Harmon (1966) examined the thermal resistance of four coagulase-positive strains of *S. aureus* in whole milk, skim milk, Cheddar cheese whey and phosphate buffer. Two of the strains (161-C and B-120) had been identified as causes of food poisoning outbreaks, and two had been isolated from milk from cows with subclinical mastitis. Heating was carried out in a laboratory apparatus that simulated batch pasteurisation on a small scale (200 mL of heating medium, continuously agitated). Temperatures were in the range 52-62°C and initial inoculum level was in the range $6-16 \times 10^6$ per mL. Most of the survival curves showed tailing, particularly at temperatures in the lower end of the range. The authors were satisfied however that the *S. aureus* strains all exhibited a logarithmic order of death through the 99.99% to 99.9999% destruction zone, at which time the surviving population numbered about 100-1000 per mL. D values based on the logarithmic (straight) part of the survivor curve are reproduced in Table 2.14.

The data of Walker and Harmon in Table 2.14 indicate that *S. aureus* strains 161-C and S-1 were usually more heat resistant in skim milk and whey than in whole milk and phosphate buffer. Thermal resistance of the four strains varied, but each strain showed a consistent pattern. The organisms would not survive batch pasteurisation at 63°C for 30 minutes. The authors did not extrapolate their data to 72°C.

Walker and Harmon (1966) also showed age of the culture used to inoculate the heating medium can have a marked effect on heat resistance. For example, the D value at 55°C was 0.95 minutes for a 12-hour culture of strain B-120, 2.7 minutes for a 60-hour culture and 3.0 minutes for a 228-hour culture, with all conditions similar.

Stumbo (1973) reported generalised data of $D_{65.6\text{C}} = 0.2-2.0$ minutes and z values of 4.4-6.7°C for *S. aureus* in pasteurised foods. Firstenberg-Eden *et al.* (1977) studied the heat resistance of a strain of *S. aureus* isolated from raw milk using a capillary tube method. The heating medium was nonfat cows' milk, the initial inoculum level was about 10^9 per mL and the heating temperatures were in the range 50-75°C. The D values obtained were as follows:

- 50°C: 9.96 minutes;
- 55°C: 3.11 minutes;

- 60°C: 0.87 minutes;
- 65°C: 0.17 minutes;
- 70°C: 0.10 minutes; and
- 75°C: 0.02 minutes.

Table 2.14. D values from the portion of the survivor curves for four strains of coagulase-positive *S. aureus* representing 99.99% destruction [after Walker and Harmon (1966)].

Strain of <i>S. aureus</i>	Heating temperature (°C)	D values in heating medium as specified (minutes)			
		Phosphate buffer	Whole milk	Skim milk	Cheddar cheese whey
161-C	58	1.80	1.85	3.50	2.70
	59	1.20	-	-	-
	60	0.60	0.75	1.30	1.33
	62	-	0.35	0.20	0.75
S-1	52	2.70	-	-	-
	53	2.10	1.40	2.90	3.00
	54	1.55	-	-	-
	55	1.12	1.25	1.55	1.75
	56	0.60	-	-	-
	57	0.15	0.55	0.70	0.75
B-120	53	1.50	1.00	-	-
	55	1.00	0.70	-	-
	57	0.40	0.40	-	-
S-18	56	2.55	-	-	-
	58	1.75	1.60	-	-
	59	1.00	1.25	-	-
	60	0.43	0.50	-	-

Firstenberg-Eden *et al.* calculated a z value for this organism from different data (but similar to the above) of 9.46°C.

From the data of Firstenberg-Eden *et al.*, it can be concluded that their test strain of *S. aureus* would be destroyed by batch pasteurisation at 63°C for 30 minutes. The D value at 72°C was estimated to be 0.025 minutes, which equates to a 10D kill with a holding time of 15 seconds, i.e. HTST pasteurisation. In relation to thermisation, $D_{62\text{C}} = 0.45$ minutes has been estimated from their data.

El-Banna *et al.* (1983) showed that *S. aureus* grown under stress had higher heat resistance in skim milk than when grown under optimal conditions. For example, the D value of a culture of *S. aureus* grown at 37°C was $D_{60\text{C}} = 2.73$ minutes, whereas when grown at 45°C, $D_{60} = 12.6$ minutes. A similar result was obtained when a number of other foods were used as the heating medium.

The heat resistance of *S. aureus* in goat milk was studied by Parente and Mazzatura (1991). Two strains of *S. aureus* were used: *S. aureus* 237 (obtained from the University of Minnesota) and *S. aureus* BP3, isolated from a raw goat milk cheese produced in Italy. Heat resistance was determined using a modified capillary tube technique, with heating in the range 55-68°C and initial population levels $>10^9$ per mL. Death generally followed first order kinetics (refer Figure 2.17). D values are reproduced in Table 2.15. A z value of $4.83 \pm 0.06^\circ\text{C}$ was calculated for *S. aureus* BP3 and $4.50 \pm 0.05^\circ\text{C}$ for *S. aureus* 237.

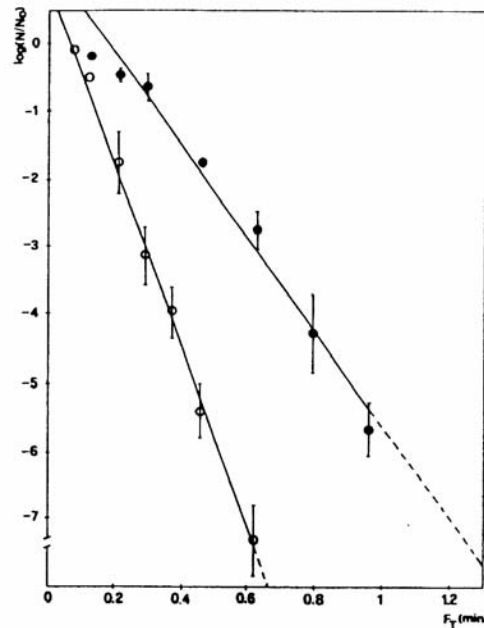


Figure 2.17. Survivor curves for *S. aureus* BP3 (○) and 237 (●) in goat milk at 63°C [after Parente and Mazzatura (1991)].

Table 2.15. D values of *S. aureus* BP3 and 237 in goat milk [after Parente and Mazzatura (1991)].

Temperature (°C)	Experimental D values (minutes)	
	Strain BP3	Strain 237
55	3.30	10.60
60	0.36	0.67
63	0.07	0.15
65	0.03	0.05
68	Not determined	0.01

Parente and Mazzatura concluded that their data confirmed that *S. aureus* is not a particularly heat resistant pathogen and that even high populations would be destroyed by both batch (62.8°C for 30minutes) and HTST (71.6°C for 15 seconds) pasteurisation. However thermisation, even at 63°C for 15 seconds, would achieve a kill of only 1.5-3.5D, depending on the strain.

Summary

S. aureus is destroyed by both batch and HTST pasteurisation with a wide margin of safety. However, a thermisation treatment at 62°C for 15 seconds would not ensure elimination of *S. aureus*; expected kill would be in the range 0.5-3.5D, depending on the heat resistance of the strains present, their numbers and the source of the reference data used to estimate kill.

2.3.11 Pathogenic *Streptococcus* spp.

Background

The significance of the streptococci as a cause of food infections has been recognised since the early 1900s, when scarlet fever and septic sore throat were traced back to the consumption of raw milk contaminated with pyogenic or Lancefield Group A streptococci. Other infections caused by Group A streptococci include tonsillitis, toxic shock syndrome, acute rheumatic fever and acute glomerulonephritis. Data on the heat inactivation of *Streptococcus pyogenes* is scarce and mostly related to commercial pasteurisation of milk. Much of it is also very old, e.g. circa 1914, and does

not conform to present day experimental guidelines (International Commission on Microbiological Specifications for Food, 1996c).

Streptococcus agalactiae, a major cause of bovine mastitis, may be transmitted to humans, especially women, who drink raw milk. It has also been implicated in the death of some infants who died from septic infections of the ileum following consumption of raw cow's milk, though it was not clear to the reviewer whether cause and effect had been unequivocally established. The role of *S. agalactiae*, a member of the Lancefield Group B streptococci, as a food pathogen remains obscure, as the mechanism of alleged foodborne illness is unknown (International Commission on Microbiological Specifications for Food, 1996c).

Juffs *et al.* (1982) found that the mean count of *S. agalactiae* in raw milk from a group of 36 Queensland farms with a history of high somatic cell counts was 2,900 per mL, with a range from <100 per mL to 600,000 per mL.

Streptococcus zooepidemicus (Group C streptococci) has been implicated in several outbreaks of severe human illness in the United Kingdom following consumption of raw milk. For example, the source of an outbreak of infection due to *S. zooepidemicus* was reported as recently as 1984. This outbreak involved the consumption of unpasteurised raw milk from a dairy herd in which mild intermittent mastitis was present. For seven of the 11 identified cases involved in the outbreak, the outcome was fatal (International Commission on Microbiological Specifications for Food, 1996c).

A study conducted in the United Kingdom during the 1980s showed that Group C streptococci accounted for 9-17% of the β -hemolytic isolates from men. About 3% of these isolates were *S. zooepidemicus*, found to have caused septicemia, pneumonia, meningitis and septic arthritis (International Commission on Microbiological Specifications for Food, 1996c).

Human infection with *S. zooepidemicus* (*Streptococcus equi* subsp. *zooepidemicus*) can usually be traced to an animal source. Outbreaks associated with ingestion of unpasteurised milk and cheese have also been described (Lee and Dyer, 2004). For example, raw milk from a house cow on a farm in South-East Queensland was shown to be the source of severe infection in a patient suffering from glomerulonephritis (Francis *et al.*, 1993). Another example is an outbreak of invasive Group C streptococcal infection that occurred in New Mexico in 1983. In this outbreak, *S. zooepidemicus* was isolated from 14 of 16 patients suffering from the infection, the source of which was traced to consumption of 'queso blanco', a homemade white cheese, made on a farm from raw cows' milk. *S. zooepidemicus* was also isolated from the cheese consumed by the patients, the milk used to make the cheese and the udders of the cows from which the milk was drawn (Anon, 2001). Two outbreaks of nephritis and pharyngitis in Europe have also been attributed to *S. zooepidemicus* infection sourced to consumption of unpasteurised milk (Anon., 2001). A point of general interest about *S. zooepidemicus* is that it has been shown to be the most common cause of infection in horses in Western Canada (Dowling 2005).

From 63 samples of milk collected from goats suspected of suffering from subclinical mastitis in India, Mallikeswaran and Padmanaban (1989) isolated *S. agalactiae* (7 strains), *S. pyogenes* (7 strains) and *S. zooepidemicus* (7 strains).

Heat resistance

Studies on the heat resistance of *S. pyogenes* in milk and dairy products up to 1996 were summarised by ICMSF (International Commission on Microbiological Specifications for Food, 1996c).

The earliest study cited by the ICMSF was by Oldenbusch *et al.* (1930). They studied the heat resistance of two strains of β -hemolytic streptococci (*S. pyogenes*) isolated from patients with scarlet fever and septic sore throat in cream and ice cream mix. Sterilised cream containing 50% butterfat was inoculated to an initial level of 9×10^4 per mL, placed in tightly-stoppered glass bottles and submerged in a water bath at the test temperature [range 135°F (57.2°C) to 145°F (62.8°C)]. On completion of heating, cooling was achieved by placing the bottles in crushed ice. The test organisms survived for four but not five minutes or longer at 57.2°C. At 60°C, 61.1°C, 62°C and 62.8°C, they survived for 30 seconds but not for one minute or longer. The same strains, with an initial inoculum level of 4.6×10^4 per mL, survived in ice cream mix for three minutes but not for five minutes or longer when heated at 62.8°C and 65.6°C. It was concluded that batch pasteurisation of cream and ice cream mix at 62°C for 30 minutes provided an ample margin of safety for destruction of β -hemolytic streptococci in cream and ice cream mix.

The ICMSF estimated that, on the basis of the above data for *S. pyogenes* in cream, $D_{60-62.8\text{C}} = 0.125-0.200$ minutes (ie more than 0.125 but less than 0.200 minutes). In ice cream mix, they estimated that $D_{62.8-65.6\text{C}} = 0.75-1.25$ minutes.

Bryan and Bryan (1944) also studied the heat resistance of *S. pyogenes* and *S. agalactiae* in cream. A source of contaminated cream was obtained by injecting the test organisms into different quarters of the udder of a dairy cow. Half of the cream obtained from the milk from this cow over a period of a week was pasteurised in the laboratory by a batch process [145°F (62.7°C) for 30 minutes]. The other half was not heat treated. Ripening and salting were additional variables. Butter was made from the cream in a small hand churn and stored at 45°F (7.2°C). Both pathogens survived in the butter made from unripened cream (both salted and unsalted) and in ripened cream (both salted and unsalted) for at least six months. Twice weekly examination of the butter made from the pasteurised cream failed to detect any surviving *S. pyogenes* or *S. agalactiae* at any stage, indicating that the organism had been destroyed by the heat treatment under the conditions described. However, it must be noted that Bryan and Bryan did not provide any indication of the population levels of the test organisms in the product prior to heat treatment.

Data on the heat inactivation of *S. pyogenes* inoculated into sterile milk reported by Nevot *et al.* (1958) was cited by the International Commission on Microbiological Specifications for Food (1996c). No survivors were detected at 60°C after 3-3.2 minutes, at 62°C after 135-140 seconds, at 65°C after 65-70 seconds, at 70°C after 8-10 seconds, at 72°C after 5-7 seconds, at 75°C after 4-5 seconds, at 78°C after 3 seconds and at 80°C after 2 seconds. Initial counts were not given.

Data on the heat inactivation of *S. pyogenes* in milk reported by Obiger (1976) allowed the ICMSF to estimate that $D_{66\text{C}} = 0.1-0.2$ minutes.

Stumbo (1973) reported generalised data of $D_{65.6\text{C}} = 0.2-2.0$ minutes and z values of 4.4-6.7°C for *S. pyogenes* in pasteurised foods.

Based on the z values and the D value at the upper end of the range as quoted by Stumbo, i.e. $D_{65.6\text{C}} = 2.0$ minutes as a worst case scenario, the ICMSF observed that pasteurisation of milk at 62.7°C for 30 minutes or at 72°C for 15 seconds would ensure only a 1.6-2.3 decimal reduction of *S. pyogenes*. However the ICMSF also observed that if the D value at 66°C was in fact 0.2 minutes, as reported by Obiger (1976), pasteurisation would give about a 20D reduction of *S. pyogenes* in milk.

Weber (1947) reported that pasteurisation of milk containing a minimum of 10^4 per *S. pyogenes* per mL in commercial HTST equipment totally destroyed this pathogen in less than six seconds at

160.8°F (71.5°C). Although the actual time to achieve complete destruction was not determined, Weber noted that the margin of safety with a 15-second holding time would be at least 9 seconds.

Evans *et al.* (1970) reported that heat treatment of milk in a commercial plate pasteuriser at 82.2°C with no holding time would ensure a 15D kill of *S. pyogenes*.

Of 116 hemolytic streptococci isolated from fresh pasteurised milk, 82% were reported to have survived heat treatment in milk at 75°C for 10 minutes⁴ (Petersson and Fonden (1974). However, the authors were uncertain as to the identity of the organisms. They differed from both *S. pyogenes* and *S. agalactiae* in some important characteristics. Also, the method of determining heat resistance and the population levels in the milk before heating were not specified.

Summary

The standard of the available studies on the heat resistance of *S. pyogenes* is variable and some of the available data is based on an empirical approach. Quantitative data where available are not consistent. The most recent report available was published in 1976. Nevertheless, there seems to be sufficient credible data available to indicate that this organism would not survive commercial batch or HTST pasteurisation of milk or cream. However, it would not be destroyed by thermisation at 62°C for 15 seconds.

Only one report on the heat resistance of *S. agalactiae* was located. This indicated - under relatively crude experimental conditions - that the organism was inactivated at unspecified population levels in cream by batch pasteurisation.

No reports on the heat resistance of *S. zooepidemicus* were located.

2.3.12 *Yersinia enterocolitica*

Background

During the early 1990s, *Yersinia enterocolitica* had all the hallmarks of a significant emerging foodborne pathogen in Australia. However, in more recent years, its perceived significance has declined (Barton and Robins-Browne, 2003).

Y. enterocolitica was first described in 1939. It has since become known as an important cause of foodborne disease in many countries, particularly those with a temperate climate. Scandinavia and northern Europe, Japan, parts of North America and New Zealand seem to be most affected. Most cases are sporadic but some outbreaks have been reported. Many foods have been incriminated as a source of infection, but it is now clear that pigs, directly or indirectly, are the primary source of human infections. Infection in humans is associated with a wide range of clinical and immunological symptoms. Self-limiting enterocolitis is the most usual syndrome in humans (Barton and Robins-Browne, 2003).

The frequent association of *Y. enterocolitica* with raw milk and the ability of this organism to grow in milk at refrigerated temperatures have been well documented. However the organism has been isolated from pasteurised milk products infrequently (Lovett *et al.*, 1982).

A report by Hughes (1979) indicating that a few strains of *Y. enterocolitica* isolated from both raw and pasteurised milk in New South Wales might survive commercial pasteurisation contributed to the interest in this organism at that time. Hughes repeatedly isolated *Y. enterocolitica* from pasteurised milk and cream from two milk treatment plants and from two raw milk receiving depots

⁴ While survival for 10 minutes seems improbable, that is the time – correctly or incorrectly - stated in the paper.

in New South Wales. Source of the organisms in all of these cases could not be established initially. However, in the case of one of the raw milk receiving depots, its source was eventually traced back to just one farm, where it was isolated from the milking equipment, milk storage vat, the environment and a farm dam that the cattle waded in and which was a source of water used for cleaning some of the milking equipment. Level of *Y. enterocolitica* in the raw milk from this farm was approximately 2,000 per mL. While isolates consistently survived 'laboratory pasteurisation' in milk containing approximately 10^9 organisms per mL (see below), there was no evidence that any of the *Y. enterocolitica* isolates from this study were pathogenic to man.

Subsequent studies by Hughes (1980) established that the source of *Y. enterocolitica* in commercial samples of pasteurised milk was in fact the pasteurised milk holding vats, i.e. it was a post-pasteurisation contaminant, not a pasteurisation-resistant organism. The organism was able to persist in certain fittings in the holding vats. Isolations of the organism from the pasteurised milk ceased as soon as the sanitizing program was upgraded. Contrary to her earlier statements, Hughes now concluded that *Y. enterocolitica* was unlikely to survive commercial pasteurisation.

The studies by Hughes are a good example of the risks involved in equating, without further investigation, the presence of an organism in pasteurised milk with its ability to survive the pasteurisation heat treatment.

Heat resistance

The heat resistance of five strains of *Y. enterocolitica* in skim milk was studied by Hanna *et al.* (1977). Three of the strains were isolated from meat and two were from the ATCC culture collection. Heating was carried out in sealed glass ampoules, using a shaker-water bath. Inoculation level was 10^{6-7} per mL. While there was considerable variation in the heat resistance of the five strains, the most heat resistant of them was completely inactivated within three minutes at 60°C.

As mentioned above, Hughes (1979) initially reported that strains of *Y. enterocolitica* isolated from both raw and pasteurised milk in New South Wales consistently survived 'laboratory pasteurisation' in milk containing approximately 10^9 organisms per mL and that some strains might survive commercial pasteurisation. Laboratory pasteurisation is in fact a variation of the tube method, shown repeatedly in this review to be a source of unreliable heat resistance data. Hughes subsequently concluded that *Y. enterocolitica* was in fact unlikely to survive commercial pasteurisation (Hughes, 1980).

Three enterotoxigenic and six non-enterotoxigenic cultures of *Y. enterocolitica* obtained from raw milk in Canada were tested for heat resistance by Francis *et al.* (1980). Cultures were inoculated into whole milk to give $3-6 \times 10^6$ organisms per mL. The inoculated milk (2mL) was sealed into glass tubes and heated by immersion in a water bath at 62.8°C for 30, 60, 90, 120 and 150 seconds. Calculated $D_{62.8\text{C}}$ values for the cultures ranged from 0.7 to 17.8 seconds. Francis *et al.* concluded that, on the basis of their data, *Y. enterocolitica* will not survive pasteurisation.

