3 REDUCTION IN MICROBIAL NUMBERS FROM PASTEURISATION

This section discusses information from the scientific literature on the inactivation of pathogens by pasteurisation, focussing on the pasteurisation conditions most commonly applied.

3.1 Measures of microbial reduction

Most assessments of heat inactivation of microorganisms are described as a log-linear relationship, i.e. a plot of the $\log_{10}$ of the number of survivors versus the exposure time at a given temperature is a straight line. This approach allows the calculation of two parameters, D time and z value.

3.1.1 D time

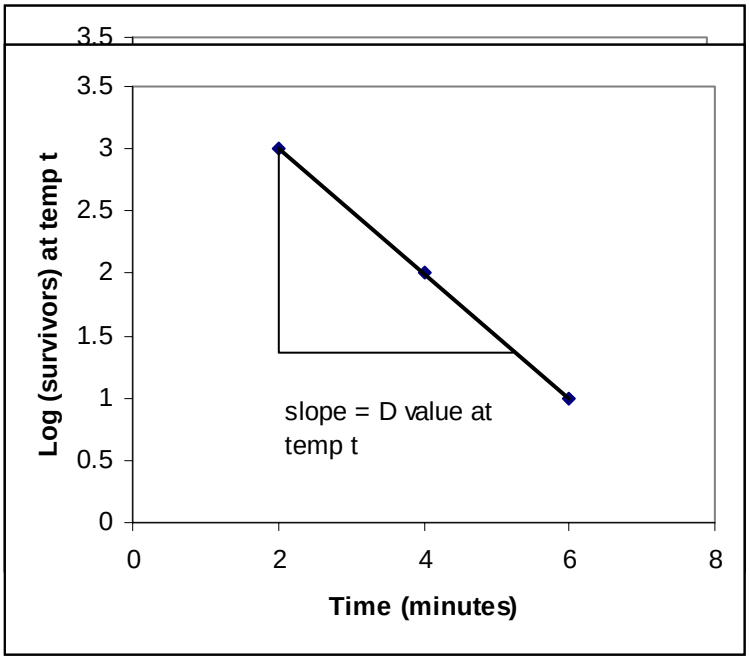
The D time is defined as the time at a given temperature required to reduce the population of organisms present by 90% or 1 $\log_{10}$ unit. Figure 1 illustrates the calculation.

Figure 1 Example of Calculation of D time at temperature t

D value at temperature t = 2/1 = 2 minutes

3.1.2 Z value: The *z* value is defined as the temperature change required to alter the D value by a factor of 10. Figure 2 illustrates the z value determination.

Figure 2 Illustration of *z* value calculation for pasteurisation



Z value for pasteurisation = 72-63/log (30x60)-log (15) = 9/2.079 = 4.33

More recently, papers have reported the presence of "shoulders" and "tails" (Ross *et al.* 1998) in thermal destruction curves that complicate the kinetics and reduce the value of easily understood concepts such as the D time. Shoulders represent an initial period of time where the organism is not being destroyed as quickly as in the subsequent section of the thermal death curve. Tails seem to represent a reduced rate of destruction (increased D value) in a hypothesised heat resistant sub-population. A method has been proposed to describe the effects of pasteurisation when considering this kind of inactivation kinetics. The method involves determining an organism's PE or "pasteurisation effect", equivalent to the log reduction that pasteurisation would deliver (Ross *et al.* 1998).

Many of the papers cited below use a value of "thermal death time", i.e. the time taken for a given number of cells to become non-detectable by the method used at a given temperature. Given a variety of reported temperatures *z* values can be calculated. A curve of thermal destruction can then be compared to the curve of pasteurisation conditions and, given an initial number of organisms, statements made about the adequacy of pasteurisation.

3.2 General pasteurisation conditions

A fuller discussion of pasteurisation standards is given in Section 4. However, to provide context for the data reported in this section, it is necessary to discuss pasteurisation times and temperatures in general terms.

The most commonly used standards are the low temperature long time (LTLT) (63.5°C for 30 minutes) method (also known as the "holding method"), and the high temperature short time method (HTST) (71.7°C for 15 seconds). The most commonly used pasteurisation method for milk products in New Zealand is the HTST method. Extended shelf life and ultra heat treated products are pasteurised at 120-124°C and 134-135°C (or higher) respectively, for short periods. The "holding method" is occasionally used for batch pasteurisation of certain products. The efficacy of pasteurisation is always checked by phosphatase enzyme based assays (Chris Erikson, Mainland Products, personal communication).

