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Heat induced inactivation of microorganisms in milk and dairy products

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ABSTRACT

It is well understood that heat treatment of milk, such as pasteurisation, allows its safe consumption in terms of foodborne illness, while failure in adequate heat treatment has resulted in both product recalls and also foodborne disease outbreaks. Aspects of different heat treatments within the dairy industry that affect relevant microorganisms, with an emphasis on bacteria, are discussed in this review. These include a description of D- and Z-values as measures of heat resistance, the factors that affect D-values, such as different dairy matrices, a discussion of some of the mechanisms associated with heat resistance of bacteria important for dairy products, different types of heating effects on microorganisms present in various dairy products, and recommendations for the most appropriate experimental design for understanding how heat affects microorganisms.

Contents

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1. Introduction – milk and heat treatment

Foods obtained from animal sources provide humans with a balance of nutrients, which are not readily available from plants (Murphy & Allen, 2003). Evidence in ancient pottery shards has indicated that humans have been preserving and possibly even processing dairy products since the 6th millennium BC (Salque et al., 2013). Milk is an example of a nutrient-rich animal-sourced food, containing lipids, proteins (casein), carbohydrates (lactose), amino acids, vitamins and minerals (calcium) and has overall dietary benefits for humans (Haug, Høstmark, & Harstad, 2007; Steijns, 2008). However, due to its nutritional properties, milk is also a growth matrix for a variety of spoilage and potentially pathogenic microorganisms.

The idea that heating milk would improve its storage life or keeping quality was recognised even before Pasteur showed that heating would inactivate the bacteria present in wine (Holsinger, Rajkowski, & Stabel, 1997). Coupled with this was the discovery by Robert Koch of the bacterial cause of tuberculosis, and by 1886, 85% of Medical Officers of Health in the UK believed that tuberculosis was spread by consumption of raw milk and undercooked meat (Savage, 1912). In the 1890s, bovine tuberculosis was also recognised as a serious human and animal health concern in New Zealand (Bryder, 1991). The uptake of pasteurisation as a technology to treat milk was rapid in Europe where Germany, followed by Denmark and Sweden, was the first to install commercial pasteurisers in the 1880s (Holsinger et al., 1997). By contrast, this technology was slow to be accepted in New Zealand, where only 20 of the 345 factories used pasteurisation by 1909 (Ford, 2013). A similar controversial view of the technology was held in the USA where the first commercial-scale pasteuriser was only installed in New York City in 1907 (Boor, Wiedmann, Murphy, & Alcaine, 2017). This was despite the study of Smith who in 1899 showed that *Mycobacterium tuberculosis* was inactivated in milk heated at 60 °C for 15 min (Holsinger et al., 1997; Smith, 1899).

Today, it is well understood that pasteurisation of milk and dairy products keeps consumers safe from foodborne illness (Costard, Espejo, Groenendaal, & Zagmutt, 2017), while failure in adequate heat treatment has resulted in both product recalls and also foodborne disease outbreaks (Table 1). Bacteria are the main focus of this review, as other microorganisms, including viruses, yeasts and moulds and protozoans, are mostly heat sensitive. For the purposes of this review, six main types of heat treatments during the manufacture of dairy products are highlighted (Table 2) and summarised in the next section.

2. Overview of types of heating in dairy manufacturing processes

During the manufacture of dairy products, milk is subjected to a variety of different heating processes. A summary of the most relevant heating types, for microbiological inactivation can be found in the factsheet produced by the International Dairy Federation (IDF, 2018). The objective of the thermal processing is to increase the keeping quality by inactivating microorganisms.

2.1. Pasteurisation

High temperature short time (HTST) pasteurisation involves heating the milk to at least 72 °C for 15 s or low-temperature long time (LTLT) to 63 °C for 30 min. Continuous flow-through, plate heat or tubular heat exchangers are used. The technical aspects of pasteurisation and its effect on the microbiological and nutritional aspects of milk are well documented in IDF Bulletin 496/2019 (IDF, 2019). Higher fat (>10%), higher solids (>18%) or added sugar products typically have a higher viscosity and lower heat transfer coefficients, and so may require higher temperatures or longer holding times.

2.2. Batch pasteurisation

In the dairy industry there are numerous different batch pasteurisation temperatures and times that are used. Generally, the ingredients are standardised or recombined into a batch tank and heat treated to inactivate at least any vegetative pathogens that may be present. Two examples are: (i) milk used for starter culture growth and yoghurt manufacture receives a significant heat treatment of 90–95 °C for 3–5 min, the objective of this heat treatment is to destroy bacteriophages, vegetative bacteria and potentially some spores, eliminate inhibitory substances, denature some protein, and expel dissolved oxygen; (ii) processed cheese batch ingredients usually receive a heat process equivalent to, or greater than, pasteurisation to inactivate vegetative cells, and there is a wide range of process parameters used to achieve this.

2.3. Thermisation

This process typically occurs between 57 and 68 °C for 5 s up to 30 min. Thermisation targets vegetative psychrotrophs (that produce heat-resistant lipases and proteinases) and is sometimes used in the manufacture of cheese (an example is described below for thermisation and raw milk cheese), or to extend the keeping quality of milk during chilled storage prior to further processing.

2.4. Extended shelf-life processing or ultra pasteurisation

Milk that is to be stored for extended periods at refrigeration prior to use can also be treated with temperatures higher than pasteurisation (125–140 °C for 1–10 s) (i.e., sub-ultra high temperature treatment). The method of heat treatment can be direct, by contact with dry steam, or indirect, involving heat exchangers. Extended shelf-life (ESL) milk can be packaged either aseptically, in which case bacteria surviving the heating process are a consideration, or under clean (but not aseptic) conditions, in which case bacteria entering the milk post-processing are a further

Table 1
Examples of cases where improper heat treatment has led directly to foodborne illness or recalls of dairy products.

Dairy category	Product details	Date	Country	Foodborne illness or recall
Liquid dairy	Milk	1986	USA	Foodborne illness (*Campylobacter)*
	Milk	1992	USA	Foodborne illness (*Campylobacter)*
	Milk	1997	UK	Foodborne illness (*Cryptosporidium*)
	Milk & Cream	2013	UK	Recall
	Milk	2015	USA	Recall
	Chocolate milk	2015	USA	Recall
	Various milk and cream products	2016	USA	Recall
	Double cream	2017	USA	Recall
	Milk	2017	USA	Recall
	Milk	2017	USA	Recall
	Milk	2018	USA	Recall
	Chocolate milk	2018	USA	Recall
	Chocolate milk	2018	USA	Recall
	Strawberry milk	2018	USA	Recall
	Milk, chocolate milk and ice cream	2020	USA	Recall
Cheese	Cheese	1986	USA	Foodborne illness (*Salmonella)*
	Mascarpone cream cheese	1996	Italy	Foodborne illness (*Clostridium botulinum*)
	Fresh cheese curd	1998	USA	Foodborne illness (*E. coli* O157 H7)
	Fresh cheese ("queso fresco")	2003	Spain	Foodborne illness (*Streptococcus equi* subsp. *zooepidemicus*)
	Morra bocconcini cheese	2004	Canada	Recall and Foodborne illness (*L. monocytogenes*)
	Cheese	2010	Australia	Recall
	Latin-style soft cheese	2015	USA	Recall and Foodborne illness (*L. monocytogenes*)
	Cottage cheese	2016	USA	Recall
	Cheese ("Margie cheese")	2018	USA	Recall
	Cheese	2019	USA	Recall
Ice cream	Ice cream and milk	2016	USA	Recall
	Ice cream	2016	USA	Recall
Yoghurt	Yoghurt	2014	USA	Recall
	Yoghurt	2016	USA	Recall

consideration. The shelf-life of ESL milk can be extended at refrigeration temperatures for between 21 and 45 days (Deeth, 2017).

2.5. In container sterilisation

Cans filled with evaporated/condensed milk are sealed and autoclaved, which can either operate continuously or in batches. The cans are kept in motion during sterilisation to distribute the heat more quickly and more evenly through the cans. Any protein precipitated during the heat treatment is uniformly distributed throughout the milk. The milk reaches the sterilisation temperature of 110–120 °C for 15–20 min, after which the milk is cooled to storage temperature. The heat treatment is intense and results in light brown colouration because of Maillard reactions (Tetra Pak, 2003).