Lovett *et al.* (1982) screened 30 Canadian strains, 6 Australian strains [including some of the strains reported to be capable of surviving pasteurisation - see Hughes (1979)] and 12 American strains of *Y. enterocolitica* for heat resistance at 62.8°C. Of these strains, three with unusual heat resistance (two Australian strains, Aus-3 and Aus-31, and one Canadian strain, C-1017) were selected for more detailed study. The three cultures were inoculated into sterile whole milk and 1.5 mL quantities were sealed into glass tubes. The sealed tubes were submerged in a water bath pre-heated to 51.7, 57.2, 62.8 or 68.3°C and removed at 30-second intervals until total inactivation occurred. $D_{57.2\text{C}}$ value for strain Aus-3 was 6.1 minutes, for strain Aus-31, 4.6 minutes, and for

strain C-1017, 13.4 minutes. $D_{62.8\text{C}}$ value for strain Aus-3 was 0.28 minutes, for strain Aus 31, 0.21 minutes, and for strain C 1017, 0.91 minutes. For the Canadian strain C-1017, it was estimated that $D_{68.3\text{C}} = 0.09$ minutes, only slightly less than that reported for *Salmonella* Seftenberg 775W. $D_{68.3\text{C}}$ values for the two Aus strains were not reported. Also, it was not stated whether or not any of the three strains were pathogenic to humans.

A chart showing the thermal death time curves for the three strains of *Y. enterocolitica* studied by Lovett *et al.* is reproduced in Figure 2.18. The z values for the three strains were 5.78, 5.22 and 5.11 °C, all within the expected range of z values for vegetative cells within the temperature range 54.4 to 71.1°C ($5.56 \pm 1.1^\circ\text{C}$). Lovett *et al.* (1982) concluded that even the most heat resistant strains of *Y. enterocolitica* that he had studied would not survive the recommended pasteurisation processes, with a wide margin of safety.

D'Aoust *et al.* (1988) studied the heat resistance of *Y. enterocolitica* in whole milk using a regenerative plate HTST pasteuriser operated in the range 60-72°C with a minimum holding time of 16.2 seconds. A 'cocktail' comprised of 15 strains of the organism obtained from various foods including raw and pasteurised milk was added to the milk. Total population of *Y. enterocolitica* in the milk before heat treatment was in the range $2.5 - 4.5 \times 10^5$ per mL in each of the three trials conducted. Survivors in the range $0.5-1.1 \times 10^1$ per mL were detected after heat treatment at 60°C for 16.2 seconds, i.e. a 4.5D kill was achieved at this temperature. No survivors were detected at 63, 66 and 72°C, i.e. a minimum 5.5D kill was achieved at each of these temperatures. D'Aoust *et al.* noted that while *Y. enterocolitica* was inactivated by recognised pasteurisation treatments, caution with sub-pasteurisation treatments was required.

Slachev (1989) reported that four reference and 42 field strains of *Y. enterocolitica* serotype 0:3, at concentrations between 2×10^4 and 2.4×10^6 per mL were destroyed by heating at 60°C for three minutes, 65°C for 30 seconds, 67.5°C for 20 seconds and 70°C for 10 seconds. In a parallel study, Pavlov (1989) compared the heat resistance of *Y. enterocolitica* with that of several other species of *Yersinia*. *Y. enterocolitica* serotype 0:3 was the most heat resistant, with $D_{55\text{C}} = 4.6$ minutes and $D_{60\text{C}} = 0.50$ minutes. Pavlov concluded however that, overall, the *Yersinia* species investigated have a low heat resistance and would be rapidly destroyed during milk pasteurisation. No other details were available for either of these studies.

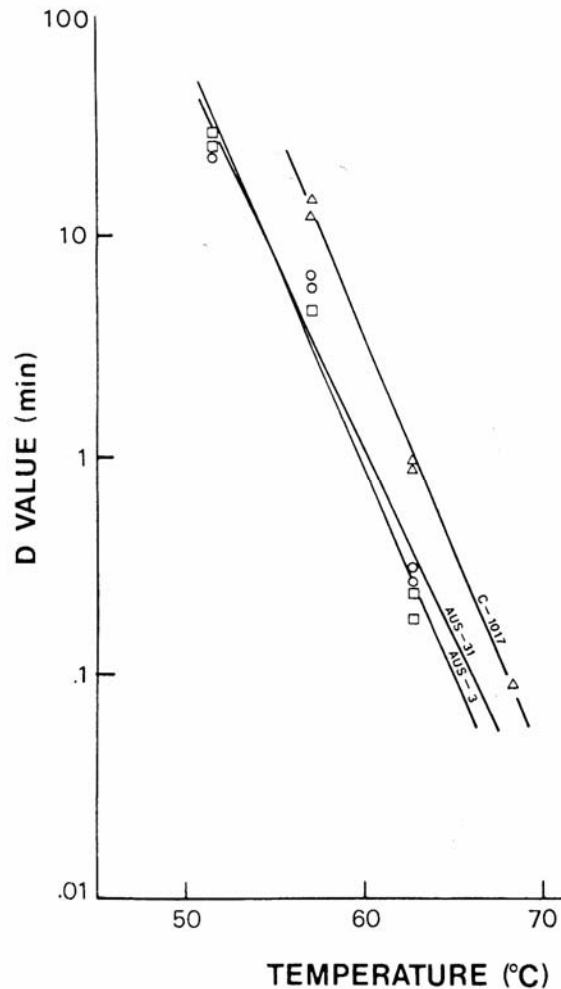


Figure 2.18. Thermal death time curves for three strains of *Yersinia enterocolitica*. Legend for strains: ○ = Aus 3; □ = Aus31; △ = C1017 [after Lovett *et al.* (1982)].

Kushal and Anand (1999) compared the heat resistance of a standard strain of *Y. enterocolitica*, MTCC-861 and of two isolates of *Y. enterocolitica* in whole and skim milk. Inoculation levels were in the range $1-8 \times 10^6$ per mL. Heating was carried out in sealed tubes immersed in water at 62.8°C for 30 minutes. No survivors were detected in whole or skim milk.

Pagan *et al.* (1999) examined factors affecting the heat resistance of *Y. enterocolitica*. For example, growth temperature in the range of 4 to 20°C did not influence the heat resistance of the organism at $54-66^\circ\text{C}$ at pH 7 in phosphate citrate buffer. However, when *Y. enterocolitica* was grown at 37°C , the $D_{62^\circ\text{C}}$ value increased from 0.04 to 0.17 minutes. This increase was consistent at all heating temperatures tested ($54-66^\circ\text{C}$). Interactions between growth temperature and pH and composition of the heating medium were also demonstrated. Hayashidani *et al.* (2005) found in their study of heat resistance among several strains of pathogenic *Y. enterocolitica* that different strains of *Y. enterocolitica* behaved differently on their heat resistance upon variation of growth temperature. One type was growth temperature-dependent including strain O:3, O:5,27 and O:8. Their $D_{60^\circ\text{C}}$ - values were larger when they were grown at 37°C than at 7 or 25°C . Another type was growth temperature-independent, such as O:9 that had similar $D_{60^\circ\text{C}}$ - values when it was grown at 7, 25 and 37°C .

Summary

Although there is evidence that strains of *Y. enterocolitica* vary widely in their heat resistance, there is also ample evidence to indicate that even the most heat resistant of them would not survive commercial batch or HTST pasteurisation, with a wide margin of safety. Strains from Australia reported to be capable of surviving pasteurisation in a 1979 study were found to be destroyed by pasteurisation in later studies. Growth temperature of the *Y. enterocolitica* cultures before heat treatment and, to a lesser extent, environmental factors such as pH and composition of the heating medium can have a marked effect on heat resistance. There exist two types of *Y. enterocolitica*. The heat resistance of one type is dependent on the growth temperature, and that of the another type is independent of growth temperature.

Thermisation at 62°C for 15 seconds would have some impact on population levels, but the available data indicates that the extent of the impact might vary widely. Different studies indicate different levels of impact, ranging from <1-20D kill, with a range of about 1-5D being most likely. The strain of *Y. enterocolitica* and the growth temperature before heat treatment have a significant influence on heat resistance.

2.4. Summary and discussion of published data on thermal death times of relevance to milk pasteurisation and thermisation and of identified gaps in knowledge

The main findings from the foregoing review of the published data on the heat resistance of the pathogens nominated for evaluation during the present study have been summarised in Table 2.16. The summary deals with two issues:

- Methods used to determine heat resistance of each pathogen, and
- Ability of the pathogens to survive/not survive commercial heat treatments.

In addition, the numbers of heat resistance studies conducted on milk from the five animal species covered by this review and on different types of milk have also been summarised, and comments made on the findings of the available comparative studies (refer Table 2.17). Some summary data is also provided on the numbers of studies reporting confidence limits for heat resistance data.

Methods used to determine heat resistance of the pathogens

As highlighted throughout this report and as further discussed below, the method of determining heat resistance is a major factor in determining (i) its reliability and (ii) its relevance to commercial pasteurisation practice. It is considered important therefore to list the methods that have been used to determine heat resistance of each pathogen, as the methodology needs to be considered when assessing the veracity of any conclusions about the ability of an organism to survive/not survive commercial heat treatments. Many different techniques and types of equipment have been used to measure heat resistance, ranging from very simple to sophisticated and from micro scale to commercial scale. However, for preparation of the summary set out in Table 2.16, each method was allocated to one of six broad categories.

Table 2.16. Summary of the main methodologies that have been used to determine the heat resistance of the nominated pathogens and their ability to survive pasteurisation and thermisation.

Organism ¹	Broad categories of methods used to determine heat resistance						Survival of heat treatment			Comments
	Lab studies: partially submerged tubes/flasks	Lab studies: submerged capillary tubes or ampoules ²	Lab studies - simulated batch pasteurisation	Lab studies - simulated HTST pasteurisation	Pilot plant scale HTST pasteuriser	Commercial scale HTST pasteuriser	Thermisation (62°C for 15 sec)	Batch pasteurization (63°C for 30 min)	HTST pasteurisation (72°C for 15 sec)	
<i>Bacillus cereus</i> (vegetative cells)	Yes	NR ³	NR	NR	NR	NR	Not known	No	No	No data available to indicate kill by thermisation
<i>Bacillus cereus</i> (spores)	NR ³	Yes	NR	Yes	NR	NR	Yes	Yes	Yes	Spores are resistant to batch and HTST pasteurisation
<i>Brucella abortus</i>	Yes	NR	Yes	Yes	Yes	NR	Yes	No	No	Thermisation would have little impact on this organism
<i>Brucella melitensis</i>	NR	NR	NR	NR	NR	NR	Yes ?	No ?	No ?	Limited data available
<i>Campylobacter jejuni</i>	Yes	Yes	Yes	Yes	Yes	NR	Yes/Partial kill	No	No	Kill by thermisation will depend on circumstances and assumptions
<i>Campylobacter coli</i>	NR	Yes	NR	NR	Yes	NR	Yes/Partial kill	No	No	Kill by thermisation will depend on circumstances and assumptions
<i>Coxiella burnetii</i>	NR	NR	Yes	No	No	Yes	Yes	No	No	Higher temperatures and/or longer times are required for milk products with higher fat or solids levels than whole milk
<i>Enterobacter sakazakii</i>	Yes	Yes	NR	NR	NR	NR	Yes/Partial kill	No	No	Kill by thermisation will depend on circumstances and assumptions
Pathogenic <i>Escherichia coli</i> (0157:H7)	NR	Yes	NR	Yes	Yes	NR	Partial kill	No	No	Kill by thermisation not assured – number and strain of organisms present and type of milk are major variables
<i>Listeria monocytogenes</i>	Yes	Yes	NR	Yes	Yes	Yes	Yes	No	No	Heat resistance of this organism has been subjected to comprehensive study
<i>Mycobacterium tuberculosis</i>	Yes	?	Yes	Yes	NR	Yes (Also batch)	Yes	No	No	Various methods and types of equipment were used to measure heat resistance (Hammer, 2004) but details not available in some cases

Organism ¹	Broad categories of methods used to determine heat resistance						Survival of heat treatment			Comments
	Lab studies: partially submerged tubes/flasks	Lab studies: submerged capillary tubes or ampoules ²	Lab studies - simulated batch pasteurisation	Lab studies - simulated HTST pasteurisation	Pilot plant scale HTST pasteuriser	Commercial scale HTST pasteuriser	Thermisation (62°C for 15 sec)	Batch pasteurization (63°C for 30 min)	HTST pasteurisation (72°C for 15 sec)	
<i>Mycobacterium bovis</i>	NR	Yes	Yes	NR	NR	Yes (Also batch)	Yes	No	No	Despite many studies on heat resistance of <i>M. tuberculosis/bovis</i> , there is only one report of kinetic data specifically for <i>M. bovis</i>
<i>Mycobacterium paratuberculosis</i>	Yes	Yes	NR	Yes	Yes	NR	Yes	No	Minimum kill >4D in worst case scenario	Conflicting data for 72°C/15 sec, but controlled studies in a HTST pasteuriser gave minimum kill at least >4D; other studies indicated >6D or 7D kills
<i>Salmonella</i>	NR	Yes	NR	NR	Yes	Yes	Partial kill	No	No	Kill by thermisation not assured – number and strain of organisms present are major variables
<i>Staphylococcus aureus</i> (organism)	Yes	Yes	Yes	NR	NR	NR	Partial kill	No	No	Kill by thermisation not assured – number and strain of organisms present are major variables
<i>Staphylococcus aureus</i> (toxin)	NR	NR	NR	NR	NR	NR	Yes	Yes	Yes	Toxin is very heat resistant
<i>Streptococcus pyogenes</i>	NR	NR	Yes	NR	NR	Yes	Yes	No	No	Most recent study was in 1976. Some of the earlier studies largely empirical. Kinetic data - where available – spans a wide range of values.
<i>Streptococcus agalactiae</i>	NR	NR	Yes	NR	NR	NR	?	No	?	Very limited data available
<i>Streptococcus zooepidemicus</i>	NR	NR	NR	NR	NR	NR	?	?	?	No data available
<i>Yersinia enterocolitica</i>	NR	Yes	NR	NR	Yes	NR	Partial kill	No	No	Kill by thermisation not assured – number of organisms present and strain are major variables

¹ Organisms listed in alphabetical order by genus, then by species if more than one.

² Includes use of a thermoresistometer.

³ NR = Use of this technique was not reported in the literature reviewed.

With regard to the methodology for determining the heat resistance of pathogenic microorganisms in the context of milk pasteurisation, Stabel *et al.* (2001) outlined the relative merits of the three general types of methodology that have been widely used, as follows:

- (a) **Batch heating in an open vial (as in the widely-used Franklin method), tube or flask.** The microbial suspension is partially immersed in a heating bath.
- (b) **Batch heating in closed systems.** The microbial suspension is fully enclosed in a sealed capillary tube, screw cap bottle or similar and fully immersed in a heating bath.
- (c) **Continuous flow heating.** The microbial suspension is continuously fed into a heat exchanger, comprised of heating, holding and cooling sections.

According to Stabel *et al.*, it is almost impossible not to contaminate parts of the holding vessel that are not fully immersed during pipetting and mixing operations with method type (a). This invariably leads to contamination of the bulk liquid with some surviving cells. The result is that the thermal death curve frequently shows tailing, apparently indicating that a fraction of the population is insensitive to heat. For this reason, Stabel *et al.* were firmly of the opinion that this type of methodology should never be used for the estimation of D values.

Stabel *et al.* further noted that method types (b) and (c) do not suffer from the drawbacks outlined above for method types (a). However, batch heating (b) and continuous heating (c) differ in important ways, particularly speed of heat transfer and the impact of laminar (ie non-turbulent) vs. turbulent flow during the holding (residence) period. Some authors have argued that batch heating in closed vials or tubes can only be used to estimate D values when the heat treatment is applied for periods of one minute or longer; batch heating for shorter periods, i.e. those measured in seconds, may not yield reliable data.

Important components of the heat treatment during commercial pasteurisation, both batch and continuous, are the come-up period before and the cool-down period after the holding period. According to Stabel *et al.*, the residence time profile in a typical HTST pasteuriser is as follows: regeneration section, incoming raw milk, 12 seconds; heating section, 5 seconds; holding tube, 15 seconds at 72°C; regeneration section, outgoing pasteurised milk, 12 seconds; and cooling section, 5 seconds.

It is perhaps ironical that, in order to estimate reliable D values, some of the researchers cited in Section 2.3 above reported that they had gone to great lengths to avoid, or to otherwise correct for, the impact of come-up period and cool-down periods on the survivor curves.

Another important feature of commercial HTST systems is turbulent flow, though even the degree of turbulence can vary between plants. Most of the laboratory scale equipment designed to simulate HTST pasteurisation even lacks this feature. In addition, homogenisation of milk for liquid milk products may also have an impact of the survival of pathogens during commercial pasteurisation, particularly where clumping of the organism might be an issue.

Thus, from a commercial perspective, it is the overall impact of the integrated heating profile, plus any other relevant inputs during processing such as homogenisation, on the survival/ destruction of any pathogens that may be present in the raw milk on any given day is what really counts. Thus, when interpreting the data summarised in Table 2.16 and outlined in detail in Section 2.3, it is recommended that the greatest weight be given to the results of heat resistance studies carried out using either pilot plant- or commercial-scale HTST pasteurisation equipment, particularly modern equipment that complies with current recognised standards for HTST equipment. This should be particularly so in any cases where

doubts about the ability of an organism to survive pasteurisation have arisen from earlier studies, e.g. *M. paratuberculosis*. However, that is not to say heat resistance data obtained using laboratory methods should be ignored; rather, the particular methodology used to obtain the data should be established and the data interpreted accordingly. Confidence limits are an importance adjunct to kinetic data but in many cases have not been reported (see below). The method used to measure heat resistance is less critical in cases where the organism is not particularly heat resistant.

Ability of the organisms to survive/not survive commercial heat treatments

For summarising the data on heat resistance of the pathogens, three commercial heat treatments were used as reference points: (i) HTST pasteurisation (72°C for 15 sec); (ii) Batch (holder) pasteurisation (63°C for 30 min); and (iii) Thermisation, arbitrarily defined as 62°C for 15 sec.

Heat resistance studies conducted using either pilot plant- and/or or commercial-scale HTST pasteurisation equipment have been reported for 12 of the 18 vegetative forms of the pathogenic species listed in Table 2.16. These were: *B. abortus*, *C. jejuni*, *C. coli*, *C. burnetii*, Pathogenic *E. coli* (0157:H7), *L. monocytogenes*, *M. tuberculosis*, *M. bovis*, *M. paratuberculosis*, *Salmonella*, *S. pyogenes* and *Y. enterocolitica*. Excluding *M. paratuberculosis*, there is ample evidence to demonstrate that each of these species is destroyed by both batch (63°C for 30 minutes) and HTST (72°C for 15 seconds) pasteurisation, with a reasonable margin of safety. However, it must be noted that even the most recent data for several of these organisms is quite dated (see below).

In the case of *M. paratuberculosis*, there appears to be ample evidence that this organism is destroyed by batch pasteurisation. However, studies on the ability of *M. paratuberculosis* to survive heating at 72°C for 15 seconds, even with pilot scale HTST equipment, have given conflicting results. However, the more credible studies have shown that a minimum log₁₀ reduction of *M. paratuberculosis* during HTST pasteurisation of at least 4D is obtained, and that given the numbers of the organism likely to be present in the raw milk, that this in fact provides a reasonable margin of safety for the consumer. More generally, population reductions in the order of 6-7D have been reported.

A fundamental question with respect to *M. paratuberculosis*, as yet unanswered, is whether the organisms is in fact a human pathogen, or whether its postulated association with Crohn's disease is just serendipitous, rather than causal.

Of the remaining six species, the available data on their heat resistance, if any, indicates the following:

- ***Bacillus cereus***. Although there is limited data available specifically on the heat resistance of the vegetative form of this organism, and none using commercial HTST equipment, it is generally accepted that the vegetative cells are readily destroyed by both batch and HTST pasteurisation. However, this is to some extent academic, as there is more than ample evidence to indicate that the spores of *B. cereus* are very heat resistant and readily survive any heat treatments in the normal pasteurisation range. The pasteurisation heat treatment is sufficient to heat activate the fast-germinating spores of *B. cereus*, but not the slow-germinating spores. Similarly, pasteurisation inactivates diarrhoeagenic toxins produced by *B. cereus*, but not the emetic toxin.
- ***Brucella melitensis***. No definitive data on the heat resistance of the organism (which is not endemic in Australia) were located. However general statements from authoritative sources indicate that the organism is destroyed by pasteurisation.

- ***Enterobacter sakazakii***. Although the data is somewhat variable, and data using commercial HTST equipment is lacking, the consensus view is that the heat resistance of this organism falls within the safety margins of commercial pasteurisation. Its presence in pasteurised milk products has been found to be due to re-contamination of the pasteurised product after the pasteurisation step.
- ***Staphylococcus aureus***. Although this organism has relatively high heat resistance for a mesophilic non-sporing bacterium, and despite the fact that data using commercial HTST equipment is lacking, there is ample evidence from laboratory studies that it is destroyed by both batch and HTST pasteurisation heat treatments with a wide margin of safety. However, the thermal stability of the enterotoxins produced by *S. aureus* greatly exceeds that of its vegetative cells, and readily survives pasteurisation by a wide margin.
- ***Streptococcus agalactiae***. Only one report on the heat resistance of *S. agalactiae* was located. This indicated - under relatively crude experimental conditions - that the organism was inactivated at unspecified population levels in cream by batch pasteurisation. That this is the extent of the data on the heat resistance of this organism is quite remarkable, given that it is a common cause of bovine mastitis and can be transmitted to humans, especially women, who drink raw milk.
- ***Streptococcus zooepidemicus***. Not a single report on the heat resistance of *S. zooepidemicus* was located. This is also remarkable, in view of the fact that human infection with this organism can usually be traced to an animal source, including ingestion of unpasteurised milk and cheese. Consumption of raw milk was shown to be the source of a severe infection with this organism in South-East Queensland.