3.3 Data on inactivation of pathogens by pasteurisation

3.3.1 *Bacillus* spp.

Pasteurisation will not inactivate *Bacillus* spores. D times for ***B. cereus*** spores at 95.0^{o}C ranged from 1.2 to 36.0 minutes, and the *z* value was 9.6^{o}C. At 100^{o}C the D time was 2.0 to 5.4 minutes (Wong *et al.* 1988). *Z* values of 8.2-8.5^{o}C have been reported elsewhere (Wescott *et al.* 1995). Most work has concentrated on heat inactivation of spores; data regarding vegetative cells are lacking.

3.3.2 *Brucella* spp.

A study of *Brucella abortus* focused on the thermal destruction of this organism under HTST and LTLT conditions (Kronenwett *et al.* 1954). It was found that the *z* value was not influenced by conditions prior to heat treatment, but that there were some differences between isolates. In this case the z value varied from 4.3 to 4.8^{o}C. Given a starting concentration of 2 x 10^{8} organisms/ml of the most heat resistant isolate, there

was a considerable margin of safety when compared with pasteurisation time/temperature combinations.

The level of detail is not present in the data provided, but some estimates on D times can be made. For the isolate 2308 the following were D times derived:

Temperature (^{o}C)	Approximate D Values (s)
61.5	< 18.7
63.3	< 6.5
66.5	< 1.8

For isolate 2016 the following D times were derived:

Temperature (^{o}C)	Approximate D Values (s)
64.3	< 8.6
65.5	< 4.8
66.6	< 2.5

N.B. These assume log linear inactivation kinetics.

Cultures containing 1.5 x 10^{8} *Bruc. abortus* per ml were destroyed by heating at 61.5^{o}C for 23 minutes and at 72^{o}C for 12-14 seconds (Foster *et al.* 1953). The calculated *z* value was 5.3^{o}C.

Experiments using guinea pig models as a detection system showed that naturally contaminated milk became unable to infect the animals after both LTLT (batch) and HTST pasteurisation (Heever *et al.* 1982).

3.3.3 *Cryptosporidium*

Where 10^{5} oocysts were heat treated at 71.7^{o}C for 15, 10 or 5 seconds in milk they were unable to infect infant mice (Harp *et al.* 1996). It was concluded that HTST pasteurisation is sufficient to destroy this organism.

3.3.4 *Campylobacter* spp.

Milk inoculated with 1.6 x 10^{6} *C. jejuni*/ml did not yield post pasteurisation survivors under HTST conditions, but the organism did withstand 10 seconds exposure at this temperature (Gill *et al.* 1981).

Data from the ICMSF give D times of 1.3-5.4 minutes in skim milk at 50^{o}C, and 0.74-1.0 minute at 55^{o}C in the same medium (ICMSF 1996). In physiological saline D times were 0.71-0.78, 0.24-0.28, 0.12-0.14 minutes at 56, 58 and 60^{o}C respectively (Sorqvist 1989).

3.3.5 *Clostridium botulinum*

As for *Bacillus* spores, pasteurisation is inadequate to inactivate spores of *Clostridium botulinum*. It appears that endospores of this organism have variable degrees of

natural heat resistance. At 100ºC, it would take 240 minutes to kill 72 x 10^9 spores compared to 40 minutes to kill 328 endospores. At 120ºC, it would need 5 minutes to kill 60 x 10^9 endospores (Jay 1986). Pasteurisation conditions of 125^{o}C for 5 seconds have been shown to be necessary for the destruction of spores of this organism (Collins-Thompson and Wood 1993) when found in low numbers.

3.3.6 *Coxiella burnetii*

It was reported in the late 1940s to early 1950s (Lennette *et al.* 1952) that this organism was capable of surviving pasteurisation conditions. Three samples showed survival, one processed at 62.2^{o}C for 30 minutes and two at 73.4^{o}C for 17 and 15.2 seconds respectively. Work was therefore undertaken under laboratory and commercial HTST conditions to examine this further (Enright *et al.* 1957). The work described produced thermal destruction times where 100,000 guinea pig units (10 times that considered the maximum possible in cow's milk) became non-infectious under the conditions used. The results showed that heating milk to 61.6^{o}C for 30 minutes would not inactivate the organism, while holding for the same time at 63^{o}C would. Under HTST conditions (72^{o}C for 15 seconds) it was concluded that these conditions are "..adequate to eliminate viable C. burnetii (sic) from whole raw milk". Because of the nature of the data provided it is not possible to calculate D times or *z* values for this organism.