For 'commercially sterile' products (i.e., some more heat resistant spores may still be present, and this is referred to in industry as 'commercially sterile') with an extended ambient shelf-life, the food safety concern is the inactivation of spores of pathogens, i.e., *Clostridium botulinum*. Because milk is a low-acid food (pH ~ 6.6), the food safety aim is to achieve 12 decimal reductions for *C. botulinum* (this is the same criterion for canned/sterile foods). The minimum botulinum cook (time/temperature combination) will produce a product that is microbiologically safe (i.e.,

Table 2
Examples of common types of heat inactivation in dairy manufacturing processes.[a]

Process	Temperature (°C)	Time	Examples of applications	Microorganism inactivated
Pasteurisation				
Low temperature long time (LTLT) milk	63	30 min	Regulatory requirement for pasteurisation,	Non-spore-forming pathogens, psychrotrophic spoilage bacteria, e.g., Gram-negative *Pseudomonas* and
High temperature short time (HTST) milk	72–75	15–20 s	Pasteurised milk, cream, cheese, powders, recombined milks	Enterobacteriaceae (not spores or thermoduric bacteria, e.g., *Streptococcus, Enterococcus*)
HTST cream	>80	1–5 s		
Batch pasteurisation				
Starter/yoghurt milk	90–95	15–30	Starter manufacture/yoghurt manufacture	All non-spore-forming bacteria and some psychrotrophic & mesophilic spores (depending on the specific heat treatment)
Cheese milk	70– > 95	4–15 min	Process cheese	
Thermisation	57–68	5 s–30 min	Further processing & manufacture of some cheese	Some lipase & proteinase producing vegetative cells of spoilage psychrotrophs (some pathogens may remain viable)
ESL or ultra-pasteurised milk	125–140	1–10 s	Extended shelf life refrigerated milk	All non-spore-forming bacteria and most psychrotrophic and mesophilic spores.
In container sterilisation	110–120	10–20 min	Evaporated/condensed milk, drinking milk with long shelf life. Nutritional dairy beverages	All bacteriophages, non-spore-forming and many spores except highly heat-resistant ones
UHT	135–150	1–10 s	Ambient drinking milk with long shelf life (6–9 months)	All non-spore-forming bacteria and all spores except highly heat-resistant spores of *G. sterothermophilus* and *B. sporothermodurans*); produces 'commercially sterile product'

[a] Adapted from IDF (2018).

C. botulinum spores are inactivated), but not necessarily sterile ('commercially sterile'). These more heat-resistant spores, which may cause spoilage, are for example *Geobacillus stearothermophilus* and *Bacillus sporothermodurans* (Lewis, 2003). Therefore, the heat treatments in dairy processes that control these more heat-resistant spores will also control *C. botulinum* spores. Note that *C. botulinum* is rarely associated with raw milk or pasteurised dairy products (Doyle et al., 2015).

2.6. Ultra high temperature

Milk that is stored for long periods of time (6–12 months) at ambient temperature prior to use is ultra high temperature (UHT)-heat treated at 135–150 °C/1–10 s, with the aim of achieving a 9-log reduction in the thermophilic spore count (e.g., *Geobacillus* species). When this 9-log reduction is achieved at a particular time and temperature combination, it is sometimes referred to as the bacteriological index (or B*). At this time/temperature combination, the B* has a value of 1 (Kessler & Horak, 1981). The UHT process involves a pre-heating stage, the high-heat stage, cooling, homogenising and aseptic packaging. There are two main types of UHT systems, direct and indirect. In the direct system the product comes into direct contact with the heating medium. The product is rapidly heated and cooled and considered to have a better flavour profile than indirectly heated product. In the indirect system the heating medium does not contact the product and heat is transferred through a heat exchanger. Indirect heating is usually more severe than the direct process (Tetra Pak, 2005).

3. Importance of understanding D-values and Z-values

Historically, the thermal death point was a concept used to describe the heat sensitivity of bacteria and has been defined as the length of time needed to completely inactivate a suspension of an organism (including prokaryotic and eukaryotic cells) at a single constant temperature (Tischer & Hurwicz, 1954). For example, Park (1927) showed that the thermal death point for the tubercle bacilli in milk was 1 min at 68 °C. The thermal death point has largely fallen out of use as it depends on the size of the population present, type of organisms, and gives the impression of an instant kill at one point in time at a set temperature. Calculation of the D-value is more useful as it provides information on the heat resistance of bacteria (Juffs & Deeth, 2007).

3.1. What are D- and Z-values?

Since the inactivation of different microorganisms by heat varies, a simple measure of how resistant they are to heating processes is needed. Hence the use of D-values (decimal reduction time; DRT). The D-value is defined as the time taken to reduce the population by 1 log colony forming unit (cfu) or 90%. D-values for bacteria are dependent on the heating matrix (e.g., liquid milk versus concentrated milk products), the processing method used (e.g., capillary tube-based versus pilot scale pasteuriser), the type and strain of bacterium tested and the temperature applied. The change in the microbial number from before heating, to after heating, is considered to be linear, which allows the D-value to be easily calculated from the slope, m, of the linear regression line of the plot of the log survivors against time at a specific treatment temperature (Berk, 2009).

The equation is:

$$D = 1/(\mu)$$

where μ is the rate of decline of the population given by the slope m.

Table 3
Examples of D-values of non-spore-forming bacteria important in dairy manufacturing, and in various dairy matrices, and dairy derivatives.

Bacterium	Inactivation temperature (°C)	D-value (min)	Type of medium	Reference
E. coli	62.8 - O104:H7	2.8	TSB with 51% milk solids	Dega, Goepfert, and Amundson (1972)
	64 - ATCC 9637	0.385	Ice-cream mix	Desmarchelier and Fegan (2003)
	66.5 - O104:H7	0.968	Non-dairy medium containing 51% milk solids	Desmarchelier and Fegan (2003)
	76 - ATCC 9637	0.00195	Milk	Read et al. (1961)
	76 - ATCC 9637	0.00265	Chocolate milk	Read et al. (1961)
	76 - ATCC 9637	0.00147	Ice cream mix	Read et al. (1961)
	76 - ATCC 9637	0.00093	40% cream	Read et al. (1961)
S. aureus	70	0.1	Milk	ICMSF (1996)
	75	0.02	Milk	ICMSF (1996)
L. monocytogenes	68 - strain 1151	0.19	Butter	Casadei et al. (1998)
	68 - strain 1151	0.15	Half cream	Casadei et al. (1998)
	68 - strain 1151	0.13	Double cream	Casadei et al. (1998)
	68	0.116	Reconstituted skim or UF whole milk (27% TS)	Szlachta et al. (2010)
	72	0.015–0.045	Milk	Sutherland & Porritt (1997)
Salmonella	57	1.4	TSB (10% milk solids)	Dega et al. (1972)
	57	26.6	TSB (51% milk solids)	Dega et al. (1972)
	60	<0.2	Milk	D'Aoust et al. (1987)
	63	1.2	Whole milk	Mañas et al. (2001)
	63	1.1	Skim milk	Mañas et al. (2001)
	63	1.3	Whey ultrafiltrate	Mañas et al. (2001)
	73–74	0.035	Retentates (4 × conc. skim or whole milk)	Kornacki and Marth (1993)
	85	0.07	Galacto-oligosaccharide syrup (74% total solids)	Bang et al. (2017)
Cronobacter spp.	58 - *C. sakazakii* DPC 6529	0.55	Reconstituted infant formula	Huertas et al. (2015)
	58	0.68	Reconstituted infant formula - whole milk	Osaili et al. (2009)
	58	0.62	Reconstituted infant formula - low fat milk	Osaili et al. (2009)
	58	0.51	Reconstituted infant formula - skim milk	Osaili et al. (2009)
	60	1.1–4.4	Reconstituted infant formula	Edelson-Mammel & Buchanan, 2004; Iversen, Lane, & Forsythe, 2004; Nazarowec-White & Farber, 1997
	70	0.07	Reconstituted infant formula	Edelson-Mammel and Buchanan (2004)
	85	0.08	Galacto-oligosaccharide syrup (74% total solids)	Bang et al. (2017)

Table 4
Examples of D-values of spore-forming bacteria important in dairy manufacturing, and in various dairy matrices, and dairy derivatives.

Bacterium	Inactivation temperature (°C)	D-value (min)	Type of medium	Reference
Bacillus cereus	90	1–13	UHT milk (2% fat)	Stoeckel, Westermann, Atamer, and Hinrichs (2013)
	95	2.6–2.9	Micellar casein concentrate (1.5–14.3% protein)	Stoeckel, Atamer, and Hinrichs (2014)
	95	2.6	Whole milk	Stoeckel et al. (2014)
	95	0.91–0.16	Cream	Mazas, Lopez, Martinez, Bernardo, and Martin (1999)
	95	6.3	Infant formula	ICMSF (1996)
	100	1.09–0.27	Skim milk	Mazas et al. (1999)
	100	1.03–0.31	Conc skim and whole	Mazas et al. (1999)
	100	2.7–3.1	Skim milk medium	Mikolajcik (1970)
	110	0.34–0.65	Infant formula (10–50% total solids)	Stoeckel et al. (2013)
B. licheniformis	100	4–8	General	Brown (2000)
	100	20–103	From raw milk, in Ringer's solution	Scheldeman, Herman, Foster, and Heyndrickx (2006)
	100	2.8–4.1	Skim milk medium	Mikolajcik (1970)
	140	0.006	Milk powder	Hill and Smythe (2004)
B. subtilis	95	2–6	Milk	Janštová and Lukášová (2001)
	121	~0.5	General	Brown (2000)
B. pumilus	95	~4	Milk	Janštová and Lukášová (2001)
	100	0.875	Skim milk medium	Mikolajcik (1970)
B. coagulans	95	4–8	Milk	Janštová and Lukášová (2001)
	100	1.97	Skim milk medium	Mikolajcik (1970)
B. sporothermodurans	125	2–13	Raw milk	Burgess et al. (2010)
	140	0.06–0.13	UHT milk	Huemer, Klijn, Vogelsang, and Langeveld (1998)
	140	~0.083	UHT milk	Scheldeman et al. (2006)
Paenibacillus	105	30	Sterile water, originally from milk powder	Sadiq et al. (2016)
	105	0.3	Low fat "requeijão cremoso" processed cheese	Oliveira et al. (2018)
	105	0.015	Full fat "requeijão cremoso" processed cheese	Oliveira et al. (2018)
Anoxybacillus	140	~3.6	Milk powder	Hill and Smythe (2004)
Geobacillus	140	0.06–1	Milk powder	Hill & Smythe, 2004; Eijlander et al., 2019
	140	0.02	UHT milk	Huemer et al. (1998)

