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Haumaru Kai Aotearoa

Safety profile of extended shelf life refrigerated milk

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Scientific Interpretative Summary

This SIS is prepared by New Zealand Food Safety (NZFS) risk assessors to provide context to the following report for NZFS risk managers and external readers.

Safety profile of extended shelf life refrigerated milk

Extended shelf-life (ESL) milk, when produced by thermal processing, applies parameters between those used for traditional pasteurisation (~72°C for 15 s) and those used for ultra-high-temperature sterilisation (~135-150°C for 1-10 s). If adequately processed, it should have a refrigerated shelf-life of more than 30 days.

ESL milk has gained substantial market share in many countries. However, to date, there is scant data on the production of ESL milk in New Zealand. The goal of this study was to identify the food safety issues that must be considered for clean filled, hermetically sealed, refrigerated milk that has been subjected to thermal treatment in the range 90-127°C. The data were sourced from the scientific literature and other resources such as materials published by overseas government agencies and/or manufacturers of equipment and packaging for ESL milk processors.

The review is focussed on thermal processing for ESL milk production. However, non-thermal processes such as microfiltration or centrifugation, usually combined with thermal processing to meet food safety requirements, are also briefly described.
Producing a product that has high bacteriological quality and safety, whilst maintaining the sought after organoleptic characteristics is challenging. Of particular concern, when manufacturing this product, is the presence of pathogenic spore-forming organisms such as *Bacillus cereus* and potentially *Clostridia* spp.

While the data presented in this review may be considered as a good starting point for process validation, specific time and temperature conditions for the production of ESL milk need to be optimised for each process to ensure shelf life and food safety limits are met. This review supports the current NZFS approach of considering production of ESL milk on a case-by-case basis, and requiring validation studies to assess the bactericidal effect of a process.

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Science for Communities

Safety profile of extended shelf life refrigerated milk

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DISCLAIMER

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1 EXECUTIVE SUMMARY

Extended Shelf Life (ESL) milk can be produced by a variety of technologies with or without heat treatment. Hence, ESL milk is commonly loosely defined as 'milk with an extended shelf life beyond that of pasteurised milk'. The shelf life of ESL milk available in overseas markets using these technologies is in the range 21-27 days with refrigeration. Once the package is opened, ESL milk has the same durability as other types of milk.

This report provides a summary of food safety considerations to produce extended shelf life refrigerated milk by thermal processing, including:

- Technologies for the production of ESL milk
- Microbial hazards associated with ESL milk
- Reduction of microbial numbers in milk by thermal treatments in the range 90-127°C
- Regulation and standards for ESL milk production

Technology

Currently, the most frequent temperature and time combinations for commercially producing ESL milk by heat alone are in the range 123-127°C for 1-3s. These temperatures result in a longer shelf life milk compared to other ESL processing technologies.

Most ESL milk is not packaged aseptically and post-production contamination of ESL milk can occur. Concentrations of microorganisms and spores in ESL milk products are influenced by:

- The quality of raw milk inputs
- The efficiency of microbial reduction technologies
- Post-processing hygienic design
- Maintenance of the cold chain during distribution and domestic storage

Microbial hazards

Raw milk can contain a variety of non-pathogenic and human pathogenic microorganisms. Analysis of overseas retail ESL milk produced by thermal treatments shows that *Bacillus* species are the main thermoduric contaminants originating from raw milk, including a foodborne hazard *B. cereus.* The majority of *Bacillus* strains isolated from overseas retail ESL milk did not grow when the milk was maintained at refrigeration temperatures highlighting the need to control the cold chain during storage and distribution. However, psychrotrophic *B. cereus* strains can grow at refrigeration temperatures, which would allow the production of emetic and diarrhoeal toxins.