Thermisation at 62°C for 15 seconds could be relied upon to give at best only partial kill of the pathogens. For 8 of the 18 species reviewed, thermisation would have no or little impact on the number of viable organisms. For 7 of the 18 species reviewed, thermisation might give a partial kill, depending upon a range of factors such as the heat resistance of the particular strains present, their numbers, the composition of the milk, the physiological state and age of the bacterial cells being heated and the particular D values used as the reference. For the remaining three species (*B. cereus* vegetative cells, *S. agalactiae* and *S. zooepidemicus*), no data were available on which to base an assessment.

This summation of the effectiveness of thermisation in reducing the populations of the pathogenic bacteria present in raw milk is consistent with the views of Pearce (2004), who noted that thermisation does not give sufficient heat stress to significantly reduce the titre of the more resistant vegetative bacterial pathogens. Pearce further noted that a thermisation treatment in the range 63-65°C for 10-15 seconds gives a 3-4 log reduction in the numbers of the psychrotrophic spoilage bacteria; it was for this purpose that thermisation was originally introduced.

Effectiveness of thermisation will always be improved by increasing the heating temperature, even just by a few degrees. However, for most organisms, the outcome would still be short of a complete kill. As demonstrated by Stabel *et al.* (2001) and Lund *et al.* (2002), it is always more efficient to increase the temperature of heating (which has an exponential influence on the log₁₀ reduction) than the time of heating (which has a linear influence). There are sufficient data provided for some of the organisms reviewed in Section 2.3 of the report to calculate D values for a range of alternative thermisation temperatures.

Identified gaps in the data on the heat resistance of the pathogens of interest include:

- Definitive evidence on whether it is valid to classify *M. paratuberculosis* as a human pathogen; and
- Quantitative heat resistance data for *Brucella melitensis*, *Streptococcus agalactiae* and *Streptococcus zooepidemicus* in milk.

Standardised methodology for the determination of heat resistance would be ideal, but probably almost impossible to achieve on an international basis. The next best option for an organisation like FSANZ is to establish and publicise its minimum requirements for the type of data that is acceptable for use in submissions on risk assessment studies. For example: milk to be used as the heating medium; confidence limits to be provided for kinetic data; preference to be given to data generated using commercial pasteurisation equipment where possible; and heat resistance data to be based on strains of test organisms known to occur in raw milk.

Numbers of heat resistance studies conducted on milk from different animal species and on different types of milk

The numbers of heat resistance studies cited in this report, categorised according to animal source and type of milk as the heating medium for each pathogen, together with comments on the findings of any comparative studies that have been reported, are summarised in Table 2.17. Numbers of studies included in composite data sets have been taken into account where appropriate. Assumptions about the number of studies used to compile the composite data had to be made in a few cases.

Approximately 265 studies on heat resistance have been reviewed in this study, either directly or via composite data compiled by other reviewers. Of these studies 94.7% were conducted using cows' milk as the heating medium (97.7% if the studies using substances other than milk as the heating medium are excluded from the data set). This is perhaps not a surprising result, given the commercial volumes of milk produced by each of the animal species world-wide.

Of the 94.7% of the studies conducted on cows' on milk, whole milk accounted for approximately 85.6%, skim milk 6.4%, flavoured milk 0.4% and cream 2.3%. Of the remaining 5.3% of studies, goat milk accounted for approximately 1.1%, sheep, buffalo and camel milk each 0.4% and non-milk heating media 3.0%.

Relatively few studies incorporating direct comparisons between the heat resistance of a particular organism in milks from different animal species or in milks of different compositions have been reported. In most of these cases, the measured heat resistance has reflected the protective effect of fat and/or total solids content of the milk, i.e. the higher the fat and/or total solids content, the higher the heat resistance. However, the effects generally have not been dramatic and there were some exceptions. In two cases, no differences in the heat resistance of the target organism in whole milk and skim milk were observed. In another case, it was established that factors other than the higher fat content of sheep's milk contributed to the greater heat resistance of the target organism in milk from this species compared with that in milk from cows or goats.

Table 2.17. Approximate numbers of heat resistance studies cited in this report categorised according to animal source and type of milk as the heating medium for each pathogen, together with comments on the findings of any comparative studies that have been reported.

Organism ¹	Animal source and type of milk										Studies in non-milk media	Approx. Total ⁷	Comments on comparative studies
	Cow					Goat	Sheep	Buffalo	Camel				
	Whole milk	Skim milk	Flavoured milk	Cream									
<i>Bacillus cereus</i> (vegetative cells)	3	1	-	-	-	-	-	-	-	-	1	5	1. No direct comparison of heat resistance in skim milk with that in other milks.
<i>Bacillus cereus</i> (spores)	C ²	?	?	?	?	?	?	?	?	?	1	22	1. No comparative data available.
<i>Brucella abortus</i>	5	1	-	1	-	-	-	-	-	-	-	7	1. No direct comparison of heat resistance in cream from cow's milk with that in other products. 2. Heat resistance in whole milk and skim milk identical.
<i>Brucella melitensis</i>	-	-	-	-	-	-	-	-	-	-	-	0	1. No quantitative data available.
<i>Campylobacter jejuni</i>	4	2	-	-	-	-	-	-	-	-	1	7	1. No direct comparison of heat resistance in skim milk with that in other milks.
<i>Campylobacter coli</i>	1	-	-	-	-	-	-	-	-	-	1	2	1. No comparative data available.
<i>Coxiella burnetii</i>	2	-	1	1	-	-	-	-	-	-	-	4	1. Heat resistance in cows' milk products with higher fat contents or added sugar or flavouring > whole cows' milk, reflecting the protective effect of the higher milk fat and/or solids contents.
<i>Enterobacter sakazakii</i>	2	-	-	-	-	-	-	-	-	-	1	3	1. No comparative data available.
Pathogenic <i>Escherichia coli</i> (O157:H7)	4	1	-	-	1	-	-	1	1	-	-	8	1. Heat resistance in buffalo milk > whole cows' milk > skim cows' milk, possibly reflecting the protective effect of milk fat. 2. No direct comparison of heat resistance in goat milk with that in milk from other species. 3. Heat resistance in cows' milk was slightly higher than in camel milk, possibly reflecting the protective effect of the milk fat.

Organism ¹	Animal source and type of milk										Studies in non-milk media	Approx. Total ⁷	Comments on comparative studies and other relevant issues
	Cow					Other							
	Whole milk	Skim milk	Flavoured milk	Cream	Goat	Sheep	Buffalo	Camel					
<i>Listeria monocytogenes</i>	C ³ , 4	C ³ , 1	-	-	1	1	-	-	-	-	-	45	1. One major study comparing heat resistance of different formulations of ice cream mix reported. 2. Heat resistance in sheep milk > whole cows' milk = goat milk; higher heat resistance in sheep milk not due solely to its higher fat content.
<i>Mycobacterium tuberculosis/bovis</i>	C ⁴ , C ⁵	-	-	-	-	-	-	-	-	-	-	100	1. More than 100 papers on the heat resistance of <i>M. tuberculosis/bovis</i> published <1973.
<i>Mycobacterium bovis</i>	4	-	-	-	-	-	-	-	-	-	-	4	1. More recent studies exclusively on <i>M. bovis</i> .
<i>Mycobacterium paratuberculosis</i>	13	-	-	-	-	-	-	-	-	-	-	13	1. No published studies in heat resistance of <i>M. paratuberculosis</i> in sheep's milk.
<i>Salmonella</i>	C ⁶ , 4	-	-	-	-	-	-	-	-	-	-	19	1. Heat resistance increases as the solids content of the milk product is increased.
<i>Staphylococcus aureus</i>	1	4	-	1	1	-	-	-	-	-	1	8	1. No direct comparison of heat resistance in goat milk with that in milk from other species.
<i>Streptococcus pyogenes</i>	5	-	-	2	-	-	-	-	-	-	-	7	1. No direct comparison of heat resistance in cream with that in other products. 2. One report on heat resistance in ice cream mix.
<i>Streptococcus agalactiae</i>	-	-	-	1	-	-	-	-	-	-	-	1	1. Limited data available.
<i>Streptococcus zooepidemicus</i>	-	-	-	-	-	-	-	-	-	-	-	0	1. No data available.
<i>Yersinia enterocolitica</i>	6	2	-	-	-	-	-	-	-	-	2	10	1. Heat resistance in whole milk and skim milk similar.
Total⁷	227 (85.6%)	17 (6.4%)	1 (0.4%)	6 (2.3%)	3 (1.1%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	1 (0.4%)	8 (3.0%)	265		

¹ Organisms listed in alphabetical order by genus, then by species if more than one.

² Composite data based on 21 published studies; heating media not specified.

³ Composite data based on 38 published studies, each using whole cows' milk and/or cows' skim milk as the heating medium.

⁴ Composite data based on numerous published studies (assume 20) related mainly to batch processing, each using whole cows' milk as the heating medium.

⁵ Composite data based on numerous published studies (assume 80) related mainly to HTST processing, each using whole cows' milk as the heating medium.

⁶ Composite data based on approximately 15 published studies using mainly whole cows' milk as the heating medium.

⁷ Totals include the number of studies incorporated into the composite studies. In the case of *Listeria*, it was arbitrarily assumed that 33 of the 38 studies had been conducted using whole milk and 5 using skim milk as the heating medium

The total number of heat resistance studies conducted on each of the pathogenic species covered by this study is also of interest (refer Table 2.16). By far the greatest numbers of studies have been conducted on *M. paratuberculosis / bovis*; however, as noted elsewhere in this report, the vast majority of these were conducted during the first half of the last century. Many of these studies were reported to be of poor quality, at least by today's standards. In more recent times, e.g. the past 20 years, *L. monocytogenes* and *M. paratuberculosis* have accounted for the majority of the heat resistance studies.

Numbers of studies reporting confidence limits for heat resistance data

Of the 91 papers directly reviewed during this study, only about 10% reported confidence limits for thermal death time curves and/or D values. These limits add considerable rigour to a data set, particularly when calculating margins of safety for a heat treatment. However, it is appreciated that while it is desirable to determine heat resistance of pathogens using commercial pasteurisation equipment, it is difficult to obtain the data required to calculate confidence limits in these cases unless the equipment has been specially modified.

2.5. Epidemiological data on disease outbreaks linked to consumption of pasteurised milk

There is a substantial body of mainly anecdotal evidence of the public health benefits of milk pasteurisation particularly during the first half of the last century, which largely drove the eventual acceptance of the process as a mandated public health measure in many countries (refer Section 2.1 of this report).

OzFoodNet has no documented reports of any outbreaks of gastrointestinal illness in Australia between 2000 and 2004 due to the consumption of pasteurised milk. However, there have been several outbreaks of enteric infection in Australia in recent years due to the consumption of unpasteurised milk (Mr Russell Stafford⁵, pers. comm., 2005).

An outbreak involving 50 cases of cryptosporidiosis amongst school children in the UK in 1995 was traced to the consumption of 'pasteurised' milk from a local farm. Upon investigation, however, it was established that the farm's pasteurisation equipment was faulty and that, as a consequence, the children had in fact consumed inadequately pasteurised milk⁵.

It should be noted that the data from OzFoodNet only relates to reported cases of gastrointestinal illness. For example, outbreaks of gastrointestinal illness due to ingestion of *Bacillus cereus* in a food might not be detected, as pathology laboratories do not routinely test stool samples from patients with symptoms of gastrointestinal illness for this organism⁵.

More detailed information supplied by Mr Stafford is provided at Attachment 2 to this report.

The detection of pathogenic microorganisms in packages of commercially–pasteurised milk is alone insufficient evidence that the organisms are resistant to the pasteurisation heat treatment. There are well-documented cases (refer above and Section 2.3 of this report) in which it has been shown that the presence of pathogens in pasteurised milk has been due to inadequate pasteurisation (eg faulty equipment or poor process control) or to re-contamination of the milk after the pasteurisation step in the processing line (eg ineffective sanitising of the equipment). Further investigation of the circumstances surrounding such incidents is always necessary.

⁵ Mr Russell Stafford, State Foodborne Disease Epidemiologist, OzFoodNet, Queensland Health Public Health Unit, Level 1, Queensland Health Scientific Services, 39 Kessels Rd, Coopers Plains, Qld 4108.

3. Current industry practices in Australia in terms of the time and temperature combinations used for the pasteurisation and thermisation of milk

Background

In addition to the review of the effectiveness of traditional milk pasteurisation methods the study required information on current industry practice in Australia with respect to methods employed for the pasteurisation of milk, in particular the time/temperature combinations used and their relationship to minimum regulatory standards.

Survey questionnaire

To obtain this information, a short questionnaire was sent to all dairy companies/plants in Australia engaged in the pasteurisation of raw milk.

The survey applied only to the 'traditional thermal methods' for the pasteurisation of milk, i.e. the HTST process as defined in Clause 1(1)(a) of Standard 1.6.2 of the Australia New Zealand Food Standards Code and the batch process where use of this process was permitted by the State regulatory agency. Hence other pasteurisation processes involving heat, e.g. ESL or UHT of liquid milks or the heat treatment of cream used for the manufacture of butter, were outside the scope of the survey. Other points about the scope of the survey were:

- It applied to milk from all of the commercial species used in Australia, i.e. cow, goat, sheep, buffalo and possibly camel.
- It applied to the pasteurisation of raw milk used for the production of liquid milk and milk products and of milk used in the production of any cream and cream products, fermented milks, yoghurt, dried, condensed and evaporated milks, ice cream and cheese, as defined in Standard 1.6.2. Information on the pasteurisation of raw cream for the fresh/table cream market was also sought.
- It did not apply to re-pasteurisation of dairy products, e.g. an ice cream mix based entirely on reconstituted milk powder, rather than on raw milk.

Respondents were asked to list each product group separately in a table, showing the species of animal, method of pasteurisation (i.e. HTST, batch, etc), the time-temperature combination used on a regular basis and an estimate of the average throughput on a weekly basis over the past year or so. If the time-temperature combination used for HTST deliberately exceeded that specified in Standard 1.6.2 (i.e. minimum of 72°C for a minimum of 15 seconds) by more than an arbitrary 0.5°C or three seconds, respondents were asked to show the time and temperatures used and explain why that particular treatment is used.

Information was also sought on the following:

- Use of the provisions of Standard 1.6.2 that permit milk used for the production of cheese to be heat treated by being held at a temperature of no less than 62°C for a period of no less than 15 seconds, provided the cheese or cheese product is stored at a temperature of no less than 2°C for a period of 90 days from the date of manufacture;

- Whether the company has been given approval by any regulatory agency to use a process, or a combination of processes, for the destruction of pathogenic organisms in milk as an alternative to traditional thermal pasteurisation.

Concurrently, respondents were asked to provide information about a list of processes that might in the future provide methods for the destruction of pathogenic organisms in milk, as alternatives to traditional thermal pasteurization. The results of this survey will be published in a subsequent report.

A copy of the questionnaire is provided at Attachment 3. A copy of a letter of introduction and support from Mr Deon Mahoney, Principal Microbiologist and Section Manager, Risk Assessment Microbiology, FSANZ, was included with the questionnaire when it was sent out.

Conduct of the survey

Distribution of the questionnaire to every dairy processor in Australia within the scope of the survey presented the reviewers with some logistical difficulties. Ideally, the reviewers would have mailed a questionnaire directly to every relevant dairy processor, with a reply paid envelope to encourage a quick and more complete response. Also, the reviewers would have been able to follow-up directly.

As anticipated, the only sources of a complete and current list of dairy processors accredited to pasteurise milk were the State regulatory agencies responsible for dairy food safety. However, confidentiality arrangements and privacy law prohibits them from releasing these details. To circumvent these restrictions, each of the relevant State agencies, i.e. Safe Food Queensland, New South Wales Food Authority, Dairy Safe Food Victoria, Tasmanian Dairy Industry Authority, Dairy Authority of South Australia and the Dairy Safety Branch of the Western Australia Department of Health, agreed to distribute the questionnaire on behalf of the reviewers. The alternative would have been for the reviewers to assemble an *ad hoc* list from various sources, including personal knowledge (largely limited to the main dairy companies), a listing of dairy product suppliers on the Dairy Australia website (many of whom are not in the business of pasteurising raw milk), a search of the Yellow Pages and 'asking around'. However, it was considered that, particularly given the relatively short time frame for the study, the latter approach might not identify all of the relevant processors, particularly the smaller and newer ones and those processing milk other than cows' milk. Hence it was decided to accept the offers of the State dairy regulatory agencies to distribute the questionnaire.

The questionnaire was distributed by the State dairy regulatory agencies over a period of about two weeks, commencing on 4 March 2005. The dairy processors in the ACT and the Northern Territory were contacted directly.

While distributing the questionnaire through the State agencies solved the problem of getting the questionnaire to every relevant dairy processor, it led to two consequent issues: managing the return of the questionnaire and follow-up of non-responders. Four of the agencies, New South Wales Food Authority, Tasmanian Dairy Industry Authority, Dairy Authority of South Australia and the Dairy Safety Branch of the Western Australia Department of Health, offered to receive the completed questionnaires and to follow-up on those who had not responded by a nominated date. However, Safe Food Queensland and Dairy Safe Food Victoria advised that while they were happy to distribute the questionnaire, they did not have the resources to receive the completed questionnaire or to follow-up non-responders.

In the case of Queensland, the reviewers were able to compile a complete list of relevant processors from public sources and industry contacts. Non-responders were contacted directly by phone and, in the case of some of the smaller processors, the questionnaire was completed via a telephone interview.

In the case of Victoria, little direct follow-up was conducted. However, this was done in the knowledge that a good set of recent data on the pasteurisation times and temperatures used by Victorian dairy processors were available from Dairy Food Safety Victoria (DFSV; see below). Other contributing factors to the lack of follow-up in Victoria were difficulties in compiling a complete list of the relevant processors in that State in the time available and limited time to conduct follow-up relative to other priorities within the project.

Response to the survey

The approximate numbers of questionnaires sent out and the number of responses are summarised in Table 3.1. Numbers have been expressed on a per plant basis. In some States, questionnaires were sent to every plant, while in others, e.g. Queensland, they were sent to the main office for each company only. Some companies with multiple sites responded on a 'one combined national response' basis, while others chose to respond separately for each site. In several States, including New South Wales, Victoria and Tasmania, responses indicated that some of the questionnaires had been sent to companies which, it was subsequently established, fell outside the scope of the survey, i.e. numbers of questionnaires sent out are overstated in some cases, but by how many is not known, as it is likely that most of those who considered themselves not eligible simply would have not responded to the questionnaire. Another point to note is that some plants process milk from more than one type of animal, e.g. cow and goat.

For convenience, responses covering the Northern Territory have been included with Queensland in Table 3.1, and similarly the ACT with New South Wales.

On a State basis, response rate ranged from 20% in Victoria (though about 90% for Victoria if the DFSV survey data are taken into account – see below) to 77% in Queensland. Nationally, the response rate was at least 44%, though this increases to approximately 71% if the DFSV data are included.

On a type of animal basis, response rate ranged from nil for 'buffalo milk only' plants to 100% for 'cow + buffalo milk' plants. For the categories with more meaningful numbers, the range was from 38% for 'goat milk only' to 73% for 'cow + goat milk' plants. In total, the survey generated 159 rows of data (refer Table 3.2).

Table 3.1. Summary of the approximate numbers of questionnaires on milk pasteurisation sent out to dairy processors and the number responses, expressed on a per plant basis.

Type of milk	Qld / NT		NSW / ACT		Victoria		Tasmania		SA		WA		Total	
	No. sent out	No. of responses	No. sent out	No. of responses	No. sent out	No. of responses	No. sent out	No. of responses	No. sent out	No. of responses	No. sent out	No. of responses	No. sent out	No. of responses
Cow only	23	18	19	14	76	13	14	8	15	9	20	9	167	71 (43%)
Cow + Goat	3	2	0	0	3	3	1	1	4	2	0	0	11	8 (73%)
Cow + Buffalo	1	1	0	0	0	0	0	0	0	0	0	0	1	1 (100%)
Goat only	2	1	5	1	2	0	1	0	3	3	3	1	16	6 (38%)
Sheep only	1	1	0	0	0	0	2	0	1	1	1	1	5	3 (60%)
Buffalo only	0	0	0	0	1	0	0	0	0	0	0	0	1	0 (0%)
Camel	0		0	0	0	0	0	0	0	0	0	0	0	0 (NA)
Total	30	23 (77%)	24	15 (63%)	82	16 (20%)	18	9 (50%)	23	15 (65%)	24	11 (46%)	201	89 (44.3%)

Despite what appears to be a low response rate in some areas, the responses nevertheless provide a well-represented stratified sample of the dairy processors in Australia involved in the pasteurisation of raw milk. All States, different animal species and all sizes of plants are all well-represented in the sample. There is also reasonable consistency in the data.