Early work by Bigelow and Esty (1920) and Bigelow (1921) demonstrated this linear relationship between the logarithm of the DRT and the temperature, and is referred to as the classical thermal death model. However, there are occasions where "shoulders" or "tailing" of survivors occurs (Ross et al., 1998). "Shoulders" are considered to be a lag in inactivation response by the cells being tested, while "tails" represent sub-populations that are reportedly more resistant to the temperature applied than the general population of cells being tested (Ross et al., 1998). In the case of "shoulders" or "tailing", the D-value is calculated from the resulting curve using models such as Weibull (Huertas et al., 2015). Besides "shoulders" and "tailing", other deviations from exponential decline have also been described. Theoretical reasons for these variations are described in the literature (Cerf, 1977; McClure, Roberts, Baranyi, & Adair, 2004; Stumbo, 1973). Tables 3 and 4 show the D-values of various bacteria of importance in dairy manufacturing, and in various dairy matrices or their derivatives.

The Z-value is used to define the temperature that would be needed to reduce the D-value by a factor of 10, and is useful for predicting how resistant bacteria may be to heat treatment for a range of temperatures. For example, if the D-value for a bacterium in milk at 65 °C is 10 s, and its Z-value is 7 °C; then it can be predicted that at 72 °C the D-value is 1 s. A treatment of 15 s at 72 °C (standard pasteurisation), would therefore result in a 15 log reduction in the population of that particular bacterium. Using a real dairy example, the D-value of *Listeria monocytogenes* strain 1151 in butter treated at 68 °C was calculated as 11.3 s, with a Z-value of 6.71 °C (Casadei, Esteves de Matos, Harrison, & Gaze de Matos, 1998). At 72.7 °C (pasteurisation), the D-value was 1.5 s (i.e., pasteurisation of butter would result in 10 log reductions of this strain of *L. monocytogenes* when carried out at the standard 15 s).

3.2. Some factors reportedly affecting the D-values of microorganisms in the dairy context

There are a number of factors that influence the heat resistance of microorganisms. One factor is the fat content of the dairy matrix. A recent study has found that oil helps to protect bacteria from thermal inactivation in two ways (i) desiccation of cells (i.e., a low water activity; a_w): and (ii) by protecting the cells by creating a moisture barrier that prevents water vapour from rehydrating the cells, thus preventing the inactivation process (Yang, Xie, Lombardo, & Tang, 2021). That study was carried out in peanut oil, but may also explain some of the phenomena seen in dairy matrices with higher fat contents. For example, higher fat whole milk formula resulted in a higher D-value for *Cronobacter* species in reconstituted infant formulae, compared with the D-value for low fat or skim milk counterparts at 58 °C under laboratory experimental conditions (Osaili, Shaker, Al-Haddaq, Al-Nabulsi, & Holley, 2009) (Table 3). An increased fat content may not always result in a higher D-value. For example, Casadei et al. (1998) showed that *L. monocytogenes* strain 1151 had a higher D-value in butter (11.30 s) compared with that in double cream (7.86 s) at 68 °C (Table 3) which was expected due to the higher fat content of butter. For a different strain, *L. monocytogenes* Scott A, there was a higher D-value in double cream (9.46 s) than in butter (6.45 s), seemingly indicating strain specific behaviour. Similarly, *Escherichia coli* ATCC 9637 was shown to have a higher D-value at 76 °C when treated in milk compared with that in 40% cream (Table 3) (Read, Schwartz, & Litsky, 1961).

Recent studies have shown that minerals contained within milk also increase or decrease the heat resistance of different bacteria. Calcium and magnesium were shown to increase the heat resistance of *Salmonella* Seftenburg 775 W at 63 °C by stabilising the cell

envelope (Mañas, Pagán, Sala, & Condón, 2001), while higher levels of phosphate were shown to decrease the heat resistance of *Geobacillus* spores at 110 °C (Kumar, Flint, Palmer, Plieger, & Waterland, 2019).

Desiccation or a change in osmotic tolerance (e.g., higher total percentage solids) are well known factors that increase the heat resistance of some pathogens in dairy products, in particular for *Salmonella* species. In liquid milk, *Salmonella* species are easily inactivated by pasteurisation (72 °C for 15s). Work carried out by Pearce et al. (2012) who tested 32 different serotypes of *Salmonella* species showed that a >6.9 log reduction was achieved at 61.5 °C for 15 s for the most heat resistant strain tested (*Salmonella* Typhumurium NZRM 4220). However, when milk is concentrated, the D-values at 57 °C for *Salmonella* species increased from 1.4 min at 10% TS to 26.6 min at 51% TS (Table 3). It has been known for many decades that *Salmonella* species are reduced during the spray drying process of milk (~4.5–6 log reduction in powder with 20% total solids and 3% moisture) (Miller, Goepfert, & Amundson, 1972). A more recent study has confirmed the work of Miller et al. (1972), and has shown that reducing the water activity of milk from 0.9 to a water activity of 0.25 resulted in a 4 log reduction in *Salmonella* species (Lang et al., 2017). However, studies have shown that desiccated *Salmonella* species are more heat resistant than their non-desiccated counterparts. Gruzdev, Pinto, and Sela (2011) showed that desiccated cells of *S.* Typhimurium, previously grown from laboratory medium, were only reduced by 3 log when heated for 1 h at 100 °C (dry heat), compared with non-desiccated cells that were completely inactivated within the 10 min testing parameters applied in that study (>8 log reduction). Similarly, in dairy media, a study by Sekhon, Singh, and Michael (2020) showed that a 6–7 log reduction was achieved in reconstituted skim milk at 65 °C for 2 min for strains of *Salmonella* Enteritidis, *Salmonella* Montevideo, *Salmonella* Newport and *S.* Typhimurium. However, a 4–5 log reduction was observed when these same strains were treated in skim milk powder at 80 °C.

The strain type also influences how bacteria respond to heat treatments, with some strains naturally more tolerant than others. *Cronobacter* spp. strain 607 was found to be more resistant to heating in the same reconstituted infant formula at 58 °C (D value of 8.8–10.4 min) compared with strain 51,329 (~0.51 min) (Edelson-Mammel & Buchanan, 2004). *L. monocytogenes* strains belonging to serogroup 1 were found to be more resistant than serogroup 4 strains when heated at 72 °C in semi-skimmed sterilised milk (Lemaire, Cerf, & Audurier, 1989). Similarly, a study on thermisation of raw milk for cheese manufacture using a pilot-plant pasteuriser showed that some strains of *E. coli* were inherently more resistant to heating than others, with *E. coli* O16:H21 (FAM21846) exhibiting a D-value of 3.3 s at 65 °C compared with *E. coli* O68:H14 (FAM21805) which had a D-Value of 93.4 s at 65 °C (Peng, Hummerjohann, Stephan, & Hammer, 2013).

The initial preparation of the culture for testing, and the heating methodology used in determining D-values, also impact the resulting D-values (see section 6). As an example, elevating the growth temperature of *L. monocytogenes* strain Scott A from 37 °C to 48 °C resulted in a higher subsequent D-value in UHT milk (Fedio & Jackson, 1989). Similarly, coupling 43 °C incubation with anaerobic storage resulted in a higher D-value for *L. monocytogenes* strain F5069 in sterile whole milk than did 37 °C aerobic storage (Knabel, Walker, Hartman, & Mendonca, 1990).

As part of the inherent tolerance to heat, sporulation of bacteria must be included. Spores from spore-forming bacteria are naturally more heat resistant because of the nature of the bacterial spore (Table 4). The proposed mechanisms of heat resistance for the various microorganisms are discussed in more detail below.

4. Mechanisms of heat inactivation or resistance for different microorganisms of dairy relevance

Russell (2003) has previously discussed the different mechanisms for heat inactivation of bacteria. While these mechanisms can be applied generally to heat inactivated bacteria in dairy processing, the possible mechanisms associated with dairy relevant microorganisms are considered.