3.3.7 *Escherichia coli* O157:H7

Very little information is available concerning the thermal inactivation of this organism in milk. Most of the data pertains to inactivation in meat and apple juice. In the only study identified, at 63^{o}C with an exposure time of 16.2 seconds and using a cocktail of isolates, D times of 4.3, 13.8 and 2.8 seconds were recorded (D'Aoust *et al.* 1988).

Z values of 4.1^{o}C in minced beef (Doyle and Schoeni 1984), 4.3 to 4.7^{o}C also in minced beef and 4.8^{o}C in apple juice (Splittstoesser *et al.* 1996) have been reported.

The similarity of this organism to *Salmonella* means that it is likely to have similar inactivation kinetics, i.e. it is not particularly heat resistant.

3.3.8 *Listeria monocytogenes*

The inactivation of *L. monocytogenes* by pasteurisation has been the subject of considerable scientific debate, especially in the mid 1980s. This followed an outbreak of listeriosis in Massachusetts in 1983 where pasteurised milk was identified as the vehicle (Fleming *et al.* 1985). Fourteen patients died, representing a case fatality rate of 29%. No fault in the pasteurisation could be identified, and the abstract of the paper comments "These results.…raise questions about the ability of pasteurisation to eradicate a large inoculum of *L. monocytogenes* from contaminated raw milk".

Even prior to this there had been controversy over the ability of *L. monocytogenes* to withstand pasteurisation. Survival of inocula in excess of 5 x 10^4 after batch (61.7^{o}C for 35 minutes) pasteurisation was demonstrated, and a D time at this temperature of 9.5-10.8 minutes calculated (Bearns and Girard 1958).

It was postulated that *L. monocytogenes* may be protected from pasteurisation after being ingested by phagocytes in raw milk (although the survival of intact phagocytes in the process has been questioned) (Fleming *et al.* 1985). Experiments using *L. monocytogenes* Scott A, isolated from the Massachusetts outbreak, however demonstrated that there was little difference in the thermal resistance of this isolate either freely suspended in milk or internalised by phagocytes (Bunning *et al.* 1986). D and *z* values are shown below:

Temperature (^{o}C)	D value (intracellular) (s)	D value (free) (s)
63.3	33.3	33.4
68.9	7.0	7.2
71.7	ND	1.3

z values: 6.0^{o}C for intracellular cells and 6.1^{o}C for freely suspended cells.

Data showing similar values have been reported in another paper (Bradshaw *et al.* 1985). These data are summarised below:

Temperature (^{o}C)	D value (s)	Range (s)
52.2	1683.7	1612.9-1754.4
57.8	289.6	269.5-309.6
63.3	19.9	13.4-28.4
66.1	7.3	6.2-10.1
68.9	3.0	2.1-4.2
71.7	0.9	0.8-1.1
74.4	0.7	0.5-0.9

The *z* value was 6.3^{o}C.

Variation in the D value at 62.7^{o}C has been shown for different isolates (Donnelly and Briggs 1986). The values ranged from 0.4 to 1.0 minute, and the *z* value for the most heat resistant isolate was 4.3^{o}C. Given a 30 minute holding time in the LTLT or batch process, this would give a 30 D reduction in numbers of *L. monocytogenes* during pasteurisation.

D times at 62^{o}C of 0.1-0.4 minute in sealed tube inactivation experiments have been determined (Donnelly *et al.* 1987). This paper also demonstrated that experiments where test tubes containing milk which are placed in waterbaths can give misleading results (see also information regarding *M. avium* subsp. *paratuberculosis* below). This phenomenon, whereby a constant number of surviving listeriae are present in the lid or on the walls of the tube above the waterline, was used to explain the results of Berans and Girard (1958). A similar finding was reported by Beckers *et al*., 1987.

In contrast to the information above, evidence was then presented which appeared to demonstrate that *L. monocytogenes* could survive the HTST process (Doyle *et al.* 1987). The organism, which was being shed in milk within polymorphonuclear leukocytes, was shown to survive pasteurisation when temperatures of 71.7-73.9^{o}C were used, but not at temperatures higher than this. Other work, also carried out using a pilot scale pasteuriser detected survivors when milk was pasteurised to 72^{o}C for 15

seconds with an inoculum of 6.5 $\log_{10}$ organisms/ml (Garayzabal *et al.* 1987). Cold enrichment was the best technique for detecting cells surviving the heat treatment.