It must be noted that some companies, both large and small, advised that they were unable to provide any information at all, because of company confidentiality policy. It is likely that some of the other non-responders had similar policies.

It was established that no plants were pasteurizing camel milk in Australia as at March 2005. A camel farm near Alice Springs has investigated the matter, but has not yet commenced milk production on a commercial basis.

Survey results – method of pasteurisation and heating times and temperatures

The information on the times and temperatures of the pasteurisation heat treatment relative to type of animal, end product and method of pasteurisation, together with the reasons for using times longer than 18 seconds or temperatures higher than 72.5°C if using the HTST process, is set out in Table 3.2.

Each of the 159 rows of data in Table 3.2 represents data from a single plant or a group of plants using the same time and temperature combination. The data was sorted in sequence, firstly by (a) species of animal, then by (b) method of pasteurisation, (c) type of product, (d) temperature of heat treatment and (e) time of heat treatment. For confidentiality reasons, information on specific product type, e.g. cheese variety, and on product throughputs have been excluded from this report.

Data gathered during the survey conducted as a component of this study were not subjected to on-site audit. However, the respondents were contacted by telephone if their data was outside the expected range.

Subsets of the time and temperature combinations from Table 3.2 are also shown as scatter diagrams in Figures 3.1 - 3.10. Each subset represents an appropriate (animal species x method of pasteurisation x end product) combination, as follows:

- Figure 3.1: Cows' milk x HTST pasteurisation x liquid milk products.
- Figure 3.2: Cows' milk x HTST pasteurisation x cheese.
- Figure 3.3: Cows' milk x HTST pasteurisation x frozen milk products.
- Figure 3.4: Cows' milk x HTST pasteurisation x yoghurt, sweetened condensed milk, concentrated milk and fermented milk.
- Figure 3.5: Cows' milk x HTST pasteurisation x table cream.
- Figure 3.6: Cows' milk x batch pasteurisation x all products.
- Figure 3.7: Cows' milk x batch pasteurisation x all products, for data sets where the holding time was <5 minutes only.
- Figure 3.8: Goat milk x HTST pasteurisation x all products.
- Figure 3.9: Goat, sheep and buffalo milk x batch pasteurisation x all products.
- Figure 3.10: Goat, sheep and buffalo milk x batch pasteurisation x all products, for data sets where the holding time was <5 minutes only.

Where time or temperature of heat treatment had been reported as a range, the lowest value of the range was used to generate the charts shown in Figures 3.1 - 3.10.

Table 3.2. *Times and temperatures of pasteurisation used by a sample of dairy processors in Australia, together with other relevant information. Source of data: Industry survey by Harley Juffs & Associates Pty Ltd, March-April, 2005.*

Species	Process	Product ¹	Temp (°C)	Holding time	Reason given by respondents for using a temperature >72.5°C and/or a time >18 sec for HTST treatments, or other relevant comments ²
Buffalo	Batch	Cheese	63	30 min	
Buffalo	Batch	Yoghurt	90	5 min	
Cow	Batch	Cheese	62	30 min	
Cow	Batch	Cheese	63	30 min	
Cow	Batch	Cheese	63	31 min	
Cow	Batch	Cheese	64	30 min	
Cow	Batch	Cheese	65.1	10 min	
Cow	Batch	Cheese	72	1 min	
Cow	Batch	Cheese	>82	15 min	Product texture and yield
Cow	Batch	Cheese	85	15 sec +	Holding time is nominal; cooling commences as soon as product reaches 85°C
Cow	Batch	Cheese	85	15 sec +	Holding time is nominal; cooling commences as soon as product reaches 85°C
Cow	Batch	Cheese	88-92	5 min	Time and temperature options restricted by equipment design
Cow	Batch	Cream	65	30 min	
Cow	Batch	Cream ³	66	30 min	
Cow	Batch	Cream	70	5 min	Long hold time used to ensure good quality product
Cow	Batch	Cream	68	30 min	
Cow	Batch	Cream	68-70	20 min	
Cow	Batch	Cream	68.5	30 min	
Cow	Batch	Gelatin	85	2 min	High solids mix
Cow	Batch	Ice cream	80	25 sec	
Cow	Batch	Ice cream	85	2 min	High solids mix
Cow	Batch	Milk ⁷	63	30 min	
Cow	Batch	Milk	64	30 min	
Cow	Batch	Yoghurt	>82	15 sec	Product texture
Cow	Batch	Yoghurt	85	20 min	Product texture
Cow	Batch	Yoghurt	85	30 min	Product texture
Cow	Batch	Yoghurt	85	30 min	Product texture
Cow	Batch ⁴	Yoghurt ⁵	85-95	7 min	Essential part of manufacturing process
Cow	Batch	Yoghurt	86	4-6 min	Product texture
Cow	Batch ⁴	Yoghurt ⁵	88	6.3 min	Essential part of manufacturing process
Cow	Batch	Yoghurt	90	5 min	Product texture
Cow	Batch	Yoghurt	90	20 min	Product texture

Species	Process	Product ¹	Temp (°C)	Holding time	Reason given by respondents for using a temperature >72.5°C and/or a time >18 sec for HTST treatments, or other relevant comments ²
Cow	Batch ⁶	Yoghurt	95	5 min	Product texture
Cow	HTST	Cheese	65	20 sec	Thermisation process plus minimum storage of the cheese
Cow	HTST	Cheese	72	15 sec	
Cow	HTST	Cheese	72	15 sec	
Cow	HTST	Cheese	72	15 sec	
Cow	HTST	Cheese	72	15 sec	
Cow	HTST	Cheese	76	22 sec	Product texture
Cow	HTST	Cheese	72-74	15 sec	Temp >72°C to allow for fluctuations in equipment operation with a margin of safety above 72°C
Cow	HTST	Cheese	72-75	15 sec	Temperature >72°C to provide safe operational margin above 72°C
Cow	HTST	Cheese	72.5	15 sec	
Cow	HTST	Cheese	72.5	18 sec	Would use 62°C for 15 sec plus specified minimum storage of the cheese if permitted by the regulatory agency
Cow	HTST	Cheese	72.5	18 sec	
Cow	HTST	Cheese	72.5	18 sec	
Cow	HTST	Cheese	72.5	18 sec	
Cow	HTST	Cheese	72.5	20.5 sec	Time >15 sec due to throughput below design capacity of pasteuriser
Cow	HTST	Cheese	72.5	20.5 sec	Time >15 sec due to throughput below design capacity of pasteuriser
Cow	HTST	Cheese	72.5	30 sec	
Cow	HTST	Cheese	72.5	30 sec	
Cow	HTST	Cheese	>72.5	15 sec +	
Cow	HTST	Cheese	72.6	15.3 sec	Minimum heat treatment to ensure cheese has good physical attributes
Cow	HTST	Cheese	72.6	39 sec	Time and temperature options restricted by equipment design
Cow	HTST	Cheese	73	18 sec	
Cow	HTST	Cheese	73-76	15 sec	Temperature >72°C to provide safe operational margin above 72°C; divert temperature is 72.5°C
Cow	HTST	Cheese	73.3	22 sec	Pasteuriser design and operational limitations
Cow	HTST	Cheese	73.3	22 sec	Pasteuriser design and operational limitations
Cow	HTST	Cheese	73.4	15 sec	Temp > 72°C for operational reasons
Cow	HTST	Cheese	74	15 sec	Temperature >72°C to provide operational margin above divert temperature of 72.5°C
Cow	HTST	Cheese	74	17 sec	Temperature >72°C for operational reasons; diversion set point 73°C
Cow	HTST	Cheese	74	24 Sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Cheese	75	15 sec	Temp >72°C to allow for fluctuations in equipment operation with a margin of safety above 72°C

Species	Process	Product ¹	Temp (°C)	Holding time	Reason given by respondents for using a temperature >72.5°C and/or a time >18 sec for HTST treatments, or other relevant comments ²
Cow	HTST	Cheese	75	25 sec	Product texture
Cow	HTST	Cheese	75	25 sec	Product texture
Cow	HTST	Cheese	75-79	22.4 sec	HTST equipment does not allow precise control of temperature, so must allow a wide safety margin
Cow	HTST	Cheese	80	15 sec	Pasteuriser limitations
Cow	HTST	Cheese	80	9.1 sec	Specific heating regime to provide curd texture and whey expulsion characteristics
Cow	HTST	Cheese	82	47 sec	High fat product mix
Cow	HTST	Cheese	84	30 sec	Product texture
Cow	HTST	Cream	75	18 sec	
Cow	HTST	Cream	75	25 sec	Time >15 sec and temperature >72°C to allow for viscosity and high fat of product
Cow	HTST	Cream	77	19 sec	Standard heat treatment for cream
Cow	HTST	Cream	78	18 sec	Temperature >72°C to provide a margin of safety; diversion set point 72.6°C
Cow	HTST	Cream	78	25 sec	Time >20 sec as recommended to cater for any organisms resistant to pasteurisation (ie MAP); Temp >72°C to allow for fluctuations in equipment operation with a margin of safety above 72°C
Cow	HTST	Cream	77-79	30 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP and to cater for high fat content
Cow	HTST	Cream	77-80	37 sec	Historical for plant
Cow	HTST	Cream	77-79	30 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP and to allow for effect of higher fat content
Cow	HTST	Cream	78-80	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP; diversion set point 80°C
Cow	HTST	Cream	78	29.7 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Cream	80	22 sec	
Cow	HTST	Cream	80	25 sec	Standard technology
Cow	HTST	Cream	80	25 sec	Greater log reduction of microbial loading
Cow	HTST	Cream	81	15 sec	Standard industry practice for cream is 80°C; divert temperature is set at 77°C
Cow	HTST	Cream	83	15 sec	Time >15 sec and temperature >72°C to allow for viscosity and high fat of product; plus market demand for longer shelf life
Cow	HTST	Cream	84	25 sec	Time >15 sec and temperature >72°C to allow for viscosity and high fat of product; plus market demand for longer shelf life
Cow	HTST	Cream	120	2 min	To improve shelf life
Cow	HTST	Frozen milk product	82	12.7 sec	High solids mix
Cow	HTST	Frozen yoghurt	82	12.7 sec	High solids mix

Species	Process	Product ¹	Temp (°C)	Holding time	Reason given by respondents for using a temperature >72.5°C and/or a time >18 sec for HTST treatments, or other relevant comments ²
Cow	HTST	Ice cream	82	12.7 sec	High solids mix
Cow	HTST	Ice cream	82	23 sec	Integral part of the blending process for the mix
Cow	HTST	Ice cream	82	45 sec	High solids mix
Cow	HTST	Soft serve ice cream mix	78	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP and to cater for high solids content
Cow	HTST	Milk	72	15 sec	
Cow	HTST	Milk	72	15 sec	
Cow	HTST	Milk	72	15 sec	
Cow	HTST	Milk	72	18 sec	
Cow	HTST	Milk	72.5	15 sec	
Cow	HTST	Milk	72.5	15 sec	
Cow	HTST	Milk	72.5	19 sec	Temperature >72°C to provide a margin of safety; diversion set point 72.4°C
Cow	HTST	Milk	72.6	39 sec	Time and temperature options restricted by equipment design
Cow	HTST	Milk	74	15 sec	Temperature >72°C to provide operational margin above 72°C; operating temperature fluctuates by ± 1°C
Cow	HTST	Milk	74	17 sec	Temperature >72°C for operational reasons; diversion set point 73°C
Cow	HTST	Milk	74	22 sec	Temperature >72°C to provide operational margin above 72°C; operating temperature fluctuates due to heating arrangement
Cow	HTST	Milk	74	25 sec	Temperature >72°C to provide safe operational margin above 72°C; time > 15 sec is due to allowance for future capacity upgrade
Cow	HTST	Milk	74	27 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	75	18 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	75	20 sec	Temperature and time options limited by equipment design plus need to provide a margin of safety
Cow	HTST	Milk	75	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP; diversion set point 73°C
Cow	HTST	Milk	75	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP; diversion set point 73°C
Cow	HTST	Milk	75.5	18 sec	Temperature >72°C to provide a margin of safety; diversion set point 72.6°C
Cow	HTST	Milk	76	15 sec	Temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	76	25 sec	Greater log reduction of microbial loading
Cow	HTST	Milk	76	25 sec	Greater log reduction of microbial loading
Cow	HTST	Milk	76	25 sec	Greater log reduction of microbial loading
Cow	HTST	Milk	76.5	26.2 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP

Species	Process	Product ¹	Temp (oC)	Holding time	Reason given by respondents for using a temperature >72.5oC and/or a time >18 sec for HTST treatments, or other relevant comments ²
Cow	HTST	Milk	76.5	27 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	76.5	28.1 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	78	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	78	25 sec	Time >20 sec as recommended to cater for any organisms resistant to pasteurisation (ie MAP); Temp >72°C to allow for fluctuations in equipment operation with a margin of safety above 72°C
Cow	HTST	Milk	78	25 sec	Time >20 sec as recommended to cater for any organisms resistant to pasteurisation (ie MAP); Temp >72°C to allow for fluctuations in equipment operation with a margin of safety above 72°C
Cow	HTST	Milk	78	25 sec	Time >20 sec as recommended to cater for any organisms resistant to pasteurisation (ie MAP); Temp >72°C to allow for fluctuations in equipment operation with a margin of safety above 72°C
Cow	HTST	Milk	78	27 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	78	27 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	78	29.7 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	>72	17 sec	
Cow	HTST	Milk	74 ± 1	34 sec	Time >20 sec as recommended for MAP; Temp >72°C to allow for calibration error
Cow	HTST	Milk	75-78	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	75-78	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP
Cow	HTST	Milk	77-79	30 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP and to allow for effect of higher solids content
Cow	HTST	Milk	78-80	25 sec	Time >15 sec and temperature >72°C as precaution for any heat resistant MAP; diversion set point 76°C
Cow	HTST	Yoghurt	76	22 sec	Product texture
Cow	HTST	Yoghurt	80	60 sec	Product texture and ensure adequate heat treatment
Cow	HTST	Yoghurt	87	25 sec	Product texture
Cow	HTST on batch heat exchanger	Sweetened condensed milk	80	2 min	Standardised process for production of sweetened condensed milk to develop flavour as well as to pasteurise the product
Cow	HTST on continuous plate heat exchanger	Milk concentrate	74	15 sec ++	Temp >72°C to allow for fluctuations in equipment operation with a margin of safety above 72°C
Goat	Batch	Cheese	63	30-35 min	Circulating hot water is cut off when the milk temperature reaches 62°C, but the temperature continues to rise to about 64°C before the milk starts to cool.
Goat	Batch + plate heat exchanger	Cheese	62	30 min	Heating of goat milk at 72°C results in soft textured curd. To minimise heat input consistent with pasteurisation, milk is brought up to 62°C in a plate heat exchanger, then circulated through the heat exchanger and a holding tank for 30 min
Goat	Batch	Cheese	65.1	10 min	

Species	Process	Product ¹	Temp (oC)	Holding time	Reason given by respondents for using a temperature >72.5°C and/or a time >18 sec for HTST treatments, or other relevant comments ²
Goat	Batch	Cheese	66.4	5 min	
Goat	Batch	Cheese	68	90 sec	
Goat	Batch	Cheese	68	5 min	
Goat	Batch	Cheese	68.1	2 min	
Goat	Batch	Cheese	70	5 min	
Goat	Batch	Cheese	72	1 min	
Goat	Batch	Cheese	72.5	15 sec	Milk continuously agitated and temperature and time automatically controlled
Goat	Batch	Cream	72.5	15 sec	Following the pasteurisation heat treatment, cream is heated to 90°C momentarily to achieve desired product texture; cream continuously agitated and temperature and time automatically controlled
Goat	Batch	Yoghurt	72.5	15 sec	Following the pasteurisation heat treatment, milk is heated to 90°C momentarily to achieve desired product texture; milk continuously agitated and temperature and time automatically controlled
Goat	HTST	Cheese	72	15 sec	
Goat	HTST	Cheese	72-75	15 sec	Temperature >72°C to provide safe operational margin above 72°C
Goat	HTST	Cheese	76	15 sec	Temperature >72°C as precaution for any heat resistant MAP
Goat	HTST	Cheese	80	15 sec	Milk is often 3-4 days old, sometimes 7 days, when processed
Goat	HTST	Milk	75	25 sec	Time > 15 sec and temperature >72°C as precaution for any heat resistant MAP; diversion set point 73°C
Goat	HTST	Yoghurt	80	60 sec	Product texture and ensure adequate heat treatment
Sheep	Batch	Cheese	63	30 min	
Sheep	Batch	Cheese	68	1 min	
Sheep	Batch	Cheese	74	15 sec	Temperature >72°C to provide operational margin above 72°C; milk continuously agitated and temperature and time automatically controlled
Sheep	Batch	Yoghurt	68	1 min	
Sheep	Batch	Yoghurt	83	30 min	Product texture; milk continuously agitated and temperature and time automatically controlled

¹

Product categories are restricted to the main categories only for reasons of confidentiality, e.g. cheese varieties, while provided by many respondents, are not shown in the table.

² The temperature of 72.5°C and the time of >18 sec for HTST treatments are arbitrary cut-off points above the standard of 72°C for 15 seconds, above which there might be a conscious decision by the operator to increase time and / or temperature for reasons other than operational considerations.

³ Cream unless specified otherwise is cream intended for consumption as cream, i.e. 'table' or 'fresh' cream.

⁴ Designated as HTST by the respondent.

⁵ Designated as Fermented Milk by the respondent.

⁶ Designated as HLLT (High-temperature long-hold) by the respondent.

⁷ Milk includes whole milk, skim milk, modified milk and flavoured milk.

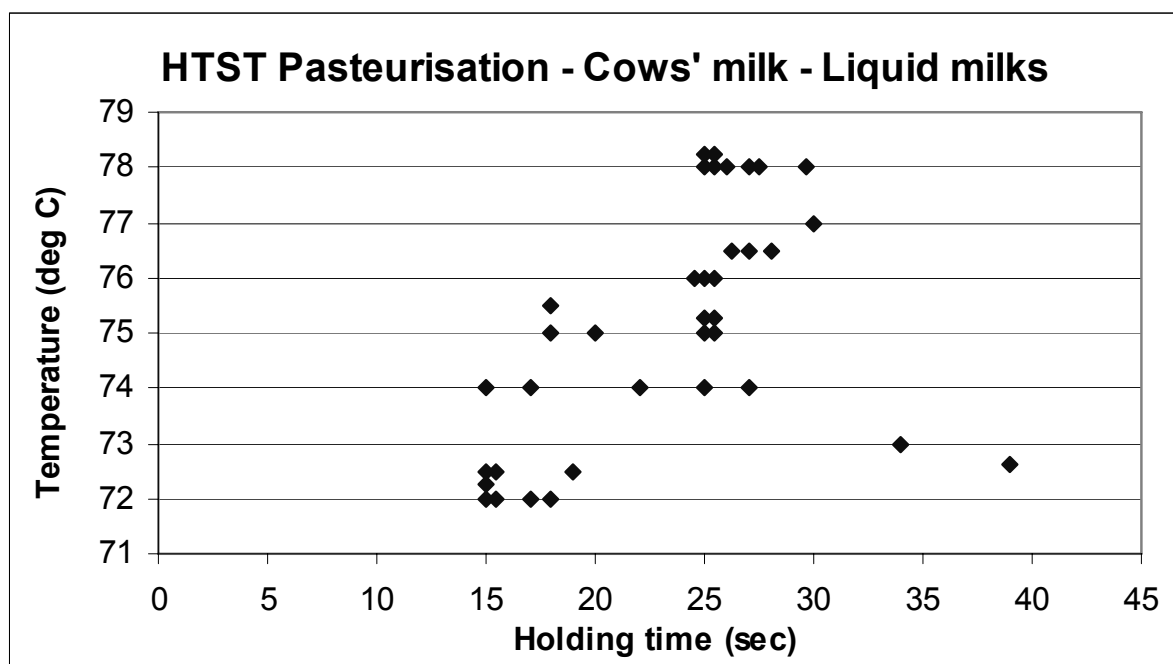
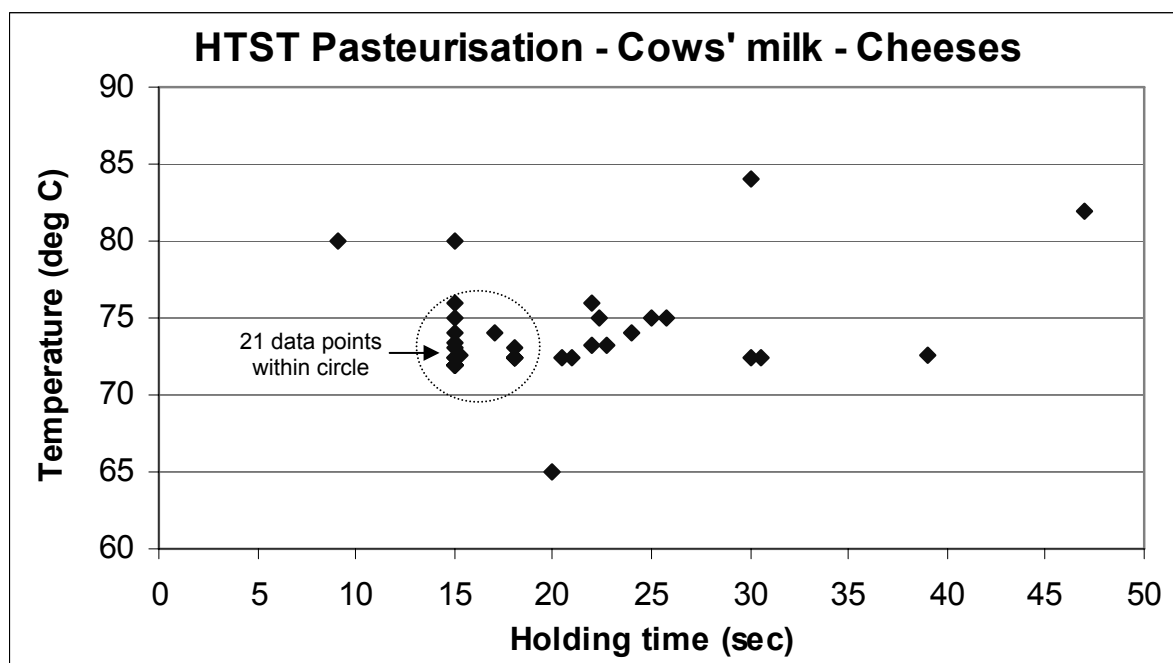


Figure 3.1. Temperatures and times of holding used by Australian dairy manufacturers for the HTST pasteurisation of raw cows’ milk used for the production of fresh liquid products (including whole milk, reduced fat milks, modified milks and flavoured milks). N = 37.