4.1. Important foodborne pathogens

The bacterial ribosome may be one explanation for why *Salmonella* species in milk powder may tend to have a higher heat resistance in the desiccated state (McDonough & Hargrove, 1968). It has been suggested that ribosomal degradation is important for inactivation of *Salmonella* species at higher treatment temperatures, and that a low water activity may inhibit this degradation by stabilising the ribosomes, perhaps by magnesium ions (Aljarallah & Adams, 2007; Tolker-Nielsen & Molin, 1996). The ribosome hypothesis is also supported by recent work indicating that the glass transition temperature (Tg) in *Salmonella enterica* serovars increased as the water activity decreased (maximum 57.5 °C at an a_w of 0.87, compared with maximum 83.3 °C at an a_w of 0.43) (Lee, Shoda, Kawai, & Koseki, 2020).

The glass transition temperature (Tg) is the temperature at which polymer chains begin to move from a rigid state, in which they act as glass, to a flexible, fluid state (Shrivastava, 2018). The higher Tg also corresponded to more cell survival at 60 °C for 10 min. It follows then that molecules in a rigid state at a lower water activity would be less vulnerable to physical or chemical interventions than molecules in a more fluid state. Thus, it seems that the observed heat resistance of *S. enterica* in a desiccated form is not due to any inherent physiology of the bacterial cell itself, but has more to do with the environment. This is supported by work showing that Salmonella species, originally isolated from milk powder, lose their heat resistance when heat tested after re-growth in laboratory medium and subsequent re-inoculation into liquid milk (Read, Bradshaw, Dickerson, & Peeler, 1968).

L. monocytogenes is an important pathogen in dairy manufacturing and continues to result in outbreaks and dairy products recalls (Bourdichon, Lindsay, Dubois, & Jordan, 2019). Unlike *Salmonella* species, ribosomal damage does not appear to be a major cause of cell death in *Listeria* species when heated between 57 and 63 °C (Skåra et al., 2011). Regarding *L. monocytogenes*, heat treatment appears to result in cell membrane damage and cell wall thinning (Bermúdez-Aguirre, Mawson, & Barbosa-Cánovas, 2011; Somolinos, Espina, Pagán, & Garcia, 2010), which is supported by studies showing a down regulation in genes that govern cell division and cell wall synthesis when *L. monocytogenes* are heat shocked (van der Veen et al., 2007). The *sigB* gene, together with a cascade of stress response mechanisms, play a role in heat tolerance (Somolinos et al., 2010). Several heat-shock genes are also activated during heat treatment, as well as the SOS response (a DNA repair mechanism in its broadest sense) (van der Veen et al., 2007). The alternative sigma factor σB is activated in *L. monocytogenes* as a general response to various types of stress, such as acid, oxidative and ethanol stresses (Chaturongakul & Boor, 2006). These environmental stresses, which could occur in dairy manufacturing environments through acid, peroxide or ethanol sanitiser use, for example, are also known to confer a cross-protection to *L. monocytogenes* when subsequently treated with mild heat (e.g., 60 °C) (Lou & Yousef, 1996). A further tolerance mechanism may be the presence of plasmids. Researchers in Finland have recently found a strain of *L. monocytogenes* reportedly tolerant to heating at 55 °C, which is conferred by the presence

of a plasmid carrying a gene for the ATP-dependent protease ClpL (Pöntinen, Aalto-Araneda, Lindström, & Korkeala, 2017). However, that isolate was found in a meat manufacturing plant, and was not dairy-associated, but it does demonstrate strain-dependant resistance mechanisms that can be found in different matrices.

Cronobacter spp. are important opportunistic pathogens, and internationally, it is recognised that all infants (<12 months of age) are the population of concern for foodborne infections with *Cronobacter* spp. (Codex, 2008). In terms of dairy manufacturing, *Cronobacter* spp. are able to survive the drying process used to manufacture infant powder products (more resistant to drying than even *Salmonella* species) (Lang et al., 2017). For *Cronobacter* spp. in broth culture, heat treatment (48 °C) damages cell membranes, and associated cell leakage of proteins, enzymes and nucleic acid occurs (Chang, Chiang, & Chou, 2009). Similar to *Salmonella* species, *Cronobacter* spp. in the desiccated form in milk powder are resistant to dry heating (Jaradat, Al Mousa, Elbetieha, Al Nabulsi, & Tall, 2014).

It has been postulated that *Cronobacter* spp. in this dried state in milk powder undergo a decrease in their respiratory ability during heat inactivation, contributing to this heat tolerance (Lang et al., 2018). Another mechanism based on a unique thermotolerant genomic island has been found in thermotolerant *Cronobacter* spp. (Gajdosova et al., 2011; Orieskova et al., 2013). The thermotolerant effect is coded for by a gene on a genomic island of other stress-response genes in strains of thermotolerant *Cronobacter* spp. (37–54 °C) (Orieskova et al., 2013). A 20-fold increase in the transcription of the thermotolerance genes occurred in such *Cronobacter* spp. cells when the heat treatment was increased from 37 to 54 °C (Orieskova et al., 2013). As for other pathogenic bacteria, there is no evidence that such thermotolerance affects the inactivation of *Cronobacter* spp. during commercial pasteurisation processes (e.g., *Cronobacter sakazakii* was reduced by > 6.7 log cfu at 67.5 °C in a pasteuriser operating under validated turbulent flow) (Pearce et al., 2012).

4.2. Potential spoilage bacteria

For the most part, spoilage bacteria of concern for dairy manufacturing are spore-formers that are able to withstand pasteurisation. The heat resistance of spores produced by spore-forming mesophilic and thermophilic bacteria is a well-established phenomenon (Davies, 1975), but the heat resistance (D-values) of the spores from these groups varies (Table 4). Membrane permeability changes, protein or enzyme damage, and DNA damage are all considered to be mechanisms of heat inactivation of bacterial spores (Russell, 2003). Dry heat is also less effective than wet heat against bacterial spores due to the dehydration of the spore core (Setlow, 2006). However, a few recent discoveries may help to shed light on the heat inactivation of bacterial spores in the dairy context.

A mobile genetic element (transposon) Tn1546-like carrying an operon called *spoVA*^{2mob}, has recently been found in strains of *Bacillus subtilis*, and has been linked to high heat resistance of the spores produced by the strains tested (Berendsen, Boekhorst, Kuipers, & Wells-Bennik, 2016). The two dairy isolates tested in that study, *Bacillus amyloliquefaciens* B425 isolated from sterilised milk and *Bacillus licheniformis* B4092 isolated from buttermilk powder, did not appear to contain the transposon, and the spores from these isolates were not particularly heat resistant. It is unknown whether other dairy *Bacillus* isolates may carry this transposon.

For thermophilic *Geobacillus* spp. a recent study using scanning electron microscopy visualised damage to the inner spore core. Heat treatment (120 °C for 10 min) caused membrane permeability and release of intracellular components (Rozali, Milani, Deed, & Silva, 2017). This correlates with studies showing that heating of *Geobacillus* spp. spores at 121 °C for 30 min released dipicolinic acid (Reyes et al., 2019). The presence of phosphate has also been shown to accelerate the loss of cations from dairy-relevant *Geobacillus* spp. spores, resulting in increased heat sensitivity (Kumar et al., 2019).

4.3. Beneficial bacteria in dairy manufacturing

Streptococcus thermophilus is a beneficial thermoduric bacterium used as a yoghurt culture in dairy manufacturing, but can also be a spoilage bacterium. In the context of its spoilage potential, wild-type *S. thermophilus* has been shown to form biofilms in the regeneration sections of pasteurisation plate heat exchangers (Flint, Brooks, & Bremer, 2015). This may result in unacceptably high bacterial counts in final dairy products, or may lead to spoilage issues (e.g., in cheese) (Flint et al., 2015). The attachment of *S. thermophilus* cells to stainless steel, and subsequent biofilm formation, has been shown to be a contributing factor to its pasteurisation survival in dairy manufacturing (Flint, Brooks, Bremer, Walker, & Hausman, 2002). Besides biofilm formation, *S. thermophilus* cells themselves exhibit a complex array of responses to heat including up-regulation of several heat shock genes, signal transduction genes, cell wall genes, iron homeostasis, ABC transporters and restriction-modification system when treated at 50 °C (Li, Bi, Dong, Yang, & Liang, 2011). Interestingly, the heat shock gene, *ClpL*, also plays important role in the physiology of *S. thermophilus* at high temperature, similar to other Gram-positive bacteria, like the heat tolerant *L. monocytogenes* carrying the plasmid for this gene (Varcamonti et al., 2006). Recently, *ClpL* in a heat-tolerant *L. monocytogenes* strain was found to share 98% nucleotide sequence identity with *ClpL2* of *Lb. rhamnosus*, with researchers suggesting the heat shock gene may have been acquired through horizontal gene transfer (Pöntinen et al., 2017).

From the probiotics perspective, some dairy manufacturers produce probiotics (by spray drying) for addition to dairy products (after heat treatment). In the context of using spray drying for probiotic manufacture (where some heating is applied), maintaining the viability of the cells is important, and ribosomal and cytoplasmic membrane damage play key roles. The ribosome and cytoplasmic membranes of *Lactobacillus delbrueckii* ssp. *bulgaricus* were shown to be damaged when heated at 65 °C in skimmed milk (Teixeira, Castro, Mohácsi-Farkas, & Kirby, 1997). Similarly, Ananta, Volkert, and Knorr (2005) showed that spray drying caused disintegration of the cell membranes in *Lacticaseibacillus rhamnosus* GG. Hence the need for molecules that protect and stabilise the cell membrane during heating (Agudelo, Cano, González-Martínez, & Chiralt, 2017; Chávez & Ledeboer, 2007; Lapsiri, Bhandari, & Wanchaitanawong, 2013; Liu et al., 2015).