Further work concerning the location of *L. monocytogenes* in milk failed to demonstrate increased heat resistance in bacterial cells located intracellularly (Bunning *et al.* 1988). The same paper reported a 33.4 D reduction in numbers during an LTLT process, but an unsafe 3.7 D reduction during HTST pasteurisation. A *z* value of 8^{o}C was derived. Work where *L. monocytogenes* was subjected to different pre-pasteurisation heat treatments indicated that the organism may survive HTST processing under some circumstances, but it was concluded that under normal conditions HTST would be effective (Farber *et al.* 1992).

Work which produced inactivation models for *L. innocua* showed that *L. monocytogenes* was more heat sensitive than the non-pathogenic species (Piyasena *et al.* 1998). This analysis of thermal inactivation took into account variability between experiments and built this into a stochastic model. The average reduction for a heat treatment of 72^{o}C for 16 seconds was 28.1 $\log_{10}$ units while the 95th percentile was 11.4 $\log_{10}$ units.

As a possible explanation of some of the discrepant findings, D values for *L. innocua* were found to be different when assessed using a pilot scale pasteuriser and a capillary tube method (Fairchild *et al.* 1994). D times at 65, 68 and 70^{o}C were 11.5, 3.5 and 1.6 s respectively when measured in the pasteuriser, and 16.5, 3.9 and 1.5 when measured in capillary tubes. Consequently the *z* values produced by each approach were different, being 5.9^{o}C for the pasteuriser and 4.8^{o}C when using capillary tubes. It was concluded that batch type lethality determinations are appropriate for batch processes (i.e. LTLT), while the laboratory scale pasteuriser is more appropriate for determining lethality in continuous flow systems.

The efficacy of HTST processing was demonstrated in four different experiments using up to 10^5 *L. monocytogenes*/ml (Lovett *et al.* 1990).

Should any cells survive pasteurisation they will most likely be heat injured. It has been shown that cells injured by pasteurisation cannot compete with surviving thermoduric organisms and do not grow in milk held under refrigerated storage (Crawford *et al.* 1989).

3.3.9 *Mycobacterium avium* subsp. *paratuberculosis (MAP)*

There has been a great degree of interest in this organism recently because of its possible link with Crohn's disease in humans (Bull *et al.* 2003;Chiodini 1989). In addition there have been reported isolations of the organism from pasteurised milk (Grant *et al.* 2001). The controversy surrounding the effect of pasteurisation on the organism has recently been reviewed (Lund *et al.* 2002) and it is apparent that much of the disagreement revolves around methodological problems which include the propensity of the organism to clump and so make the preparation of dilutions difficult, and the slow rate of growth of the organism in culture.

Methodological problems also exist with the ways in which heat treatments are delivered to inoculated milk. For example there are many cases where not all of the milk may have reached the target temperature, and where pilot scale pasteurisers are used the milk flowing through the holding tube shows laminar flow rather that the

turbulent flow found in commercial machines (this has an impact on the variability of time that any particle may take to pass through the holding tube).

As a number of different methods have been used, the reported decimal reductions that LTLT and HTST treatments achieve varies (Lund *et al.* 2002). For LTLT the values ranged from <2 $\log_{10}$ units to >10 $\log_{10}$ units, while for HTST the range was <2 $\log_{10}$ units to 5.6-6 $\log_{10}$ units.

A publication has appeared since the review was published from New Zealand workers (Pearce *et al.* 2001). They used a validated pilot plant pasteuriser operating with turbulent flow in the holding tube. The data presented in this paper gave mean D times of 15.0 +/- 2.8 s at 63°C and 5.9 +/- 0.7 s at 66°C, with a mean *z* value of 8.6°C. Extrapolation to 72°C indicated a D time of <2.03 s, equivalent to a >7D kill under HTST conditions.

3.3.10 *Mycobacterium bovis*

Death kinetics of three isolates of *M. bovis* have been examined (Kells and Lear 1960). *Z* values of 4.8, 4.9 and 5.2°C were calculated. D times calculated from the data presented are as follows;

Isolate 12621	Temperature (°C)	D time (s)
	64	6.6
	65	3.4
	66	2.3
	67	1.4
	68	0.9
	69	0.6
Isolate 11756	64	4.8
	65	3.3
	66	2.4
	67	1.3
	68	0.8
	69	0.4
Isolates 854	64	4.8
	65	2.3
	66	1.8
	67	1.3
	68	0.9
	69	0.6

It was concluded that pasteurisation conditions gave a considerable margin of safety when the organism is present at the "maximum probable concentration" in raw milk (taken here to be 10^4/ml).