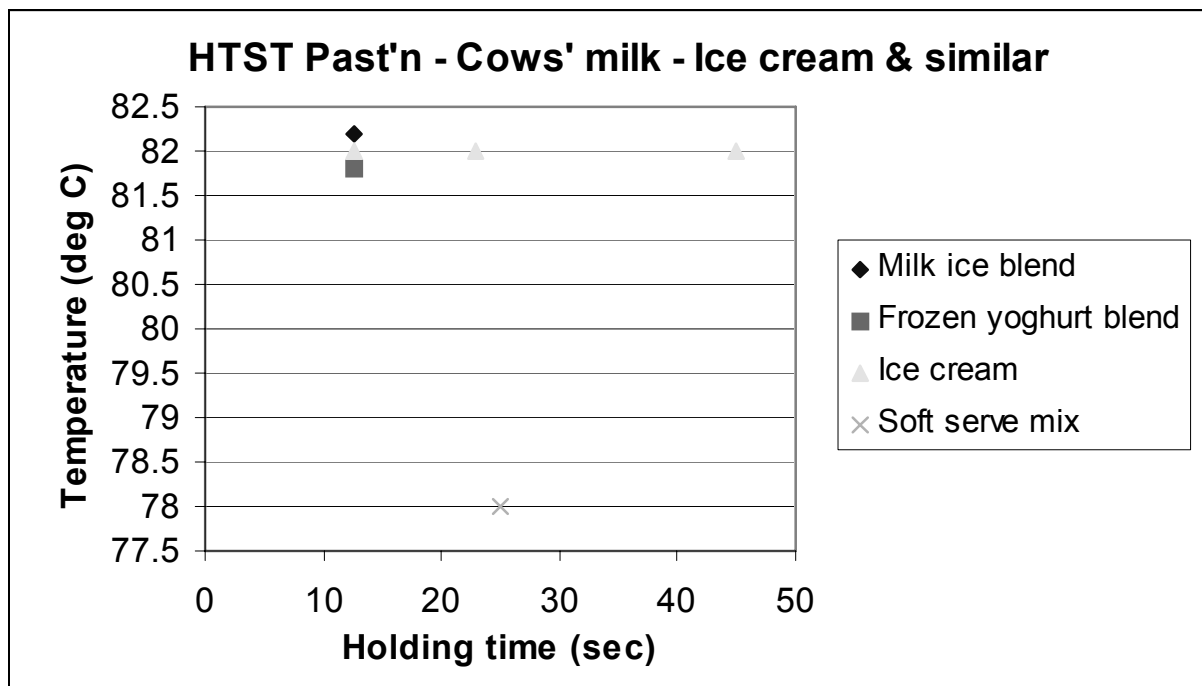


Figure 3.3. Temperatures and times of holding used by Australian dairy manufacturers for the HTST pasteurisation of raw cows' milk for the manufacture of milk ice, frozen yoghurt, ice cream and soft serve mix. N = 7.

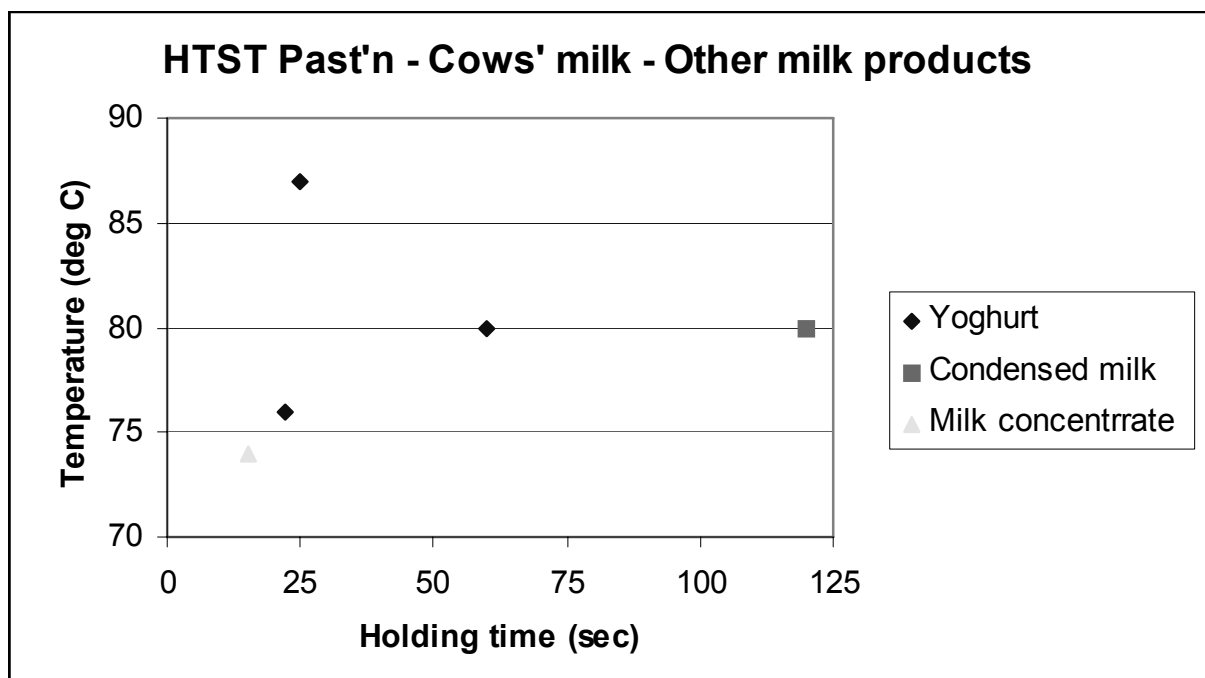


Figure 3.4. Temperatures and times of holding used by Australian dairy manufacturers for the HTST pasteurisation of raw cows' milk used for the manufacture of yoghurt, sweetened condensed milk, milk concentrate and fermented milks. N = 5.

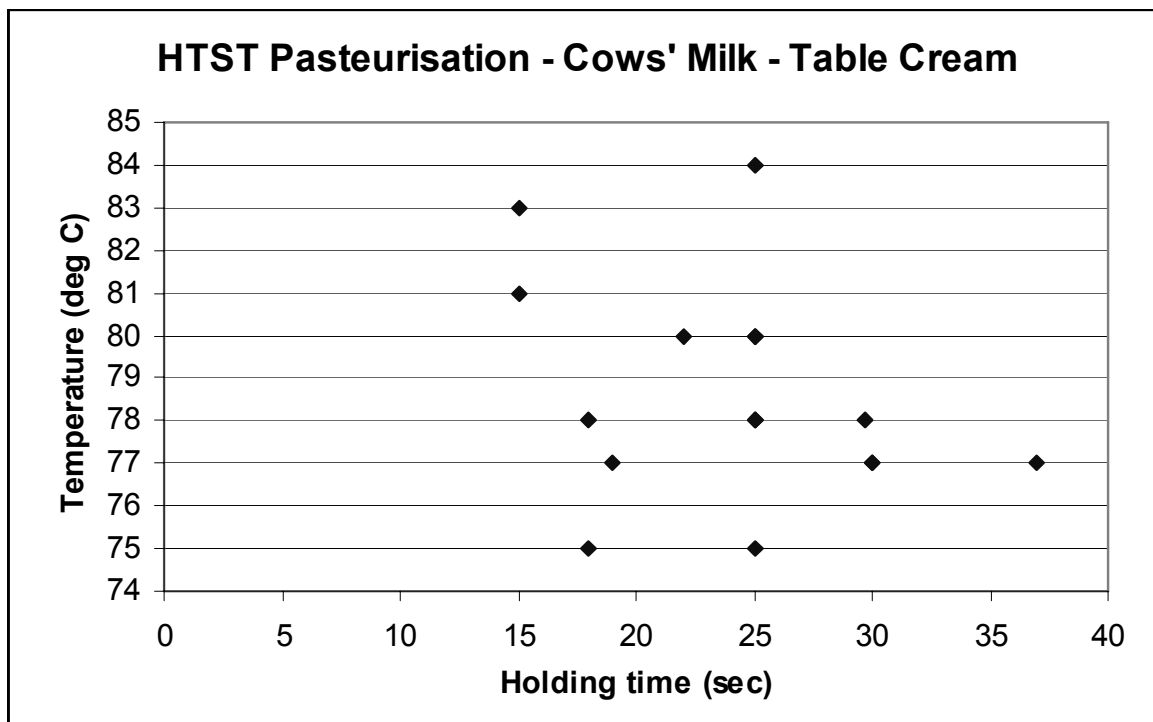


Figure 3.5. Temperatures and times of holding used by Australian dairy manufacturers for the HTST pasteurisation of raw cream from cows' milk for the table cream market. N = 16. Data for heat treatments in the ESL range have not been included in the chart.

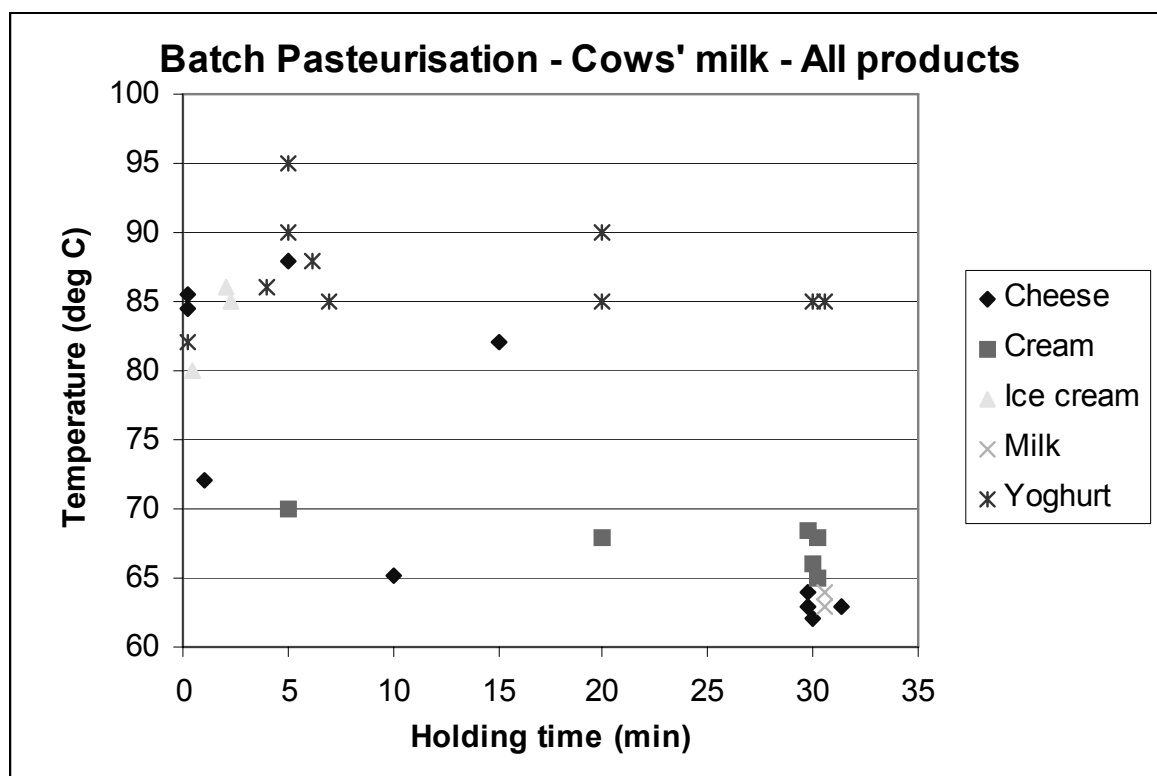


Figure 3.6. Temperatures and times of holding used by Australian dairy manufacturers for the batch pasteurisation of raw cows' milk used for the manufacture of products as indicated. N = 31.

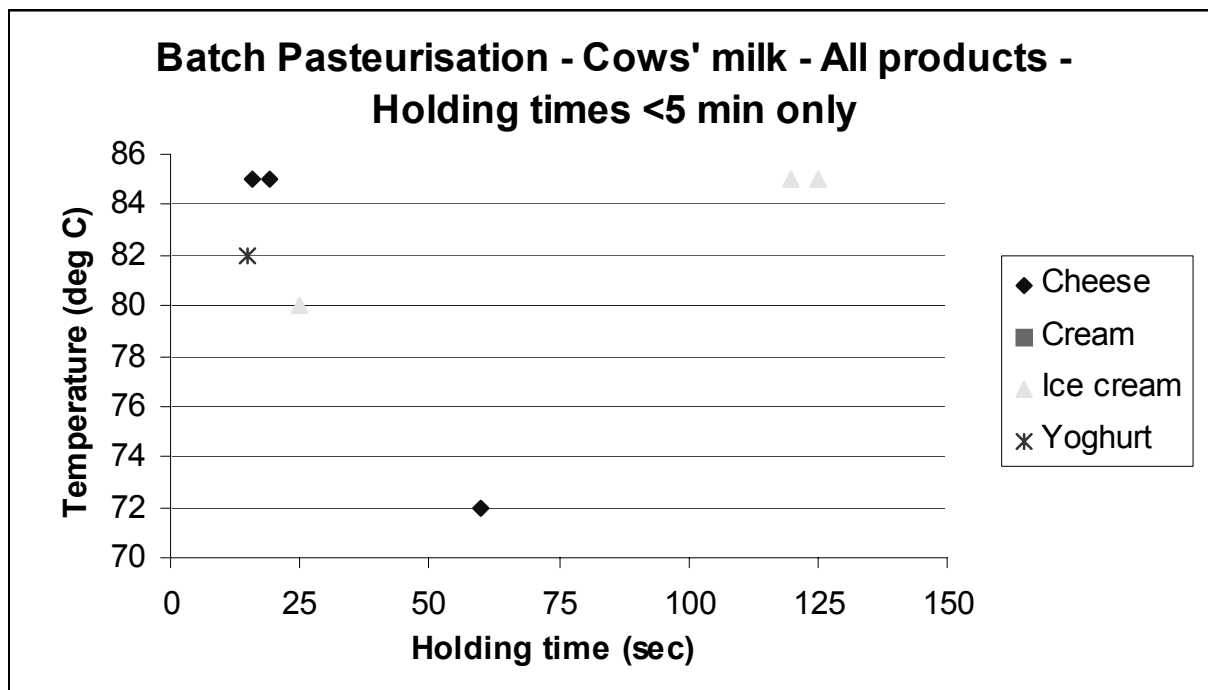


Figure 3.7. Temperatures and times of holding used by Australian dairy manufacturers for the batch pasteurisation of raw cows' milk used for the manufacture of products as indicated, showing data points where holding time was less than five minutes only. N =7.

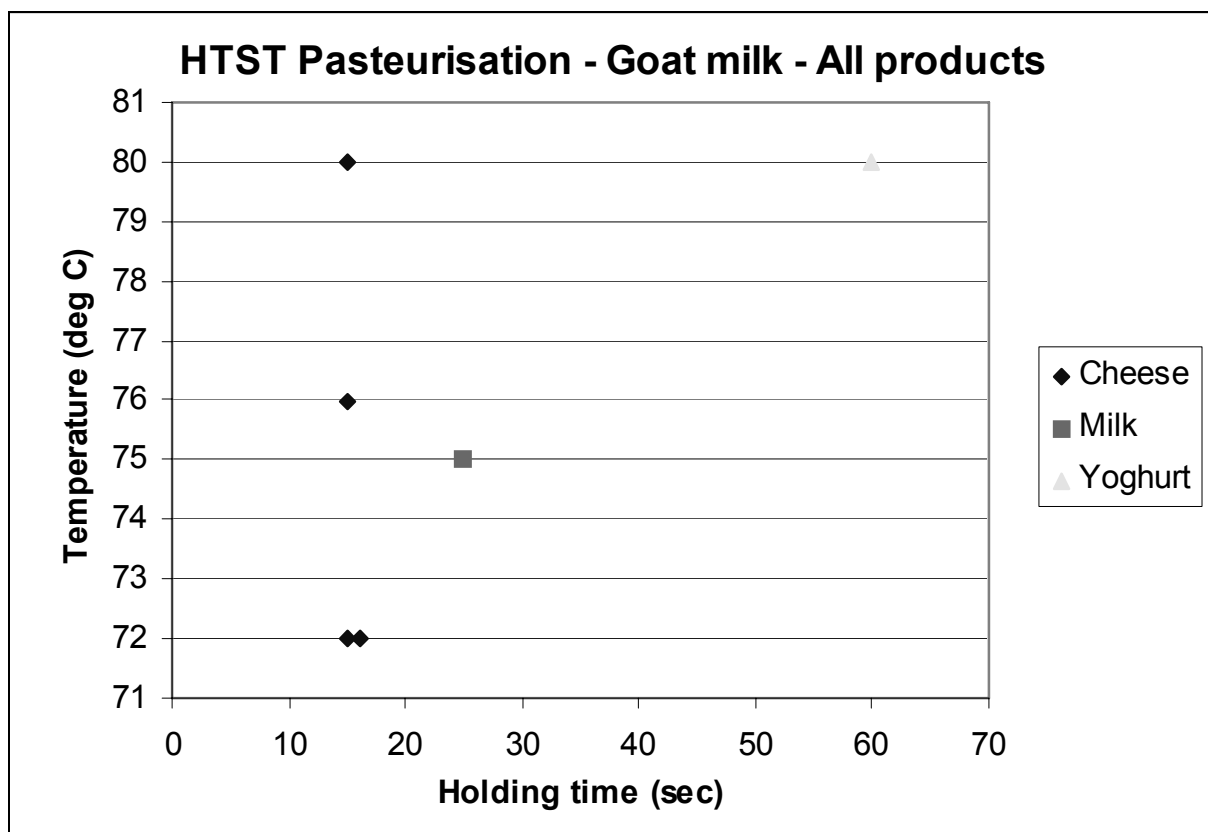


Figure 3.8. Temperatures and times of holding used by Australian dairy manufacturers for the HTST pasteurisation of raw goat milk used for the manufacture of products as indicated. N = 6.