5. Heat inactivation in dairy products – examples

5.1. Thermisation to improve safety of raw milk cheeses

The Centers for Disease Control and Prevention has reported that unpasteurised milk dairy products cause 840 times more illnesses and 45 times more hospitalisations than pasteurised milk dairy products in the USA (Costard et al., 2017). A review of 1993–2006 USA foodborne disease outbreaks associated with raw milk products found cheese to be the causative agent in 27 of those outbreaks (Langer et al., 2012). The safety of raw milk cheeses is primarily dictated by the microbiological quality of the milk itself and not the ability of the cheesemaking process to inactivate pathogens (Condron et al., 2009; Donnelly, 2018). The exceptions are if cheese varieties are made with raw milk, and they include a curd-cooking step (such as Swiss or pasta filata styles) or feta cheese (in which the pH is reduced to 4.4 and curd cooked to 48 °C during manufacture).

Table 5
Cheese milk thermisation definitions and predicted decreases in *L. monocytogenes* based on literature model given in Fig. 1.[a]

Heat treatment (°C)	Duration	Notes	Predicted *L. monocytogenes* decrease (log cfu mL^{-1})	Reference
55	2–16 s	"Much of the aged raw milk cheese produced in the US is subjected to some form of heat treatment, generally thermisation. This treatment generally consists of heat treatment at 55 °C for a period ranging from 2 to 16 s"	0.0 to 0.0	Donnelly (2004)
57	30 min	Swiss alpine-style specific; suggested to be equivalent to thermisation at 60 °C for 5 min or 65 °C for 15 s	3.6	Jakob and Menéndez Gonzâlez (2015)
57–68	5 s–30 min	Noted for psychrotroph control as well as destruction of some non-spore-formingpathogens; "Some pathogens may remain viable"	0.0 to > 8.0	IDF (2018)
57–68	10–20 s	Noted for psychrotroph control; "Not sufficient to reduce significantly the population of vegetative cells of the more heat resistance bacterial pathogens but creates a suitable environment for the multiplication of selected starter cultures"	0.0 to 3.6	Condron et al., 2009; Eugster & Jakob, 2019; Rukke, Sorhaug, & Stepaniak, 2011; Stepaniak & Rukke, 2002
57–68	≤15 s	Specification for certain Swiss PDO cheeses; *L. monocytogenes* reductions of <0.1 log, 0.2 log, 0.7 log, and 2.0 log for 15 s treatment at temperatures 57, 62, 65, and 68 °C, respectively, expected	≤0.0 to ≤2.7	Eugster & Jakob, 2019; Sorqvist, 2003
57–68	≥15 s	"At the lower end of the temperature range for thermisation, there is little if any destruction of *L. monocytogenes*"	≥0.0 to ≥2.7	Condron et al., 2009; EU, 2004
57–68	15–20 s	Noted for psychrotroph control; *L. monocytogenes* noted to be able to survive	0.0 to 3.6	Fernandes (2009)
57–68	15–30 s	Noted for psychrotroph control	0.0 to 5.4	Panthi, Jordan, Kelly, and Sheehan (2017)
60–62.8	15–20 s	"Used to kill most bacteria found in milk; does not kill all pathogenic bacteria, spores or most non-starter lactic acid bacteria"	0.1 to 0.5	Johnson (2002)
60–68.5	≤15 s	Noted for psychrotroph control	≤0.1 to ≤ 3.1	Johnson (1988)
60	5 min	Swiss alpine-style specific; suggested to be equivalent to thermisation at 57 °C for 30 min or 65 °C for 15 s	2.5	Jakob and Menéndez Gonzâlez (2015)
61	15 s	Blue specific; allows for survival of some yeasts	0.2	Dines Cantor, van den Tempel, Kronborg Hansen, and Ardo (2017)
61–62	15 s	Blue specific	0.2 to 0.3	Cantor, van den Tempel, Hansen, and Ardo (2004)
≥62	≥15 s	Australian thermisation requirement with ≥90 days aging at ≥2 °C; "Could inactivate up to 3 log of pathogenic *E. coli*" (with 2 log attributed to thermisation, 1 log to ageing); "*L. monocytogenes* likely to survive the combined treatment in some cheeses, e.g., Camembert"	≥0.3	Condron et al. (2009)
≥62	≥16 s	Canadian thermisation guideline with ≥60 days aging at ≥ 2 °C and cheese pH ≤ 5.5 and $a_w \leq 0.95$ required at the end of the manufacturing process	≥0.3	Government of Canada (1996)
62–65	10–15 min	For selective pressure for natural milk starter cultures in Italian traditional cheese manufacture	>8.0	Parente and Cogan (2004)
62–67	20–90 s	Industrial thermisation conditions (France)	0.4 to > 8.0	Levieux, Geneix, and Levieu (2007)
62–68	15 s	"Practiced widely" for thermisation	0.3 to 2.7	Rukke et al. (2011)
63	10–15 s	Noted for psychrotroph control; milk may be subsequently pasteurised before cheesemaking	0.3 to 0.4	McSweeney (2007)
63	15 s	Camembert, Brie, and Limburger specific	0.4	Farkye (2002)
~63	15 s	Noted for psychrotroph control	0.4	Chambers (2002)
63	15 min	For selective pressure of natural milk starter cultures in Traditional Specialty Guaranteed Mozzarella	>8.0	Parente, Cogan, Powell, and Fox (2017)
63–65	15 s	"Typical" thermisation	0.4 to 1.0	Panthi et al. (2017)
63–65	15–20 s	Noted for psychrotroph control with enzymes unaffected; "Likely to result in 2–5 log reduction of *E. coli*"	0.4 to 1.3	Condron et al., 2009; Fox, 1993; IDF, 1981; Johnson, 1991
63–65	16 s	Cheddar specific	0.5 to 1.0	Hirvi and Griffiths (1998)
64.4	16 s	Proposed as a guideline for minimum thermisation	0.8	Bishop, 2001; Johnson et al., 1990
64.4	17.5 s	"Can achieve at least a five-log reduction of *E. coli* O157:H7"	0.9	Boyd et al. (2021)
64.6–65	15.5 s	Shown to eliminate coliforms, reduce APC by 1.71 log cfu mL^{-1}	0.8 to 1.0	Johnson (1991)
65	15 s	Noted for psychrotroph control; milk may be subsequently pasteurised before cheesemaking; suggested to be equivalent to thermisation at 57 °C for 30 min or 60 °C for 5 min; recommended minimum thermisation for control of STEC in mature cheese	1.0	Dusterhoft, Engels, & Huppertz, 2017; Eugster & Jakob, 2019; Fox & Cogan, 2004; Fox & McSweeney, 2017; Jakob & Menéndez Gonzâlez, 2015; Martin, Schlimme, & Tait, 2011
65	16–18 s	"Will destroy pathogenic organisms that threaten cheese safety"	1.0 to 1.1	Wendorff and Smukowski (2007)
65	20 s	Upper limit for thermisation; > 50% of alkaline phosphatase remains active	1.3	Panthi et al. (2017)
65–66	16–18 s	"Lethal for virtually all pathogenic microorganisms present in milk that pose major threats to cheese safety"	1.0 to 1.6	Marcos (2004)
65–70	15–20 s	Noted for cheese milk (general)	1.0 to 6.6	Fernandes (2009)
65–70	16–20 s	Typical thermisation for raw milk cheeses	1.0 to 6.6	Johnson (2001)
66	10–15 s	Noted for psychrotroph control	0.9 to 1.4	Van der Berg, Meijer, Dusterhoft, and Smit (2004)

Table 5 (*continued*)

Heat treatment (°C)	Duration	Notes	Predicted *L. monocytogenes* decrease (log cfu mL^{-1})	Reference
66	15 s	Noted for psychrotroph control	1.4	Bennett & Johnston, 2004; Legg, Carr, Bennett, & Johnston, 2017
66.7	16 s	Cheddar specific	1.9	D'Aoust et al. (1985)
67	20 s	For reduction of amino acid decarboxylase-positive bacteria	2.6	Martin et al. (2011)
67–70	20 s	Cheddar specific	2.6 to 6.6	Johnson (2001)
67.7–70	≤15 s	Swiss specific	≤2.4 to ≤ 5.0	Johnson (1988)

[a] For predictions giving >8.0 log cfu mL^{-1} reduction, a value of >8.0 is assigned.