3.3.11 Pathogenic *Streptococcus*

Streptococci are not unusually heat resistant. The following are D times for *Strep. pyogenes* in milk (International Commission on Microbiological Specifications for Foods 1996):

Temperature (°C)	D time at stated temperature (mins)
60	0.44
62	0.33
65	0.15
70	0.02
72	0.01
75	0.01
78	0.007
79	0.005
65	0.1-0.2
66	0.1-0.2

3.3.12 *Salmonella* spp.

In an analysis of multiple isolates of *Salmonella*, D times of between 3.5 and 5.9 seconds were recorded at 60°C, and from 2.2 to 3.7 seconds at 63°C. *S.* Senftenberg was more thermally resistant, with D times of 3 to 3.2 seconds at 66°C (D'Aoust *et al.* 1987).

A review on the thermal resistance of salmonellae has been published recently (Doyle and Mazzotta 2000). Table 4 in that paper records D times for inactivation of salmonellae in raw milk, and the data are summarised below:

Temperature (°C)	D time at stated temperature (mins)
51.8	22.6 (mean of two values)
57.2	1.7
60.0	0.084 +/- 0.026 (mean and standard deviation of six values)
61.5	0.063 +/- 0.025 (mean and standard deviation of five values
62.8	0.11
63.0	0.05 +/- 0.015 (mean and standard deviation of five values)
64.5	0.051
67.5	0.046
68.3	0.015
71.7	0.004

Z values from the same paper are 5.3°C for two isolates

A D time of 147.0 s at 55.5°C was reported as the only datapoint in one paper (Moore and Madden 2000).

Salmonellae isolated from dried milk (7 serotypes) had D times of 3.6-5.6 s, 1.1-1.8 s and 0.28-0.52 s at 62.8, 65.6 and 68.3°C respectively. For *S.* Senftenberg D values of 34.0, 10.0, 1.2 and 0.55 s were recorded at 65.5, 68.3, 71.7 and 73.9°C respectively (Read *et al.* 1968).

Factors found to influence the thermal resistance of *Salmonella* in milk include total solids (higher total solids gave increased *z* and D values), pressure (reduced pressure

decreases heat resistance), and inoculum growth temperature (higher temperature increases heat resistance). The effects of other factors, such as water activity are beyond the scope of this report.

3.3.13 *Staphylococcus aureus*

D times for inactivation of this organism in milk are as below (International Commission on Microbiological Specifications for Foods 1996):

Temperature (^{o}C)	D time (mins)
50	10.0
55	3.0
60	0.9
65	0.2
70	0.1
75	0.02

A *z* value of 9.5^{o}C was reported.

3.3.14 *Yersinia enterocolitica*

There appears to be little information available on the thermal inactivation of *Y. enterocolitica*. D times obtained in physiological saline have been reported as 1.4-1.8, 0.40-0.51 and 0.15-0.19 minutes at 58, 60 and 62^{o}C. The *z* values reported ranged from 4.00 to 4.52^{o}C (Sorqvist 1989).

Considerable variability has been demonstrated in heat resistance of different isolates of the organism (Hanna *et al.* 1977). When five isolates, inoculated at 10^{6}-10^{7} cfu/ml were heat treated at 60^{o}C in skim milk, all became undetectable within 3 minutes.

The efficacy of LTLT pasteurisation (62.8°C for 30 minutes) has been shown in brain heart infusion broth, skim milk and whole milk with three different isolates of the organism added at levels of 10^{5} to 10^{6} per ml (Kushal and Anand 1999). None of the isolates survived pasteurisation treatment. However, these authors also reported recovery of injured *Y. enterocolitica* cells after post-pasteurisation storage at 10^{o}C for 8-10 days (but not before this time). While pasteurised milk is unlikely to experience these conditions the observation indicates that some cells survived pasteurisation, albeit in a sub-lethally damaged form.

D times at 62.8^{o}C varied from 0.7 to 17.8 s when 21 different *Y. enterocolitica* isolates were tested (Francis *et al.* 1980). Parallel inactivation curves were obtained for three *Y. enterocolitica* isolates, but for one isolate the D times were higher than for the other two with values at 62.8^{o}C of between 0.24 and 0.96 minute reported (Lovett *et al.* 1982). The *z* values reported were higher than those cited above, being in a range of 5.11 to 5.87^{o}C. Similar values for *z* were obtained in skim milk where data read from the graphs presented indicated *z* values of 5.3^{o}C when cells were incubated at 37^{o}C prior to testing and 6^{o}C when pre-incubation was at 4^{o}C (Pagan *et al.* 1999).