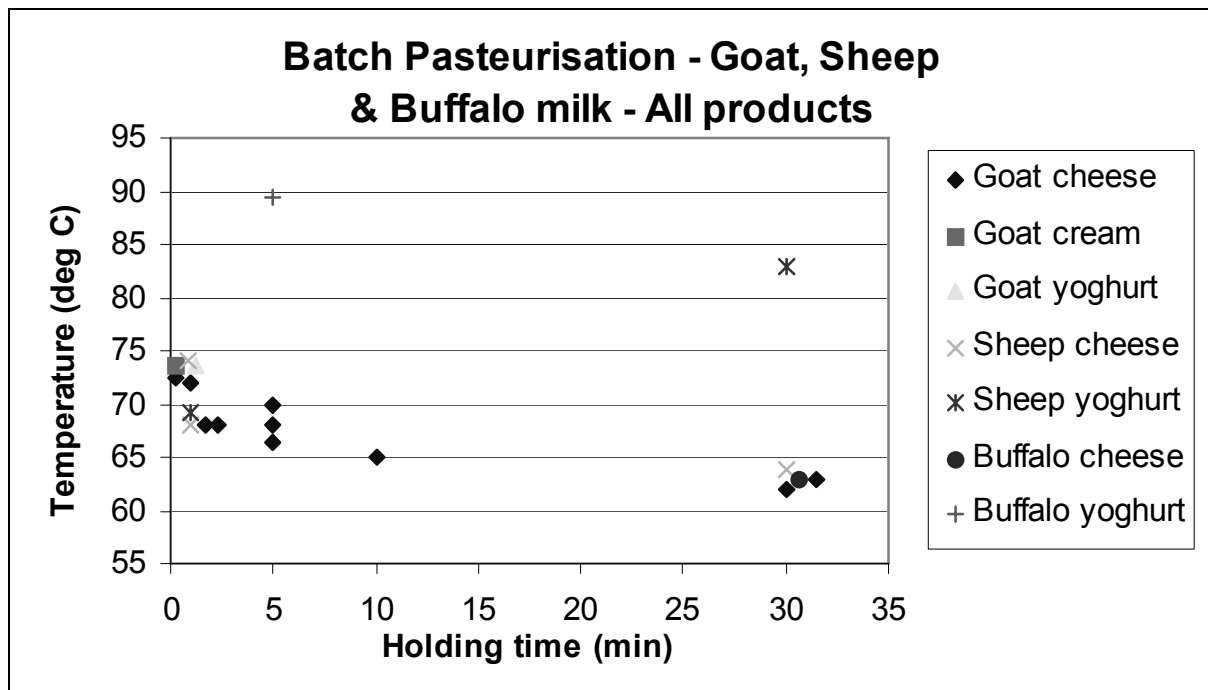


Figure 3.9. Temperatures and times of holding used by Australian dairy manufacturers for the batch pasteurisation of raw goat, sheep and buffalo milk used for the manufacture of products as indicated. N = 18.

Note 1. Data points with holding times <5 min are in an expanded format in Figure 3.10.

Note 2. Refer to Table 3.2 for further details on some of the heat treatments.

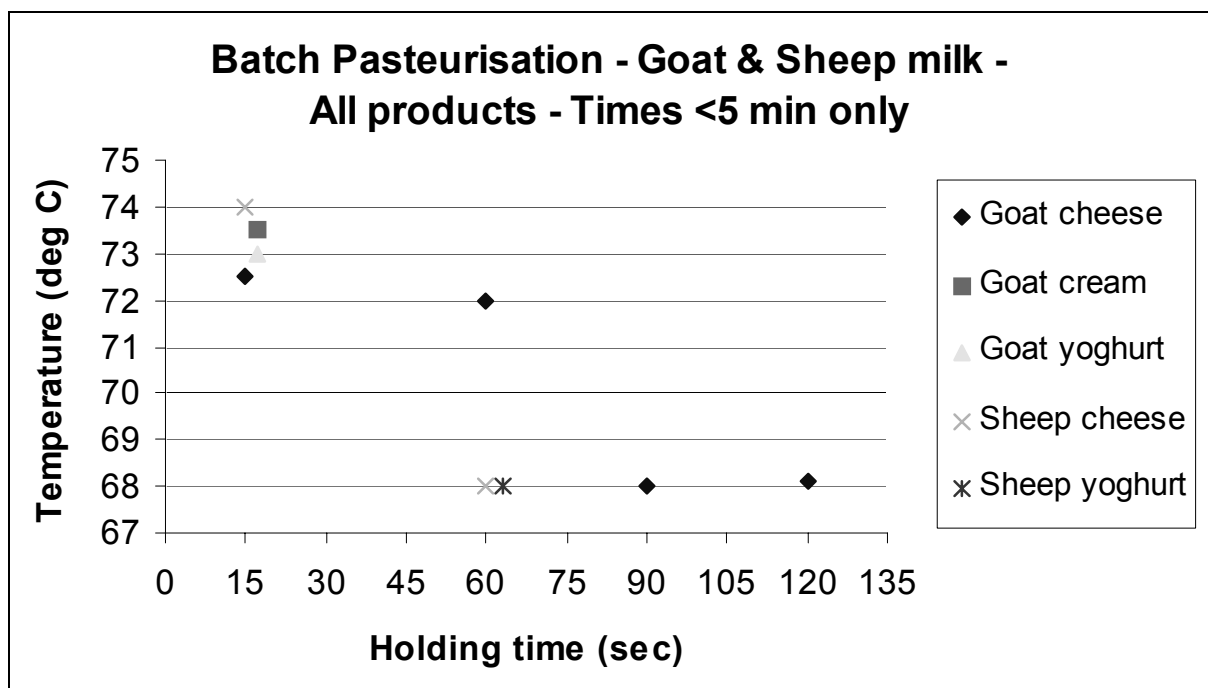


Figure 3.10. Temperatures and times of holding used by Australian dairy manufacturers for the batch pasteurisation of raw goat and sheep used for the manufacture of

products as indicated, showing data points where holding time was less than five minutes only. N =9.

Note. Refer to Table 3.2 for further details on some of the heat treatments.

In some cases, designation of the pasteurising process as ‘batch’ or ‘HTST’ was a source of some confusion among some of the respondents. This was particularly so in the case of yoghurt manufacture, in which case some of the manufacturers, especially the larger ones, use various ‘hybrid’ systems that incorporate both continuous flow heat exchangers and jacketed holding vats to heat treat the yoghurt milk. In a few cases, pasteurizing processes that had been designated as ‘HTST’ or ‘HTLT’ (high-temperature long-hold) by the respondents were arbitrarily re-designated as ‘batch’ for the purposes of this study.

To aid clearer presentation of the data, minor adjustments were made in a few cases to the values in the charts where points based on the reported values were superimposed one upon another. Thus, if the actual values as reported are required, they should be taken from Table 3.2, not from the scatter diagrams.

The main points from an analysis of the data presented in Table 3.2 and in Figures 3.1 – 3.10 are as follows:

- Batch pasteurisation is widely used, particularly by the smaller processors, many of whom are processing the milk in on-farm situations.
- Temperatures and times of heat treatment for batch pasteurisation covered a wide range, from 62 to 95°C and from 15 seconds to 30 minutes.

As discussed in Section 2.1, the recognised standard for batch pasteurisation is 63°C for 30 minutes; however some processors reported using 62°C for 30 minutes, a heat treatment that *Coxiella burnetii* can survive if present in large numbers (see Section 3.3.4). The reasons for using 62°C were not entirely clear, but may have been related to historically-accepted practice in some States or perhaps to the interpretation of rounding when converting temperatures from °F to °C.

Also of concern was that several processors reported using what is essentially a HTST treatment, e.g. 72°C for 15 seconds and similar, under batch conditions. However, it was beyond the scope of this study to determine if an adequate level of process control was in place to ensure that every particle of milk received the minimum heat treatment in these cases.

- Several processors of goat and sheep milk for cheese manufacture indicated that they needed to use the very minimum of legal heat treatments to make a satisfactory product; excessive heat treatment of the milk results in a weak curd.
- In many - though certainly not all - cases, HTST treatment of milk for cheese manufacture was as close to the standard of 72°C for 15 seconds as the pasteurizing equipment would reliably allow. Cheeses manufactured with milk so treated include the hard varieties, e.g. Parmesan, and the semi-hard varieties, e.g. Cheddar, for which excessive heating has a deleterious effect on the cheesemaking process and on the physical properties of the cheese. In contrast, at the other end of the heat treatment scale, cheese varieties such as cream cheese and Ricotta (the latter of which can be manufactured from cheese whey only or a mixture of whey and milk) require a more severe heat treatment to precipitate the whey proteins as an integral part of the process.

Only one processor reported using the permitted 'thermisation option' for the heat treatment of milk for cheese production (see below).

- HTST treatment of milk for liquid milk products, at least by the large processors and some of the smaller ones, was mostly in the range 74-78°C for 15-30 seconds as a precaution against the presence of any pathogens that might be resistant to pasteurisation, notably *M. paratuberculosis*. Elevation of temperatures and lengthening of holding times for the HTST pasteurisation of milk for the liquid milk market in Australia was recommended by the Australian Dairy Industry Council in about the year 2000 as a precautionary measure, pending more definitive data on whether or not *M. paratuberculosis* was in fact able to survive a minimum heat treatment of 72°C for 15 seconds.
- It was of interest that some processors of liquid milk products reported that they had been advised to use elevated temperature and longer times for HTST treatment as a precaution against the presence of any *M. paratuberculosis* organisms that 'might be resistant to heat treatment at 72°C for 15 seconds', even though they were located in regions which, according to Animal Health Australia, are free of Johne's disease in cattle, the common source of *M. paratuberculosis* in raw milk.
- A wide range of time and temperature combinations for HTST treatment of table cream was reported, with the majority in the range 75-80°C for 20-30 seconds, which is broadly consistent with internationally-recognised heat treatments for the pasteurisation of cream⁶ (ie for cream with 10-20% fat, 65°C for 15 seconds and for cream with >20% fat, 80°C for 15 seconds).
- HTST treatments of yoghurt milks (some of which would contain added milk solids) were in the range 76-87°C for 22-60 seconds. The primary purpose of applying a reasonably severe heat treatment for yoghurt milk is to precipitate the whey proteins, thereby increasing viscosity and thickening the product.
- HTST treatments for ice cream mixes and similar were all in the range 78-85°C for 13-45 seconds. Apart from food safety considerations, it is understood heat treatments of this order are required to activate the stabilizers in the mixes.
- A number of processors, particularly those in the small and medium size categories, reported that design of their pasteurisers and operational considerations largely dictated the times and temperatures of heating that they could use in practice. For example, some processors reported that their operating temperatures fluctuated by $\pm 1^\circ\text{C}$, with the result that to operate the pasteuriser safely and without risk of activating flow diversion, the minimum operating temperature that could be used in practice was about 74°C. Other examples reported included limitations in heating capacity of the equipment, homogeniser capacity lower than the rated capacity of the pasteuriser and other 'mismatches' of capacity of equipment in the pasteurising line, each of which generally had the effect of extending the holding time.

Use of thermisation for production of extra hard grating cheese

Standard 1.6.2 of the Australia New Zealand Food Standards Code permits milk used for the production of cheese to be heat treated by being held at a temperature of no less than 62°C for a period of no less than 15 seconds, provided the cheese or cheese product is stored at a

⁶ Bogh-Sorensen T (1992). Cream pasteurisation technology. In *Bulletin of the International Dairy Federation No. 297/1992, Monograph on the pasteurisation of cream. Chapter 7*. International Dairy Federation, Brussels. pp 32-39.

temperature of no less than 2°C for a period of 90 days from the date of manufacture. This heat treatment is termed ‘thermisation’ by some writers, but not by others.

Only one processor reported that they were using the thermisation option (in this case, 65°C for 20 seconds) for production of cheese (in this case, Gruyere, not a hard grating cheese), as permitted by Standard 1.6.2.

Three processors specifically reported that they were manufacturing extra hard grating cheese, but none was using the thermisation option as permitted by Standard 1.6.2. Instead they were using conventional HTST treatment, i.e. 72.5°C for 15 seconds, 73.4°C for 15 seconds and 74°C for 24 seconds. One processor reported that they had sought approval, unsuccessfully, from the State regulatory agency to use the thermisation option for extra hard grating cheese.

From comments received from various sources, the reviewer gained the impression that the interpretation and application of Clause 2 of Standard 1.6.2., Australia New Zealand Food Standard Code, which deals with the processing of cheese and cheese products, has been the subject of some confusion and frustration.

Approved uses of any other processes for the destruction of pathogenic organisms in milk other than traditional pasteurisation

Respondents were asked whether their company has been given approval by any regulatory agency to use a process, or a combination of processes, for the destruction of pathogenic organisms in milk as an alternative to traditional thermal pasteurisation. Responses indicated that there had been no such approvals granted, at least among the respondents. Some respondents reported, for example, that they had been granted approval to use heat treatments in the ESL/UHT range for bulk starter milk. However these treatments still fall within the broad definition of pasteurisation.

DFSV survey data on pasteurisation times and temperatures used in Victoria

Dairy Food Safety Victoria undertook a comprehensive survey of the design and operation of pasteurisers used for the processing of milk in Victoria during February and March 2004 (Hempenstall, 2004). The survey covered all pasteurising plants, both batch and continuous, used for heat treatment of cows’ milk only in that State. Some of the survey questionnaires were completed independently by respondents, while others were completed via interview (Chris Hempenstall – DFSV, pers. comm.).

Questions on the survey form related to the times and temperatures used for pasteurisation and the capacity of the pasteurisation equipment. Of the 14 manufacturers who were using batch pasteurisation equipment, 10 (71%) provided information on their pasteurisation times and temperatures (Chris Hempenstall - DFSV, pers. comm.). The temperatures and times of holding used by the 10 respondents are shown in Table 3.3 and in Figure 3.11 as a scatter diagram. The capacity of the equipment is shown in Table 3.4.

Table 3.3. Temperatures and times of holding used by 10 Victorian dairy manufacturers for batch pasteurisation of cows' milk. *Source: Chris Hempenstall -DFSV, pers. comm. Data current as at February-March 2004.*

Temperature of milk during the holding period (°C)	Time of holding (minutes)
62.5	30
63.0	30
63.0	30
63.5	34
65.0	30
65.1	10
66.0	22
68.0	17
76.5	20
80.0	5

Table 3.4. Capacity of batch pasteurisers used for cows' milk by 10 Victorian dairy manufacturers. *Source:* Chris Hempenstall -DFSV, pers. comm. Data current as at February-March 2004.

Capacity of batch pasteurisers (L)	No. of units
<100	1
100-500	3
500-1000	4
>1000	2

The temperatures and times used for batch pasteurisation in Victoria, as shown in Table 3.3 and Figure 3.11, are within the expected range.

A total of 59 dairy manufacturers were using 73 continuous flow heat exchangers for pasteurisation of milk and milk products in Victoria as at February March 2004 (Hempenstall, 2004). Of these plants, approximately seven were used for UHT and 66 for HTST. For the latter category, 61 sets of data on time and temperature of heating were obtained, representing a response rate of at least 92%. These data are shown in tabular form in Table 3.5 and as a scatter diagram in Figure 3.12. The capacity of the equipment, expressed in terms of average operating flow rate, is shown in Table 3.6.

Table 3.5. Time and temperature of heating for each of 61 continuous flow HTST heat exchangers used to pasteurise milk in Victoria. *Source:* Chris Hempenstall -DFSV, pers. comm. Data current as at February-March 2004.

Temperature of milk during the holding period (°C)	Time of holding (seconds)	Temperature of milk during the holding period (°C)	Time of holding (seconds)	Temperature of milk during the holding period (°C)	Time of holding (seconds)
72	15	75	21	79.5	30
72	18	75	21	80	20
73	15	75	22	80	25
73	15	75	25	80	30
73	15	76	15	81	15
73	16	76	16	81	17
73	16.49	76	17.53	81	17
73	18	76	18	81	18
73	20	76	18	81	20
74	15	76	18	82	18
74	16	77	15	82	25
74	17	77	22	82	30
74	18	77	24	83	15
74	19	77	25.36	84	15
74	20	77	28.02	84	15
74	30	78	17	85	15
74.5	18	78	19	85	30
74.5	18	78	28	86	15
74.5	30	78.9	28	86	15
75	15	79	32	86	15
→			→	86	15

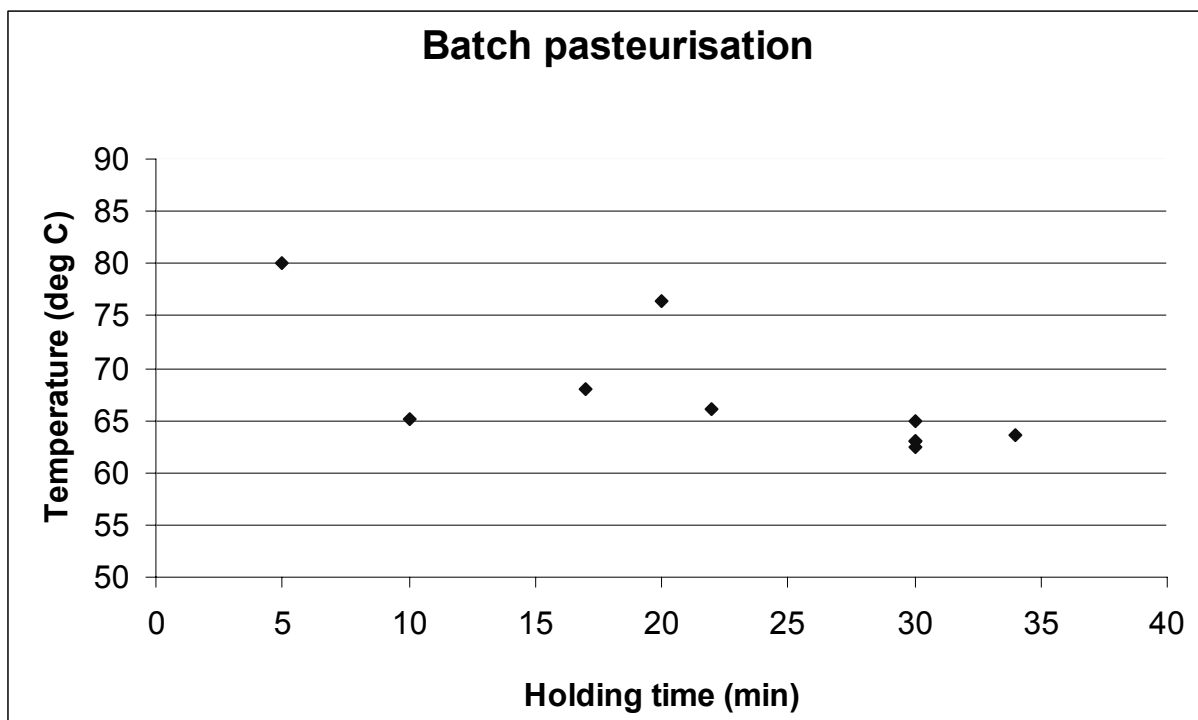


Figure 3.11. Temperatures and times of holding used by Victorian dairy manufacturers for batch pasteurisation of milk. N = 10. (Source: Chris Hempenstall -DFSV, pers. comm. Data current as at February-March 2004.)

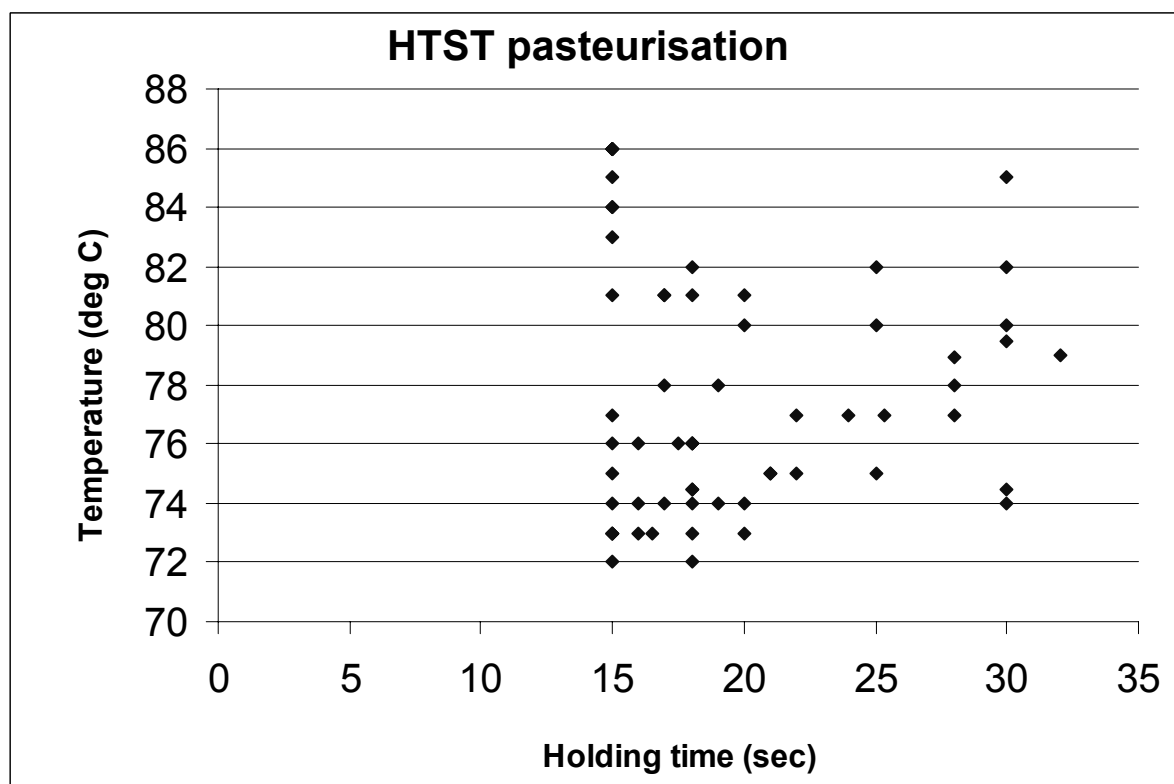


Figure 3.12. Temperatures and times of holding used by Victorian dairy manufacturers for HTST pasteurisation of milk. N = 61. (Source: Chris Hempenstall -DFSV, pers. comm. Data current as at February-March 2004.)