While raw milk cheeses may be technically in compliance with FDA regulations mandating a minimum of 60 days' aging at ≥ 2 °C, this ageing period has been found to be insufficient in eliminating foodborne pathogens from certain cheese varieties (Reitsma & Henning, 1996; Ryser & Marth, 1987). For this reason, some countries have set a minimum aging time of 90 days for such raw milk cheese products, such as Food Standards Australia New Zealand (Condron et al., 2009). To improve the food safety confidence in reducing bacterial counts in raw cheese milk, thermisation has been used in the USA. The thermisation step allows the final product to still meet the labelling requirements for the raw milk cheese designation (Johnson, Nelson, & Johnson, 1990), and has been recommended by Canadian regulators to increase the microbial safety of Gouda or Gouda-like cheeses (Boyd et al., 2021). Among >900 USA artisan, farmstead, and specialty cheese producers surveyed in 2018, 50% used raw (no heat treatment) and 17% used thermised (some heat treatment but below legal pasteurisation requirements) milk in cheesemaking, up from 32% to 6%, respectively, as reported in 2016 (American Cheese Society, 2016, 2018). Several working definitions of thermisation exist (Table 5), but no established definition in USA regulations has been declared.

L. monocytogenes and Shiga toxin-producing *E. coli* (STEC) have been identified as especially high-risk pathogens in raw milk cheeses due to the severity of illness associated with each as well as their ability to grow or survive during the aging process of certain cheeses, even when initially present in milk at very low levels (Condron et al., 2009; Donnelly, 2018; Johnson et al., 1990). Montel et al. (2014) found STEC to be more resistant to naturally occurring lactic acid bacteria (LAB) in unpasteurised milk compared with *L. monocytogenes*, *Salmonella* species and *Staphylococcus aureus*, while Pereira, Graca, Ogando, Gomez, and Malcata (2009) found *Listeria innocua* and non-Shiga toxin-producing *E. coli* to be the most difficult contaminants to control by co-inoculation with LAB isolated from raw milk cheeses when tested in a raw milk model cheese (Pereira et al., 2009). *L. monocytogenes* has been shown to be more heat-tolerant than most other non-spore-forming pathogens (Doyle, Mazzotta, Wang, Wiseman, & Scott, 2001); however, as STEC strains are less heat-tolerant than *L. monocytogenes*, thermisation treatments to reduce *L. monocytogenes* should sufficiently reduce STEC at equal or higher rates (Betts, 2000; Fernandes, 2009; Sorqvist, 2003; Van Asselt & Zwietering, 2006).

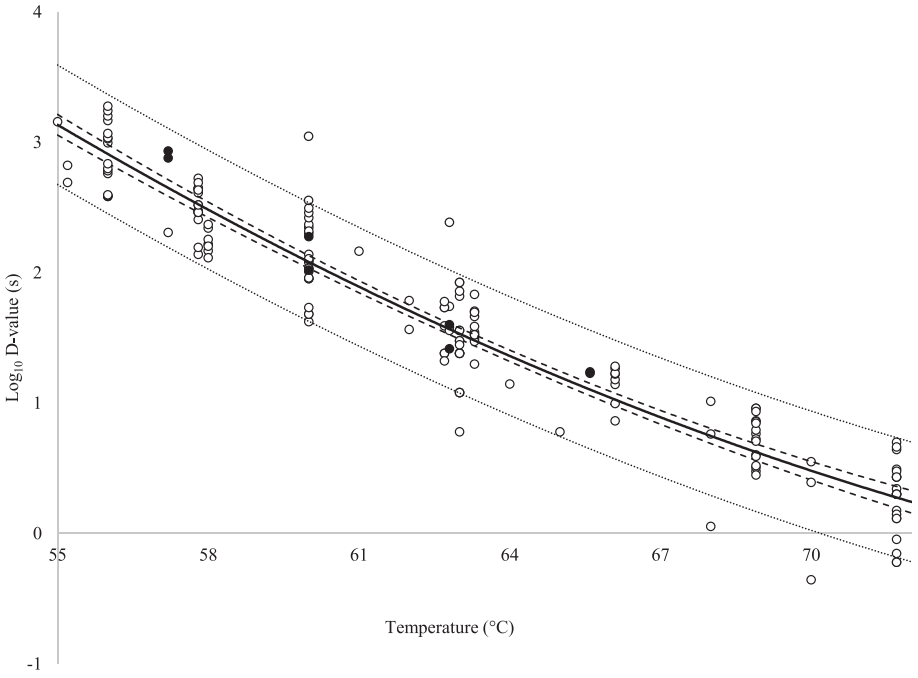

Fig. 1. Log D-value versus heating temperature for *L. monocytogenes*-inoculated fluid whole bovine milk samples in the scientific literature. For relevance to thermization of raw milk intended for cheesemaking, dataset omits non-*monocytogenes Listeria* species, reconstituted milks, and temperatures < 55 °C or ≥ 72 °C. Dataset includes whole (standardized or raw) milks at all pasteurization levels with all methods of culture preparation, inoculation, and heating: - -, confidence at 95% interval; •••, prediction; black dots, researcher data (Bradshaw, Peeler, Corwin, Hunt, & Twedt, 1987, 1991, 1985; Bunning, Crawford, Tierney, & Peeler, 1992, 1986; Crawford, Beliveau, Peeler, Donnelly, & Bunning, 1989; Donnelly & Briggs, 1986; Farber & Pagotto, 1992; Fedio & Jackson, 1989; Holsinger, Smith, Smith, & Palumbo, 1992; Kamau, Doores, & Pruitt, 1990; Kenney et al., 2004; Knabel et al., 1990; Pearce et al., 2012; Rowan & Anderson, 1998; Szlachta, Keller, Shazer, & Chirtel, 2010; van der Veen, Wagendorp, Abee, & Wells-Bennik, 2009).

A significant body of research on the thermal reduction of *L. monocytogenes* in liquid dairy and other food products exists, with predictive models constructed from meta-analyses by several authors (Mackey & Bratchell, 1989; Sorqvist, 2003; Van Asselt & Zwietering, 2006; van Lieverloo, de Roode, Fox, Zwietering, & Wells-Bennik, 2011, 2013). Differences in methods limit the usefulness of these models to appropriately facilitate a definition of thermisation conditions necessary for the thermal reduction of *L. monocytogenes* in cheese milk. Differences which affect the results between models include incorporation of reconstituted and/or skim milks (Mackey & Bratchell, 1989; van Lieverloo, de Roode, Fox, Zwietering, & Wells-Bennik, 2013), inclusion of high fat or high solids dairy products (e.g., butter, cream, ice cream mix) (Van Asselt & Zwietering, 2006), pooling of milk D-values with other foods and microbiological media (Sorqvist, 2003; Van Asselt & Zwietering, 2006; van Lieverloo et al., 2011), and inclusion of temperatures higher than HTST pasteurisation (Mackey & Bratchell, 1989; Sorqvist, 2003; van Asselt 2006; van Lieverloo et al., 2011, 2013). Predicted *L. monocytogenes* Z-values of 5.7–7.0 °C and D-values at 60 °C of 87–140 s were recorded in those published predictive models. Comparatively, a predicted *L. monocytogenes* Z-value of 6.1 °C and D-value at 60 °C of 130 s were found by a meta-analysis of 162 whole milks inoculated with *L. monocytogenes* and heated to temperatures 55 °C–71.7 °C (Fig. 1).

Fewer studies investigating STEC in liquid dairy and other products exist in the scientific literature, with an additional limitation for cheese thermisation application due to the inclusion of non-STEC general *E. coli* D-values in two existing published meta-analyses (Sorqvist, 2003; Van Asselt & Zwietering, 2006). Predictive models published by Sorqvist (2003) and Van Asselt and Zwietering (2006) predicted *E. coli* Z-values of 6.0 °C and 10.6 °C, and D-values at 60 °C of 39 and 113 s, respectively. Comparatively, a predicted STEC Z-value of 4.5 °C and D-value at 60 °C of 115 s were found in a meta-analysis of 25 whole milks inoculated with STEC and heated to temperatures 55–65 °C (S. Engstrom, unpublished results).

5.2. Bacteria surviving heating of extended shelf-life milk

Extended shelf-life (ESL) milk can be produced by thermal processes, or non-thermal processes such as microfiltration or bactofugation. Only thermally processed ESL is considered in this review. ESL heat treatment is undertaken with the aims of extending the shelf-life of the milk (beyond that of pasteurised milk) and maintaining optimum flavour.

Production of ESL milk is a balance among microbiological quality, organoleptic acceptability and chemical damage. The higher the temperature and the longer the time the safer is the milk microbiologically, but there is more chemical injury to the milk and it is less organoleptically acceptable. The measurement of chemical change in ESL milk is best measured by the degree of denaturation of the whey protein β-lactoglobulin (Deeth, 2017). The volatile sulphur compounds formed during degradation of β-lactoglobulin contribute to the 'cooked' flavour of heat-treated milk. Approximately 50% of the native β-lactoglobulin should remain in ESL milk; otherwise the flavour may be affected. There are no international standards for this heat treatment, but generally temperatures of between 125 and 130 °C for 2–6 s are used, with the aim of achieving a 6-log reduction in the thermophilic spore count (Deeth, 2017).

The production process of ESL milk will inactivate all vegetative cells, but spore-forming bacteria can survive the heat treatment process. The quality of the raw milk used should also be considered. If protease producing bacteria in raw milk (for example, species of *Pseudomonas*) get the opportunity to grow before processing, the proteases produced can be heat-stable, surviving the heat treatment and causing spoilage in the processed milk.

The diversity of bacteria in ESL milk has been studied (Deeth, 2017; Mayr, Gutser, Busse, & Seiler, 2004; Mugadza & Buys, 2017, 2018). When spoilage occurred in 191 samples, Mayr et al. (2004) found that 76 of the samples contained aerobic spore-formers while 31 contained Gram-negative bacteria. The contamination included species of *Rhodococcus*, *Anguinibacter*, *Arthrobacter*, *Microbacterium*, *Enterococcus*, *Staphylococcus* and *Micrococcus*. The presence of non-spore-forming bacteria in the ESL milk indicates that there was post-heat treatment contamination. Non-spore-forming bacteria can enter the ESL milk after the heat treatment, from filling equipment and packaging (Mugadza, Owusu-Darko, & Buys, 2019) if aseptic filling is not used.

Spore-forming bacteria can also survive ESL milk processing (Mugadza & Buys, 2017), and similar to spore-presence in other dairy products, spores can enter raw milk from the surface of animals' teats, bedding, fodder, pasture or milking equipment, among other sources on the farm (Gleeson, O'Connell, & Jordan, 2013).

As with other dairy products, *Bacillus cereus* is a consideration in ESL milk due to its ability to produce emetic and/or diarrhoeal toxins. A wide range of D- and Z-values, as measures of this heat resistance, have been reported for spores of *B. cereus* strains (Deeth, 2017), which are difficult to compare because of the different temperatures used. For example, D-values at 100 °C from 3 to 36 s have been reported, presumably depending on the strain tested, but it could also depend on the method used. This range of temperatures does not include particularly heat resistant strains of *B. cereus*, such as that reported to have a D-value at 129.4 °C of 14.4 s (Bradshaw, Peeler, & Twedt, 1975). The range of Z-values reported is also variable (for review see Deeth, 2017), with values from 6.7 to 13.8 °C reported. All of these show that there is a wide range of heat resistance of spores among different strains of *B. cereus* (Table 4). *B. cereus* has been found in ESL milk (Mugadza & Buys, 2017), but refrigerated storage of ESL milk will slow its growth and toxin production capability (Deeth, 2017).

Similarly, other species of *Bacillus* may be present in ESL milk, and many of these species have also shown a diversity in D- and Z-values (Janštová & Lukášová, 2001) (Table 4). Other species of *Bacillus* will cause spoilage rather than safety issues in ESL milk. *Paenibacillus* species, which were formerly part of the *Bacillus* genus, have emerged as a unique spoilage concern in ESL milk. Spores of some species of *Paenibacillus* can survive heat treatment in raw milk up to 130 °C (MPI, 2020), and these bacteria have the ability to grow to high numbers during refrigerated storage (7.6 log cfu mL^{-1}) (Mayr et al., 2004; Schmidt, Kaufmann, Scherer, Kulozik, & Wenning, 2012). *P. macerans* isolated from milk powder also showed the highest level of protease compared with other spore-formers present in a study by Sadiq et al. (2016), highlighting their spoilage potential (Table 4).

In terms of clostridia, spores are prevalent in the farm environment, but most dairy associated species are not pathogenic to humans as only a few strains carry toxin genes (MPI, 2014). *C. botulinum* strains of Groups I and II are the main concerns for human health. *C. botulinum* is rarely associated with pasteurised dairy products, and anaerobic conditions are needed for its growth. On the occasion it does occur, Group I strains are most common (Doyle et al., 2015). The minimum temperature for growth of strains of *C. botulinum* Group I is about 12 °C, and under anaerobic conditions. Therefore, *C. botulinum* Group I strains in ESL milk are unlikely to grow and produce toxin if the heat-treated milk is not subjected to temperature abuse, and anaerobic conditions.

C. botulinum Group II strains are psychrotrophs with the ability to grow and produce neurotoxin at temperatures as low as 3 °C, but the spores are only moderately heat-resistant (inactivated below 100 °C) and, if present, will probably be inactivated during the ESL heating process (MPI, 2020).

5.3. Bacteria in UHT milk

The aim of the UHT process is to inactivate bacteria and spores so that the milk can be stored at ambient temperature. UHT milk is a 'commercially sterile' product as some spores can survive the heat treatment and cause spoilage, particularly if there is temperature abuse, such as storage at ambient temperature of above 40 °C. The heat-treated milk is packaged aseptically so the main concern is bacterial spore survival of the heating process, rather than post-processing contamination.

At the processing temperatures used, all vegetative bacterial cells will be inactivated. Additionally, many bacterial spores will be inactivated and those that survive are referred to as high-heat resistant spores. There is no standard method for the isolation of high-heat resistant spores but they can be isolated by heating the sample to 100 °C for 30 min and incubating the agar plates aerobically or anaerobically at 30 °C for mesophilic or 55 °C for thermophilic spores (ISO/IDF, 2009; Wehr & Frank, 2004).

Spores surviving UHT heat treatment are usually of a quality concern, such as the spores of *B. sporothermodurans*, first isolated from UHT milk (Pettersson, Lembke, Hammer, Stackebrandt, & Priest, 1996), and *G. stearothermophilus*. These spores are present at low numbers in raw milk, and are selected for by manufacturing conditions where high temperatures during processing favour their growth (Burgess, Lindsay, & Flint, 2010). Some strains can exhibit proteolytic activity, thus causing spoilage of the UHT product (Pinto et al., 2018). Occasionally other *Bacillus* species causing spoilage have also been isolated from UHT milk, such as *Paenibacillus lactis* (Scheldeman et al., 2004). *B. cereus* spores are not known to survive UHT heating, and spoilage of UHT product from *B. cereus* growth is associated with post-heat re-contamination issues.

6. Conducting industrially applicable bacterial heat inactivation experiments

6.1. Experimental design - why is it important?

In the commercial context, the D- and Z-values for bacterial heat inactivation are important to understand the risks posed by these pathogens in different processes, i.e., they inform the quantitative risk assessments for industry. The availability of better tools to take into account processing conditions, more advanced software, and better mathematical models are an aid in modelling inactivation kinetics of microorganisms (Smelt & Brul, 2014). However, robust scientific data must underpin this information. The D- and Z-values of a variety of bacteria of dairy importance have been studied as illustrated in Tables 3 and 4 But comparing results from heat inactivation studies is often difficult as different methods (e.g., heating in open vials versus heating in sealed capillary tubes), equipment (e.g., laboratory-scale setup versus pilot-scale), bacteria (e.g., strain differences and environmental or type culture isolates), and heating media (e.g., in sterile water in capillary tubes versus in milk in capillary tubes) etc. can be used. Data for the heat inactivation of bacteria in real food systems is scarce when compared with work done in buffer or simple liquid systems, and can vary by a factor of 10 in different food matrices (Smelt & Brul, 2014). It is not always known whether this is due to interlaboratory variation or due to differences in the food matrices. Experiments measuring heat inactivation should be performed with the commercial process in mind, and mimic the effects as closely as possible.

Condron et al. (2015) outlined the considerations that underpin developing a harmonised and carefully designed protocol for the study of the heat resistance of pathogens, with an understanding and knowledge of commercial and/or epidemiological data. These guidelines list a variety of factors for consideration when designing heat inactivation studies, and some of the key aspects, based on experience, are highlighted below.

6.2. Experimental design – what needs to be considered?

6.2.1. Culture selection, preparation and testing medium

Inactivation studies would ideally use key bacteria of concern with the greatest resistance to the heating process being tested, such as *Mycoplasma bovis* in the case of milk pasteurisation, or *G. stearothermophilus* and *C. botulinum* in the case of a UHT treatment. However, using pathogens in a real food manufacturing process is not always possible. In such instances, surrogates can be used, provided they have a similar heat resistance when compared with the pathogen of interest. For example, an attenuated strain of *E. coli* O157:H42, demonstrated to have similar resistance to the most heat resistant virulent O157 strains screened was selected for heat inactivation trials in milk (Pearce et al., 2012).

Pearce et al. (2012) also recommended using single strain isolates that show the most robust heat resistance, rather than using a cocktail when evaluating inactivation kinetics, as a mixture of several different heat tolerances would distort the survival curves. Different strains may also respond differently to recovery conditions, potentially requiring growth media suited to the recovery of more than one strain (i.e., may not be the optimum growth medium for particular strains tested). In the absence of certainty that the most heat resistant strain has been isolated, a mixture or "cocktail" of strains may on the other hand increase confidence that the upper limit of heat resistance is established (Condron et al., 2015).

Growing cultures for heat treatment experiments should take into account the temperature, pH, age of culture, spores, stage of growth and nutrients in the medium (Condron et al., 2015). The culture conditions should mimic the actual test medium, and the most likely physiological state of the cells in the dairy process or product as closely as possible to ensure culture variables are removed. For example, cells in the stationary phase of growth are typically more resistant to a range of stresses, including heat, than corresponding exponential phase cells (Jørgensen, Hansen, & Knøchel, 1999; Lou & Yousef, 1996; McMahon et al., 2000), or alkaline growth conditions (pH 10) can result in increased heat resistance (56 °C) in *L. monocytogenes* (Taormina & Beuchat, 2001).