Table 3.6. Flow rate capacity of 61 continuous flow HTST heat exchangers used to pasteurise milk in Victoria. *Source: Chris Hempenstall -DFSV, pers. comm. Data current as at February-March 2004.*

Average operating flow rate (litres/hr)	No. of units
500-2000	7
>2000-5000	9
>5000-10,000	17
>10,000-20,000	14
>20,000-50,000	7
>30,000-60,000	7

It is understood that a few of the data sets shown in Table 3.5 and in Figure 3.12 apply to pasteurisers that are used for re-pasteurisation of milk products, e.g. ice cream mix based on reconstituted dried milk (Chris Hempenstall - DFSV, pers. comm.). As far as the reviewers are aware, re-pasteurisation of reconstituted milk does not require any special consideration *per se*. However, many of the products based on milk powder, or to which milk powder is added to boost the milk solids content, will have a higher solids content than a standard raw milk and this would need to be taken into account when selecting a heat treatment that will ensure effective pasteurisation.

Data provided by the manufacturers of milk powder are not included in Table 3.5 or Figure 3.12. They reported that they were pasteurising milk for this purpose within a range from 116°C for 3.1 seconds to 121°C for 1.8 seconds. Milk is superheated by direct steam injection before it is passed through an evaporator before drying.

The data presented in Table 3.5 and Figure 3.12 showed that the minimum heat treatment for HTST pasteurisation as per Standard 1.6.2, i.e. 72°C for 15 seconds, was being achieved in all cases. Temperatures ranged from 72-86°C and times from 15-32 seconds with many different combinations within those ranges. Several manufacturers were using temperatures from the upper end of the temperature range, e.g. 86°C, in combination with times from the lower end of the time range, e.g. 15 seconds.

Summary and discussion of survey data on industry pasteurisation practices

A specified component of the study was to report on current industry practice in Australia with respect to methods employed for the pasteurisation of milk, in particular the time/temperature combinations used and their relationship to minimum regulatory standards.

To obtain this information, a short questionnaire was sent to all dairy companies/plants in Australia engaged in the pasteurisation of raw milk. The Government agency in each State responsible for regulating food safety in the dairy industry assisted with distribution of the questionnaire and, in some cases, also with collection of the completed questionnaires and follow-up of non-responders.

Information was sought from industry on the pasteurisation of raw milk from all of the animal species currently used for commercial milk production in Australia, i.e. cow, goat, sheep and buffalo. The pasteurisation of raw milk used for the production of liquid milk and milk products and of raw milk used in the production of any cream and cream products, fermented milks, yoghurt, dried, condensed and evaporated milks, ice cream and cheese, as defined in

Standard 1.6.2, were both within the scope of the study. Information on the pasteurisation of raw cream for the fresh table cream market was also sought. Respondents were also asked to provide information on method of pasteurisation and type of product being manufactured, and to comment on why each particular heat treatment, if different from the Standard, was being used.

Completed questionnaires from 71 companies, representing 87 processing sites, were received. With a total of 159 individual data sets provided, the responses provided a good stratified sample of the dairy processors in Australia involved in the pasteurisation of raw milk. All States, different animal species and all sizes of plants were all well-represented in the sample. There was also reasonable consistency in the data.

Additional information on pasteurisation times and temperatures from a comprehensive survey of the design and operation of pasteurisers used for the processing of milk in Victoria undertaken by Dairy Food Safety Victoria (DFSV) during February and March 2004 (Hempstall, 2004) was made available to the reviewers and is summarised in the report. The DFSV survey covered all pasteurising plants, both batch and continuous, used for heat treatment of cows' milk in Victoria. The DFSV data covers 10 (71%) of the 14 manufacturers who were using batch pasteurisation equipment in that State at that time and, similarly, 61 (92%) of the 66 manufacturers who were using HTST pasteurisation.

From the Australia-wide survey conducted during this study, it is clear that batch pasteurisation is widely used in Australia, particularly by the smaller processors, many of whom are processing the milk in on-farm situations. However, batch pasteurisation would account for only a very small percentage of all milk pasteurised in Australia. Temperatures and times of heat treatment for batch pasteurisation covered a wide range, from 62 to 90°C and from 15 seconds to 30 minutes. Some processors reported using heat treatments with a temperature slightly lower than in the recommended standard for batch pasteurisation, 63°C for 30 minutes. Also, several processors reported that they were using what is essentially a HTST treatment, i.e. 72°C for 15 seconds or similar, under batch conditions.

For technological reasons, cheese variety has a major influence on the heat treatment applied to milk used for manufacture of cheese. For example, HTST pasteurisation of milk for the manufacture of the hard and semi-hard varieties of cheese is conducted as close to the standard of 72°C for 15 seconds as the pasteurizing equipment will reliably allow. In contrast, at the other end of the heat treatment scale, cheese varieties such as cream cheese and Ricotta require a more severe heat treatment.

Several processors of goat and sheep milk for cheese manufacture indicated that they needed to use the very minimum of legal heat treatments to make a satisfactory product.

HTST treatment of milk for liquid milk products, at least by the large processors and some of the smaller ones, was mostly in the range 74-78°C for 15-30 seconds. The peak dairy industry organisation in Australia recommended in 2000 that the times and temperatures for HTST pasteurisation of milk for the liquid milk trade be increased as a precaution against the presence in the raw milk of any pathogens that might be resistant to pasteurisation, notably *M. paratuberculosis*. Whether use of this enhanced heat treatment is still warranted in the light of more recent studies on the heat resistance of this organism that have been conducted using commercial HTST equipment (refer Section 2.3.8 of this report), particularly in areas of

Australia where Johne's disease in cattle is reported to be not endemic, is a matter of conjecture.

Heat treatments applied to yoghurt milk, ice cream mixes and cream were generally within in the expected range.

A number of processors, particularly those in the small and medium size categories, reported that design of their pasteurisers and operational considerations largely dictated the times and temperatures of heating that they could use in practice.

The DFSV survey showed that the minimum heat treatment for HTST pasteurisation as per Standard 1.6.2, i.e. 72°C for 15 seconds, was being achieved by all respondents to that survey. Temperatures ranged from 72-86°C and times from 15-32 seconds with many different combinations within those ranges.

4. General discussion and conclusions – pasteurisation and thermisation

History of pasteurisation

Pasteurisation of milk is now taken for granted. However an understanding of the history of milk pasteurisation, which is long and highlighted by periods of considerable controversy, is an essential prerequisite to a scientific evaluation of the process.

Recommendations on the heating of milk in the home before it was fed to infants were recorded as early as 1824, 40 years before Pasteur's first experiments. In 1911, the National Milk Standards Committee in the United States was the first professional body to recommend a minimum time-temperature combination for the pasteurisation of milk: 62.8°C (145°F) for 30 minutes (now known as the batch or holder method). This heat treatment was slightly above what many people at the time considered to be adequate exposure for the destruction of *Mycobacterium tuberculosis*, one of the main milk-borne pathogens of concern in that era.

However, it was not until after further research and investigation of the capabilities of the available commercial equipment, that the 'holding method' of milk pasteurisation was first officially and legally recognised as an adequate method of pasteurisation in the United States where, in 1924, the first Pasteurised Milk Ordinance was published. In the Ordinance, pasteurisation was defined as '*a heating process of not less than 142°F (61.1°C) for 30 minutes in approved equipment*'. However, it is noteworthy that a temperature 3°F lower than that which had been recommended earlier, in 1911, was officially adopted.

Following further studies on the thermal destruction of *M. tuberculosis* and other pathogens, a High Temperature Short Time (HTST) pasteurisation standard - 161°F (71.7°C) for 15 seconds - was included in the 1933 edition of the U.S. Public Health Service Milk Ordinance and Code. The effect of HTST treatment on the creaming ability of milk was also taken into account in setting the standard.

In the late 1930s, it became apparent that *Coxiella burnetii*, the causal agent of Q Fever, was more heat resistant than *M. tuberculosis/bovis*. Studies reported in 1956 showed that if *C. burnetii* cells were present in raw milk in large numbers, some might survive 143°F (61.7°C) for 30 minutes. These studies resulted in a recommendation by the U.S. Public Health Service to increase the standard for the 'holding method' of pasteurisation to 145°F (62.8°C) for 30 minutes. It was also suggested that at least an additional 5°F (2.8°C) be added to the holding temperature for products with a fat content higher than whole milk or with added sugar.

Apart from some rounding of numbers to take account of Fahrenheit-Celsius conversions, the above standards for pasteurisation have remained unchanged to the present day. According to the International Dairy Federation, the minimum time-temperature combinations now recognised world-wide are 63°C for 30 minutes or 72°C for 15 seconds.

The phosphatase test has been widely used in quality control and food safety programs as an indicator of the efficiency of the milk pasteurisation process. Alkaline phosphatase is an enzyme that is naturally present in raw milk and which, by coincidence, is inactivated when heated at 71.7°C for 15 seconds.

Thermisation is a loosely-defined sub-pasteurisation heat treatment applied to raw milk, typically in the range 62-65°C for 10-20 seconds, first introduced in the late 1950s. There are two schools of thought on its application:

- To extend the storage life of the raw milk before normal pasteurisation, by controlling the psychrotrophic bacteria at an early stage. In this case, the milk is cooled to refrigerated storage temperatures immediately following the thermisation treatment, pending pasteurisation at a later date, i.e. it is not intended to be a replacement for pasteurisation.
- To allow '*cheesemaking to proceed with the positive bacteriological effect of pasteurisation, but without its disadvantages for cheese ripening and whey protein degradation*'. In this case, the milk is not subjected to later pasteurisation and would usually be cooled directly to the cheesemaking temperature only. Some have argued that the application of a sub-pasteurisation heat treatment for this purpose is not 'thermisation'.

As clearly demonstrated by this evaluation, thermisation cannot be relied upon to destroy any pathogenic bacteria that might be present in the raw milk.

Typical minimum time-temperature combinations used for the pasteurisation of table cream are as follows:

- Holding (batch) method: 65°C for 30 minutes;
- HTST method for cream with 10-20% fat: 75°C for 15 seconds; and
- HTST method for cream with >20% fat: 80°C for 15 seconds.

Requirements for pasteurisation - Australia New Zealand Food Standards Code

Standard 1.6.2 of the Australia New Zealand Food Standards Code specifies that, for the pasteurisation of milk in Australia, the minimum heat treatment is no less than 72°C for no less than 15 seconds, or any other time and temperature combination of equal or greater lethal effect. Batch pasteurisation of milk is covered by the latter provision. Sub-pasteurisation heat treatment of milk for the manufacture of certain types of cheese is permitted under specified conditions.

Methods for determination of heat resistance and interpretation of the data

Many different techniques and types of equipment have been used to measure heat resistance of milk-borne pathogens, ranging from the very simple to the very sophisticated and from micro scale to commercial scale. However, there is ample evidence to indicate that the method used to determine heat resistance is a major factor in determining (i) the reliability of the heat resistance data generated, and (ii) its relevance to commercial pasteurisation practice. Hence methodology should always be considered when assessing the veracity of any conclusions about the ability of an organism to survive/not survive commercial heat treatments.

From a commercial perspective, it is the overall impact of the integrated heating profile, plus any other relevant system inputs, on the survival/destruction of any pathogens that may be present in the raw milk on any given day is what really counts. Other system inputs during commercial processing include turbulent flow and, in some cases, homogenization. Thus

greatest weight should be given to the results of heat resistance studies carried out using actual HTST pasteurisation equipment, be it either pilot plant- or commercial-scale.

Ability of the nominated bacterial pathogens to survive pasteurisation

Heat resistance studies conducted using either pilot plant- and/or or commercial-scale HTST pasteurisation equipment, together with additional data from studies using various laboratory techniques, have confirmed that the vegetative forms of 11 of the 18 pathogenic species nominated for review are destroyed by both batch (63°C for 30 minutes) and HTST (72°C for 15 seconds) pasteurisation, with a reasonable margin of safety. These species are:

- *Brucella abortus*
- *Campylobacter coli*
- Pathogenic *Escherichia coli* (O157:H7)
- *Mycobacterium tuberculosis*
- *Salmonella enterica* serotypes
- *Yersinia enterocolitica*
- *Campylobacter jejuni*
- *Coxiella burnetii*
- *Listeria monocytogenes*
- *Mycobacterium bovis*
- *Streptococcus pyogenes*

However, it must be noted that the most recent heat resistance data for several of the above pathogens is now quite dated (see below under 'Identified Gaps in the Data'). In addition, the available data for *Streptococcus pyogenes* is of poor quality.

The situation with respect to each of the remaining seven organisms nominated for study is as follows:

- ***Mycobacterium paratuberculosis***. The heat resistance of this organism has been subject to extensive study during the past decade using various laboratory techniques and pilot scale HTST equipment. For a number of reasons, obtaining definitive heat resistance data for this organism has proved to be difficult. While there appears to be ample evidence that this organism is destroyed by batch pasteurisation, studies on the ability of *M. paratuberculosis* to survive heating at 72°C for 15 seconds, even with pilot scale HTST equipment, have given conflicting results. However, the more recent, well-controlled studies have shown that a minimum 4-log₁₀ reduction is obtained during HTST pasteurisation. In view of the numbers of *M. paratuberculosis* likely to be present in the raw milk, this level of kill in fact provides a reasonable margin of safety for the consumer. More generally, however, population reductions in the order of 6-7D have been reported. The fact that it is necessary for operational reasons to operate HTST equipment at temperatures slightly higher than 72°C - apart from any decision to use higher temperatures for other reasons - provides an additional margin of safety.

A fundamental question with respect to *M. paratuberculosis*, which as yet remains unanswered, is whether the organism is in fact a human pathogen, or whether its postulated association with Crohn's disease is just serendipitous, rather than causal. If studies eventually establish that there is no causal connection between *M. paratuberculosis* and Crohn's disease, any concerns that this organism might be able to survive HTST pasteurisation will prove to have been unfounded. Consideration of this issue was beyond the scope of the present review.

- ***Bacillus cereus***. Although there is limited data available specifically on the heat resistance of the vegetative form of this organism, and none using commercial HTST equipment, it is generally accepted that the vegetative cells are readily destroyed by both batch and HTST pasteurisation. However, this is to some extent academic, as there is

more than ample evidence to indicate that the spores of *Bacillus cereus* are very heat resistant and readily survive any heat treatments in the normal pasteurisation range. The pasteurisation heat treatment is sufficient to heat activate the fast-germinating spores of *B. cereus*, but not the slow-germinating spores. Similarly, pasteurisation inactivates diarrhoeagenic toxins produced by *B. cereus*, but not the emetic toxin.

- ***Brucella melitensis***. No definitive data on the heat resistance of the organism (which is not endemic in Australia) were located. However general statements from authoritative sources indicate that the organism is destroyed by pasteurisation.
- ***Enterobacter sakazakii***. Although the data is somewhat variable, and data using commercial HTST equipment is lacking, the consensus view is that the heat resistance of this organism falls within the safety margins of commercial pasteurisation. Its presence in pasteurised milk products has been found to be due to re-contamination of the pasteurised product after the pasteurisation step.
- ***Staphylococcus aureus***. Although this organism has relatively high heat resistance for a mesophilic non-spore-forming bacterium, and despite the fact that data using commercial HTST equipment is lacking, there is ample evidence from laboratory studies that it is destroyed by both batch and HTST pasteurisation heat treatments with a wide margin of safety. However, the thermal stability of the enterotoxins produced by *S. aureus* greatly exceeds that of its vegetative cells, and readily survives pasteurisation by a wide margin.
- ***Streptococcus agalactiae***. Only one report on the heat resistance of *S. agalactiae* was located. This indicated - under relatively crude experimental conditions - that the organism was inactivated at unspecified population levels in cream by batch pasteurisation. That this is the extent of the data on the heat resistance of this organism is quite remarkable, given that it is a common cause of bovine mastitis and can be transmitted to humans, especially women, who drink raw milk.
- ***Streptococcus zooepidemicus***. Not a single report on the heat resistance of *S. zooepidemicus* was located. This is also remarkable, in view of the fact that human infection with this organism can usually be traced to an animal source, including ingestion of unpasteurised milk and cheese. Consumption of raw milk was shown to be the source of a severe human infection with this organism in South-East Queensland about 12 years ago.

Approximately 95% of the 265 studies on heat resistance reviewed during this study, either directly or via composite data compiled by other reviewers, were conducted using cows' milk as the heating medium. Limited numbers of studies comparing heat resistance in milk from the different animal species or in different formulations of cows' milk have been reported. In most of these cases, the measured heat resistance has reflected the protective effect of fat and/or total solids content of the milk, i.e. the higher the fat and/or total solids content, the higher the heat resistance. However, the effects generally have not been dramatic and there were some exceptions.

Of the 91 papers directly reviewed during this study, only about 10% reported confidence limits for thermal death time curves and/or D values. These limits add considerable rigour to a data set, particularly when calculating margins of safety for a heat treatment.

Ability of the nominated bacterial pathogens to survive thermisation

Thermisation at 62°C for 15 seconds is generally insufficient to destroy any of the bacterial pathogens likely to be present in raw milk with a reasonable margin of safety. For 8 of the 18 species reviewed, thermisation would have no or little impact on the number of viable organisms (see Table 2.16). For 7 of the 18 species reviewed (see Table 2.16), thermisation might give a partial kill, depending upon a range of influencing factors, such as the heat resistance and numbers of the particular strains present in the milk (and, for predictive purposes, the particular D values chosen from the literature for calculation of expected kill). For the remaining three species (*B. cereus*, vegetative cells; *S. agalactiae* and *S. zooepidemicus*), no data were available on which to base an assessment of impact of thermisation on them.

Epidemiological data on disease outbreaks linked to consumption of pasteurised milk

There is a substantial body of mainly anecdotal evidence of the public health benefits of milk pasteurisation particularly during the first half of the last century, which largely drove the eventual acceptance of the process as a mandated public health measure in many countries (refer Section 2.1 of this report).

OzFoodNet has no documented reports of any outbreaks of gastrointestinal illness in Australia between 2000 and 2004 due to the consumption of pasteurised milk. However, there have been several outbreaks of enteric infection in Australia in recent years due to the consumption of unpasteurised milk (Mr Russell Stafford⁷, pers. comm., 2005).

An outbreak involving 50 cases of cryptosporidiosis amongst school children in the UK in 1995 was traced to the consumption of 'pasteurised' milk from a local farm. Upon investigation, however, it was established that the farm's pasteurisation equipment was faulty and that, as a consequence, the children had in fact consumed inadequately pasteurised milk⁵.

It should be noted that the data from OzFoodNet only relates to reported cases of gastrointestinal illness. For example, outbreaks of gastrointestinal illness due to ingestion of *Bacillus cereus* in a food might not be detected, as pathology laboratories do not routinely test stool samples from patients with symptoms of gastrointestinal illness for this organism⁵.

More detailed information supplied by Mr Stafford is provided at Attachment 2 to this report.

The detection of pathogenic microorganisms in packages of commercially-pasteurised milk is alone insufficient evidence that the organisms are resistant to the pasteurisation heat treatment. There are well-documented cases (refer above and Section 3.3 of this report) in which it has been shown that the presence of pathogens in pasteurised milk has been due to inadequate pasteurisation (eg faulty equipment or poor process control) or to re-contamination of the milk after the pasteurisation step in the processing line (eg ineffective sanitising of the equipment). Further investigation of the circumstances surrounding such incidents is always necessary.

Times and temperatures used by industry for the pasteurisation of milk in Australia

From the Australia-wide industry survey conducted during this study and additional data from a survey of the Victorian dairy industry by Dairy Food Safety Victoria in 2004, it was clear that batch pasteurisation is widely used in Australia. However, as the batch method is mainly

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used by the smaller processors, it would account for only a very small percentage of all milk pasteurised in Australia. Temperatures and times of heat treatment for batch pasteurisation covered a wide range, from 62 to 90°C and from 15 seconds to 30 minutes. Type of product being manufactured was a major influence on the temperature-time combination used.