The density of the inoculum used in the heat challenge study should reflect levels that would be expected to occur naturally, and the inoculum should be evenly distributed in the lowest amount of test medium. Inocula of lower cell densities ($<10^5$ cfu), which may better mimic real levels in the product under consideration, can be used if large sample volumes can be processed, e.g., using most probable number (MPN) methods (Duquet, Trouvat, Mouniqua, Odet, & Cerf, 1987), or more sensitive methods are applied, e.g., based on staining and flow cytometry (viable and injured cells can be detected) (Khan, Pyle, & Camper, 2010).

Clumping is a further factor highlighted by Condron et al. (2015). Some bacteria naturally form clumps or pellicles, and certain growth media can exacerbate this phenomenon. *Bacillus* species (spores and vegetative cells) are well known examples of bacteria that naturally form aggregates (Furukawa et al., 2005). Clumping can lead to an underestimation of cell numbers by plate count methods (Cerf, Griffiths, & Aziza, 2007; Condron et al., 2015;

Hastings, Blackburn, & Crowther, 2001; Klijn, Herrewegh, & de Jong, 2001).

When considering the heat inactivation medium, i.e., the milk, it is well known that changes in milk fat and protein contents are major elements in the seasonal compositional variation of milk (Auldist, Walsh, & Thomson, 1998) that can influence heat inactivation in other systems (Keller et al., 2008; Ma, Kornacki, Zhang, Lin, & Doyle, 2007). To eliminate this possible variable, standardisation of the milk substrate fat and protein levels to relevant levels across experiments are required. Other test media, such as non-thermised whey, will contain a high level of naturally occurring background microorganisms. Enumeration of the test bacterium may become difficult, and selective media may be required (Condron et al., 2015). But selective growth media contain agents (e.g., antibiotics) that can prevent the growth of stressed and/or injured surviving cells. As a result, there is a risk of overestimating the efficacy of the heating treatment applied, and underestimating the survival of the pathogen present (Miller, Brandão, Teixeira, & Silva, 2006). For this reason, some investigators (e.g., Pearce et al., 2012) have preferred to use heat treated milk (e.g., UHT).

The optimum method to revive heat injured cells for enumeration must be established (Teo, Ziegler, & Knabel, 2001). For example, 0.8–1% (w/v) yeast extract was important for the maximum recovery of *E. coli* cells after heat treatment (Russell & Harries, 1968), blood agar in combination with incubation temperatures of 20–25 °C recovered heat-injured *L. monocytogenes* (Mackey, Boogard, Hayes, & Baranyi, 1994), and pyruvate has also been identified as a key ester for improving recovery of heat injured cells, such as *Salmonella* (Kobayashi et al., 2005).

6.2.2. How the experimental design reflects commercial reality

Condron et al. (2015) has highlighted the practical issues with attempting to test the heat inactivation of pathogens in a real world dairy setting. As a result, heat treatment efficacies must be extrapolated from model systems (e.g., test-tubes, vials, capillary tubes, submerged coils, heating blocks and pilot-scale pasteurisers) even though some of these test systems are known to create artefacts, and can give conflicting results (Cerf et al., 2007; Kou et al., 2016). Donnelly, Briggs, and Donnelly (1987) found that a test tube heating methodology overestimated the thermal resistance of *L. monocytogenes* in reconstituted non-fat dry milk compared with values derived using sealed glass tubes. This finding was substantiated by Sorqvist (1989), who found *Yersinia enterocolitica* D-values at 60 °C in physiological saline to be 8 to 29 times greater using 9-mm test tubes than when using capillary tubes. Nevertheless, van Lieverloo et al. (2011) found insignificant difference in *L. monocytogenes* D-values obtained using four different heating methodologies (lab-scale pasteuriser, large volume pre-heated menstruum, submerged capillary tubes, or submerged vials); their analysis of 735 published D-values obtained from dairy and non-dairy liquid foodstuffs and microbiological media demonstrated a high overall R^2 value of 0.88. Even though results from tube-based heating experiments should be treated with caution, the information they produce can be used as an indicative screening tool for further work.

One way some researchers have attempted to minimise artefacts in heat treatment studies is by verifying an existing heat step in a commercial setting. This type of experiment would use naturally contaminated milk, but it can only give information on the absence of survivor(s) in the treated and tested volume. It gives no indication of the probability of survivor presence in larger volumes and there will be little certainty that the process would be reliable under all realistic pathogen loads and operating conditions (i.e., data collected are true only for that time and those parameters) (Condron et al., 2015). Such an approach cannot be used to optimise a process or to develop an alternative process. This requires a more fundamental and detailed knowledge of the effects of time and temperature on the kinetics of inactivation. The change in microbial numbers as a function of heating time, and survivors under conditions encompassing the full range of likely processing or formulation conditions is needed.

The most appropriate approach is the design of a model system to replicate commercial continuous HTST pasteurisation conditions as much as possible, and where the method is scientifically reproducible (Pearce et al., 2001, 2012). Under laboratory-controlled conditions, some researchers have employed commercial-type conditions with turbulent flow in pasteurisation experiments to better mimic commercial processing (Pearce et al., 2012; Piyasena, Liou, & McKellar, 1998). Extrapolation from batch with laminar flow, to continuous HTST pasteurisation with turbulent flow, cannot be done, as batch systems do not include shear force (turbulence or Reynold's number) and other physical stresses, which can induce physiological changes in bacteria. Bacteria under laminar flow are exposed to the heating step for a shorter time (called the “hold time”) than what really occurs in a commercial process. As evidenced by results of Fairchild, Swartzel, and Foegeding (1994) significantly different *L. innocua* Z-values were obtained when raw skim milk was heated in sealed capillary tubes (batch system), as compared with values obtained with a laboratory-scale pasteuriser (turbulent flow).

Robust kinetic data collected from a standardised, repeatable, practical, safe, and cost-effective model system replicating commercial conditions, with appropriate turbulent flow, has been successful for investigating the heat inactivation of various non-spore-forming pathogens from raw milk (Pearce et al., 2012).

6.3. What about the testing of alternate technologies?

Food processing using non-thermal processes such as high-pressure processing (HPP), cold plasma, membrane filtration, pulsed electric fields (PEF), irradiation or a combination of any of these through a hurdle approach represents a change from the traditional heat processes that are relatively well characterised. The need for novel or alternative processing technologies in food industries, many without a traditional and recognised kill-step, is a direct result of consumer demand for fresh, high quality and healthy products that are also safe.

Within dairy manufacturing, regardless of the long and successful history of milk pasteurisation, consumers believe that the heat treatments applied in dairy manufacturing negatively affect some aspects of the final products, such as reduced nutritional properties. This is despite many scientific publications disputing this belief. For example, as early as 1934, a study by Elvehjem, Hart, Jackson, and Weckel showed that rats fed on mineralised raw milk and mineralised pasteurised milk showed no differences in growth or development, indicating no significant changes to the nutritional properties of the pasteurised milk. Other international bodies also agree that pasteurisation has little impact on the nutritional, and other properties, of milk (CDC, 2017; IDF, 2019; MPI, 2013). However, dairy manufacturers must also keep abreast of progress in technology, and consider consumer trends.

Consequently, how to verify and validate such a non-traditional process, requires consideration of microbial reduction requirements and the conditions necessary to achieve this. The key microorganism(s) of concern for the new process/product must be established from the food safety and spoilage perspectives. Alternative process technologies, e.g., HPP or PEF, bring about different microbial kill mechanisms that can influence the type of organism likely to be of concern and be most resilient to that alternative process (e.g., *B. amyloliquefaciens* for HPP versus *Geobacillus* spp. for

UHT treatments). As the microbial kill mechanism(s) are not necessarily directly comparable with heat treatment (Zhang et al., 2011) establishing microbial equivalence to heat treatment is not a simple process. To robustly validate an alternative method still requires identification of the food safety objective (FSO) required to confidently achieve an appropriate level of protection (ALOP) and adherence to the elements described in this paper.

7. Conclusions

Pasteurisation of milk was a step change technology that allowed nutritious dairy products to be safely consumed by mankind. Research has shown that different microorganisms respond in different ways to heat depending on a variety of factors. Evaluating the risks of pathogens and microorganisms that compromise quality in heat treated dairy products needs a clear understanding of the dairy matrix, type of heat used, genetics of the microorganism present and methodology used to collect data on D- and Z-values. Unlike the use of antibiotics and the associated development of antibiotic resistance over time, microorganisms remain sensitive to pasteurisation and heat treatments applied in the dairy industry. Future technologies would need to meet the same inactivation abilities as current heating knowledge. Despite the developments of alternative technologies for destroying microorganisms, such as HPP and PEF technology, thermal treatments remain the preferred option for most processes in the dairy industry. Heat treatment of milk is a proven reliable technology for the production of microbiologically safe, quality dairy products.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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