Of concern was that several processors reported using what is essentially a HTST treatment, e.g. 72°C for 15 seconds and similar, under batch conditions.

All of the temperatures and times for the pasteurisation of milk by the HTST method reported during the industry surveys showed that the minimum heat treatment for HTST pasteurisation as specified in the Australia New Zealand Food Standards Code, i.e. 72°C for 15 seconds, was being achieved by all respondents to the surveys. Beyond that, however, temperatures ranged from 72-86°C and times from 15-50 seconds, with many different combinations within those ranges. As with batch pasteurisation, type of product was again a major influence on the heating regime used, with the time and temperatures reported generally being within the expected range for the type of product.

Of particular note is that HTST treatment of milk for liquid milk products, at least by most of the large processors and some of the smaller ones, was mostly in the range 74-78°C for 15-30 seconds. This reflects a recommendation by the peak Australian dairy industry organisation in 2000 that the times and temperatures for HTST pasteurisation of milk for the liquid milk trade be increased as a precaution against the presence in the raw milk of any *M. paratuberculosis* organisms that might be resistant to minimum pasteurisation treatment of 72°C for 15 seconds. Whether use of this enhanced heat treatment is still warranted in the light of more recent studies on the heat resistance of this organism that have been conducted using commercial HTST equipment (refer Section 3.3.8 of this report), particularly in areas of Australia where Johne's disease in cattle is reported to be not endemic, is a matter for conjecture.

Some processors, particularly those in the small and medium size categories, reported that design of their pasteurisers and operational considerations largely dictated the limits on the times and temperatures of heating that they could use in practice.

Dairy industry knowledge of and interest in alternative processes for the destruction of pathogenic organisms in milk

From the Australia-wide industry survey conducted during this study, it was established that, on average, 22% of respondents had 'some knowledge' of each of 10 nominated alternative processes that have potential application for the destruction of pathogenic organisms in milk. This can be regarded as a relatively high number, given the fact that some of the processes are still in the early stages of development, are quite 'high tech' or have not been well publicised in the industry at large.

There is real industry interest in the application of alternative technologies, for a range of reasons, some purely economic (eg reduced costs), some technological (eg making a better cheese) and some philosophical (eg keeping milk in its natural state). Conversely, some respondents also had concerns about the alternative technologies, e.g. technical feasibility, effects on manufacturing process and product quality, capital and operating costs, food safety and operator safety.

In the context of this review, a number of the respondents did recognise that validation of an alternative process in terms of food safety outcomes was a prerequisite to the commercial application of that process. How this might be done is addressed in Appendix 1 to this report.

Identified gaps in the data on heat resistance of pathogens

The main gaps in data and knowledge - with respect to traditional pasteurisation - identified during this study include:

- Definitive evidence on whether it is, or is not, valid to classify *M. paratuberculosis* as a human pathogen; and
- Quantitative heat resistance data for *Brucella melitensis*, *Streptococcus agalactiae* and *Streptococcus zooepidemicus* in milk.

In addition, it must be noted that:

- Heat resistance data obtained using commercial HTST pasteurisation equipment appears to be lacking for the vegetative cells of several of the pathogenic species covered in this review, e.g. *Bacillus cereus*, *Enterobacter sakazakii* and *Staphylococcus aureus*;
- The available data for *Streptococcus pyogenes* is of particularly poor quality; and
- The most recent heat resistance data obtained by any method that is available for some of the pathogens is quite dated, e.g. *M. tuberculosis* (1927), *C. burnetii* (1956 & 1961), *M. bovis* (1960), *S. agalactiae* (1974) and *S. pyogenes* (1976).

While the available data are not necessarily in dispute, it would nevertheless be reassuring to have more current data for every pathogen of concern; especially data obtained using modern commercial HTST equipment that complies with current design and operational standards.

Standardised protocols and methodologies for the determination of heat resistance appear to be lacking. Sadly, studies using methodologies known to give unreliable results, e.g. open tubes, are still being reported in the literature. Realistically, however, it would probably be almost impossible to achieve general adherence to such a protocol, particularly on an international basis, even if one did indeed exist. The next best option for an organisation like FSANZ is to establish and publicise its minimum requirements for the type of data that is acceptable for use in submissions on risk assessment studies. For example: milk to be used as the heating medium; confidence limits to be provided for kinetic data; preference to be given to data generated using commercial pasteurisation equipment where possible; and heat resistance data to be based on strains of test organisms known to occur in raw milk.

Overall conclusions with respect to traditional pasteurisation treatments

It is concluded that consumers of pasteurised milk and dairy products in Australia can be assured that pasteurisation continues to be a very effective public health measure. Three complementary observations allow this conclusion to be drawn:

- (a) Ample heat resistance data to indicate that the vegetative cells of the most significant milk-borne pathogens are destroyed by pasteurisation, with a reasonable margin of safety [though it is recognised that there are still some gaps in the data for some organisms and that there are other forms (eg spores) or products (eg toxins) of some species that can withstand pasteurisation];
- (b) With a small number of exceptions, (which are related more to process control or the interpretation of what constitutes an equivalent treatment, rather than significant

deficiencies in the actual times and temperatures used), pasteurisation of milk and liquid milk products in Australia meets the minimum time and temperature standards prescribed in the Australia New Zealand Food Standards Code, or recognised equivalents; in many cases, the product is heated to a temperature and/or a time often well in excess of the prescribed minimums; and

- (c) Lack of epidemiological data indicating that pasteurised milk products have been implicated in any outbreaks of gastrointestinal illness in Australia in recent years whereas, in contrast, such outbreaks continue to be associated with consumption of raw milk, both in Australia and in other countries.

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ATTACHMENT 1: Extract from standard 1.6.2, processing requirements (Australia only) of the Australia New Zealand Food Standards Code**Clause 1. Processing of milk and liquid milk products**

- (1) Milk must be pasteurised by -
- (a) heating to a temperature of no less than 72°C and retaining at such temperature for no less than 15 seconds and immediately shock cooling to a temperature of 4.5°C; or
 - (b) heating using any other time and temperature combination of equal or greater lethal effect on bacteria;
- unless an applicable law of a State or Territory otherwise expressly provides.
- (2) Liquid milk products must be heated using a combination of time and temperature of equal or greater lethal effect on the bacteria in liquid milk than would be achieved by pasteurisation or otherwise produced and processed in accordance with any applicable law of a State or Territory.

Editorial note:

For the purposes of Clause 1 of this Standard (refer above), milk and liquid milk products includes milk and liquid milk products used in the production of any cream and cream products, fermented milks, yoghurt, dried, condensed and evaporated milks, butter and ice cream.

Clause 2. Processing of cheese and cheese products

- (1) Cheese and cheese products must be manufactured -
- (a) from milk and milk products that have been heat treated -
 - (i) by being held at a temperature of no less than 72°C for a period of no less than 15 seconds, or by using a time and temperature combination providing an equivalent level of bacteria reduction; or
 - (ii) by being held at a temperature of no less than 62°C for a period of no less than 15 seconds, and the cheese or cheese product stored at a temperature of no less than 2°C for a period of 90 days from the date of manufacture; or
 - (b) such that -
 - (i) the curd is heated to a temperature of no less than 48°C; and
 - (ii) the cheese or cheese product has a moisture content of less than 36%, after being stored at a temperature of no less than 10°C for a period of no less than 6 months from the date of manufacture; or
 - (c) in accordance with clause 3 of Standard 2.5.4.

Editorial notes:

1. Cheese under paragraph 2(1)(b) is generally known as 'extra hard grating cheese' – see the Codex International Standard for Extra Hard Grating Cheese (CODEX STAN C-35-1978).
2. Clause 3 of Standard 2.5.4 refers to the production of Gruyere, Sbrinz or Emmental cheese according to the specific provisions of the Ordinance on Quality Assurance in the Dairy Industry of the Swiss Federal Council of 18 October 1995.

ATTACHMENT 2: Outbreaks of enteric infection associated with the consumption of pasteurised and unpasteurised milk in Australia and overseas

Notes prepared by Russell Stafford⁸
OzFoodNet (Qld)
March 2005

1. Introduction

OzFoodNet was established in 2000 to conduct enhanced surveillance of foodborne disease. OzFoodNet has the responsibility of collating national data on all gastrointestinal outbreaks due to foodborne or other modes of transmission. These data are summarised to provide information on common causes of outbreaks and contribute to the development of policy on food safety.

2. Outbreaks of enteric infection associated with consumption of pasteurised milk in Australia (2000-2004)

OzFoodNet has no documented reports of any outbreaks of enteric infection in Australia between 2000 and 2004 due to the consumption of pasteurised milk.

3. Outbreaks of enteric infection associated with consumption of pasteurised milk in other countries

There was a reported outbreak of 50 cases of cryptosporidiosis in the UK in 1995 among school children who drank pasteurised milk supplied to the school by a local farm. The investigation identified a faulty pasteuriser at the farm during the same period as the outbreak and it was suspected that the illness among children was caused by inadequately pasteurised milk (Gellietlie *et al.*, 1997).

4. Outbreaks of enteric infection associated with consumption of unpasteurised milk in Australia

There have been several outbreaks of enteric infection in Australia in recent years due to the consumption of unpasteurised milk. These include:

- Two outbreaks of campylobacteriosis were recorded in Victoria among school students visiting farms. One occurred in 2000 involving approximately 25 cases. The other occurred in November 2003 involving 13 cases. Based on the epidemiological information, it was suspected that both outbreaks were associated with the consumption of unpasteurised milk. However, no microbiological testing was done to confirm the source of infection.
- An outbreak in Queensland (2001) of 8 cases (all children) of *Cryptosporidium* infection linked to the consumption of commercially-obtained unpasteurised cow's milk. *Cryptosporidium* oocysts were detected in milk samples (Harper *et al.*, 2002).
- One outbreak of *Campylobacter* infection occurred in South Australia during 2000 (total of 12 cases) associated with unpasteurised milk purchased from a farm; and

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- An outbreak of 12 cases of *Salmonella* Typhimurium phage type 44 infection in South Australia in 1999 associated with unpasteurised milk purchased from a farm.

While the sale of unpasteurised cows' milk is now prohibited in all states and territories, this does not prevent people drinking samples of this product in organised settings such as farm visits by school students.

5. Overseas outbreaks of enteric infection associated with consumption of unpasteurised milk

There have been numerous reports in the literature of outbreaks of salmonellosis and more commonly *Campylobacter* infection associated with consumption of unpasteurised milk. There have also been outbreaks caused by *Listeria monocytogenes* and *E. coli* O157. These include:

United Kingdom:

- 1983-84: Twenty-seven outbreaks of disease – associated with consumption of raw milk – *Salmonella*, *Campylobacter* and *Yersinia*;
- 1992-94: Three different *Campylobacter* outbreaks linked to consumption of raw milk (72, 22, and 23 cases respectively); and
- 1996: One *E coli* O157 outbreak – 9 cases – drinking raw cow's milk.

USA:

- 1981-1990: Twenty outbreaks of *Campylobacter* associated with drinking raw milk. Attack rate was 45% i.e. 458 of 1013 persons who drank milk were infected;
- 1983: One *Campylobacter* outbreak – 6 cases – raw goat milk;
- 1992-1993: One *E coli* O157 – 16 cases – drinking raw cow's milk;
- 1997: One *Salmonella* typhimurium outbreak – 54 cases – cheese made from raw milk;
- 2001: One *Listeria* outbreak – 3 cases – cheese made from raw milk; and
- 2003: One *Campylobacter* outbreak – 13 cases – drinking raw cow's milk.

A bibliography of the published reports on the above outbreaks is included in Section 9 of this Note.

6. Potential problems for dairy manufacturers due to *Bacillus cereus* (and *Clostridium perfringens*)

Clinical pathology laboratories in Australia do not routinely test for *Bacillus cereus* or *Clostridium perfringens* toxins nor conduct routine culture for these organisms. Therefore, a small outbreak of gastroenteritis in the community caused by these potential foodborne pathogens may go undetected if these outbreaks are not reported to public health authorities by doctors or the public. Laboratory confirmation for these infections requires specialised testing by a NATA-accredited public health reference laboratory. (Larger outbreaks should eventually be reported to health authorities.)

There are two types of clinical illness that may be caused by *B. cereus* gastrointestinal infection. One is an emetic illness with a short incubation period characterised by vomiting while the second syndrome has a longer incubation period and is characterised by diarrhoea. Both are caused by different toxins. The emetic illness is caused by the ingestion of pre-

formed toxin in the foods while the diarrhoeal illness is caused by the ingestion of cells and spores followed by production of toxin *in vivo* (Andersson *et al.*, 1995).

Reference [3] contains more information on this topic.

7. Comments

Persons who consume unpasteurised milk are at increased risk of infection due to several different enteric pathogens capable of causing severe illness and potentially death. This was demonstrated with the recent *Cryptosporidium* outbreak in the Sunshine Coast in which three children required hospitalisation. The International Journal of Infectious Diseases recently reported two cases of Haemolytic Uraemic Syndrome (HUS) due to *E. coli* O26 in two young children in Europe in which transmission through unpasteurised cow's milk was positively identified.

Outbreaks associated with consumption of unpasteurised milk are uncommon in Australia because the sale of raw cow's milk to the public is illegal. If the proportion of persons consuming raw milk were to increase, then we could expect to see more outbreaks of disease occurring because of this practice. A study of raw milk associated outbreaks in USA between 1973 and 1992 identified 46 outbreaks of illness; 40/46 (87%) occurred in states where the sale of raw milk was legal.

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ATTACHMENT 3: Copy of proforma industry questionnaire and covering letters

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4 March 2005

Attention: The Manager

Survey of processing methods and heat treatments used for the pasteurisation of milk by the traditional thermal processes in Australia and of interest in alternative methods

As outlined in the attached copy of a letter dated 25 February 2005 under the signature of Deon Mahoney, Principal Microbiologist, Food Standards Australia New Zealand (FSANZ), we have been engaged by FSANZ to conduct a study titled '*Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products*'. The purpose of the study, which is part of the process for development of a Primary Production and Processing (PPP) Standard for Dairy, is also summarised in the attached letter.

We seek your assistance with two specific aspects of our study:

- Determining current industry practice in Australia with respect to methods employed for the pasteurisation of milk, in particular the time/temperature combinations used and their relationship to minimum regulatory standards; and
- Dairy industry interest in the uptake of alternative processes and methods, including thermisation, for the destruction of pathogenic organisms in milk intended for human consumption or further processing into dairy products.

Attached is a short questionnaire related to the above aspects of our study. An edited extract from Standard 1.6.2 of the Australia New Zealand Food Standards Code, which sets out the minimum requirements for the pasteurisation of milk, is attached to the questionnaire for your information and reference.

You will greatly assist us by answering the questions and returning the completed document to us by mail or fax, using the contact details above. If you would prefer an electronic copy of the questionnaire, please let me know.

It would be appreciated if the completed questionnaire was returned to us by Thursday 24 March. FSANZ has stipulated that our interim report is to be submitted to them by 15 April.

Please note that information supplied will not be reported at the individual plant or company level. All data will be aggregated into common data sets at the national industry level for reporting purposes.

Please contact me if you have queries about the study or the questionnaire.

Many thanks

Harley Juffs



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To whom it may concern,

Food Standards Australia New Zealand (FSANZ) is a statutory authority which ensures safe food for consumers in Australia and New Zealand by developing effective food standards for the entire food supply chain, from primary production through to manufactured foods and retail outlets.

FSANZ has engaged **Harley Juffs & Associates Pty Ltd** to conduct the project '*Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products*'.

The purpose of the project is to provide information on:

- the effect of pasteurisation on pathogenic microorganisms in milk;
- how current industry pasteurisation practices compare to regulatory requirements; and
- possible alternative methods and processes for the destruction of pathogenic microorganisms in milk and milk products.

FSANZ request your cooperation in the provision of information about your company's pasteurisation practices and experience of or interest in alternative methods of ensuring food safety in dairy products. Any information provided will be treated as commercial in confidence and please ensure that where relevant it is marked as such. FSANZ would like to be able to use this information as part of its risk assessment report relating to the Primary Production and Processing Standard for Dairy Products. Any information or data submitted will be not be used in a format to enable identification of individual companies or plants.

Any assistance you can give Dr Juffs and his team is greatly appreciated and will help us in developing standards that meet the needs of consumers, industry and government.

Sincerely

A handwritten signature in black ink that reads 'Deon Mahoney'. The signature is fluid and cursive, with a long horizontal stroke extending to the right.

Deon Mahoney
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25 February 2005

Commercial - in - confidence

Questionnaire – Milk Pasteurisation and Alternative Methods

Part A. Company information

Dairy company/enterprise:

Plant(s) covered by this response:

.....

Contact details for follow-up if required:

Name

Phone

Email

Part B. Application of the traditional thermal process for the pasteurisation of milk in the above plants on a commercial basis

Explanatory notes

1. The survey applies only to the ‘traditional thermal methods’ for the pasteurisation of milk, i.e. the HTST process as defined in Clause 1(1)(a) of Standard 1.6.2 of the Australia New Zealand Food Standards Code (refer Extract attached) and the batch process where use of this process is permitted by the State regulatory agency. The survey does not apply to other pasteurisation processes involving heat, e.g. ESL or UHT of liquid milks or the heat treatment of cream used for the manufacture of butter.
2. The survey applies to milk from all of the commercial species used in Australia, i.e. cow, goat, sheep, buffalo and camel.
3. The survey applies to the pasteurisation of raw milk used for the production of liquid milk and milk products and of milk used in the production of any cream and cream products, fermented milks, yoghurt, dried, condensed and evaporated milks, ice cream and cheese (refer extract of Standard 1.6.2 attached). It does not apply to re-pasteurisation of dairy products, e.g. an ice cream mix based on milk powder rather than raw milk. Pasteurisation of table cream should however be included in your response.
4. Please list each product group separately in Table 1 below, showing the species of animal, method of pasteurisation (ie HTST, batch, etc), the time-temperature combination used on a regular basis and an estimate of the average throughput on a weekly basis over the past year or so. If the time-temperature combination used for HTST deliberately exceeds that that specified in Standard 1.6.2 (minimum of 72°C for a minimum of 15 seconds) by more than 0.5°C or three seconds, please show the time and temperatures used and explain why that particular treatment is used. If different treatments are used for batches of the same product to meet market requirements or for other reasons, please list separately.

Part C. Use of thermisation for production of extra hard grating cheese

Standard 1.6.2 of the Australia New Zealand Food Standards Code permits milk used for the production of extra hard grating cheese to be heat treated by being held at a temperature of no less than 62°C for a period of no less than 15 seconds, provided the cheese or cheese product is stored at a temperature of no less than 2°C for a period of 90 days from the date of manufacture. This heat treatment is termed thermisation.

Does your company manufacture extra hard grating cheese? Yes..... No.....

If No, proceed to Part D.

If Yes, please record the heat treatments applied to the milk used in the manufacture of the product in the table below. If more than one time –temperature combination is used, please list separately.

Treatment temperature (°C)	Holding time (secs)	Estimated average weekly throughput of milk during the past year (L)

Part D. Approved use of any other processes for the destruction of pathogenic organisms in milk

Has your company been given approval by any regulatory agency to use a process, or a combination of processes, for the destruction of pathogenic organisms in milk as an alternative to traditional thermal pasteurisation? Yes..... No.....

If No, proceed to Part E.

If Yes, please outline process:

Type of product:.....

Regulatory agency granting approval:

Part E. Knowledge of and interest in alternative processes that have potential application for the destruction of pathogenic organisms in milk

A number of processes that might in the future provide methods for the destruction of pathogenic organisms in milk, as alternatives to traditional thermal pasteurisation, are presently undergoing development. The main processes under development are listed in Table 2 below.

Please include the following information about each process in the table:

- Whether or not you have some knowledge of the process, however limited that knowledge might be?
- Whether the process might be of interest to your company, and why?
- Whether you have any concerns about the process from the perspective of your company, and why (eg doubts about effectiveness from a food safety viewpoint, cost, technological reasons)?

Table 2. Your knowledge of alternative processes for the destruction of pathogenic organisms in milk and your views on their possible future application within your company.

Possible alternative process	Do you have some knowledge of the process (Yes/No)?	Is the process of any interest to your company?		Do you have any concerns about the process from the perspective of your company?	
		Yes/No	If yes, explain why?	Yes/No	If yes, please comment?
High pressure					
Pulsed electric field technology					
Ultrasonication					
Microfiltration					
High intensity light – UV or white					
Ohmic heating					
Microwave heating					
Other heating, e.g. radiofrequency, induction, electric tube (Actijoule®)					
Hydrogen peroxide					
Peroxide / Lacto-peroxidase system					
Other – please specify					