Clostridium botulinum may be present in raw milk, but numbers of spores are thought to be low. Group I and Group II *C. botulinum* strains are the main concerns for human health. Group I *C. botulinum* are proteolytic, can metabolise casein, have heat-resistant spores (though less so than *Bacillus*), and are the most common group associated with dairy products. The minimum growth temperature for Group I *C. botulinum* is likely to be in the range 10-12°C. As such, any surviving *C. botulinum* are unlikely to proliferate and produce toxin during refrigerated storage of heat-treated milk, provided temperature abuse does not occur. Group II *C. botulinum* strains are psychrotrophs with the ability to grow and produce neurotoxin at temperatures as low as 3°C. However, the spores of Group II *C. botulinum* are only moderately heat-resistant and they have not been associated with dairy products. It is important to note that data on the behaviour of *C. botulinum* and/or botulinum toxin in ESL milk, including growth and toxin production, have not been reported.

Emerging spore-forming species such as *Paenibacillus*, which can cause spoilage and opportunistic infections, may become a more significant concern in the future, but at present insufficient data are available.

Milk flavourings such as cocoa can naturally harbour heat-resistant spores such as *Bacillus* spp. Cocoa and other flavourings may also be cross-contaminated with spores during processing, so this should be considered when designing ESL thermal processes.

There are incomplete data for microbial hazards that could potentially occur in ESL milk, particularly so for Clostridia. Milk processors commissioning new ESL milk processes should, therefore, ensure that systems are validated to ensure adequate control of these hazards in their specific process.

Reduction of microbial numbers in milk by thermal treatments

The temperature-time conditions reported to be commercially used to prepare ESL milk should be sufficient to destroy non-spore-forming pathogens and all but the most heat-resistant psychrotrophic spore-formers. Researchers have recommended using temperature-time conditions to give at least a 6 log reduction in the psychrotrophic spore count to maintain the safety and quality of ESL milk. While this is the ideal, most published ESL milk thermal processes do not achieve this goal.

The large variability in reported D-values exhibited by *B. cereus* isolates from milk make generalisations of effective time-temperature ESL processing conditions difficult for this organism, so conditions should be validated for each ESL process. Similarly, there are a range of published D-values for Group I *C. botulinum* spores, some of which are compatible with existing ESL practice. For example, heat treatment at 125°C for 5s inactivated 10^3 type A and B spores. The spores of Group II *C. botulinum* are only moderately heat-resistant and are inactivated at temperatures below 100°C.

Paenibacillus spores can survive heat treatment up to 130°C, but data for ESL thermal treatments are lacking.

Addition of milk stabilisers appears to have only minor effects on inactivation rates for thermotolerant spores.

Regulation and standards for ESL milk production

Specific guidance on ESL milk production and regulatory controls is sparse. The US FDA Grade 'A' Pasteurized Milk Ordinance standard describes the thermal processing of ESL milk, although at 138°C for ≥2 s this would be considered as a UHT treatment in other countries. The Austrian Food Codex provides guidance that ESL milk processing should use a treatment of a few seconds at least at 85°C or a time/temperature combination with the same effect. The Canadian Food Inspection Agency suggests that high heat ESL processing generally occurs above 100°C and that the ESL process should be designed to achieve a minimum lethality value of F0=0.1. The reported Austrian and Canadian processing conditions would likely not inactivate all spore-forming microorganisms.

Conclusion

ESL milk is not a sterile product at purchase and slow-growing microorganisms, spore-formers and post-production contaminants may cause unpredictable spoilage and food safety concerns over time due to the variety of microorganisms present. Therefore, while there are guidance data, specific time/temperature conditions for ESL milk processing need to be optimised for each process to ensure shelf life and safety targets are met.

2 INTRODUCTION

2.1 EXTENDED SHELF LIFE REFRIGERATED MILK

Pasteurisation is a thermal process that has proven to be a key technology for controlling pathogens in raw milk* (Hudson *et al.* 2003), thereby providing significant public health benefits. A limitation of the pasteurisation process is the relatively short shelf life of the milk; typically 7-10 days in Europe (Tetra Pak 2016), 14 days in New Zealand (Alothman *et al.* 2018), and up to 20 days in the US (Koutchma and Barnes 2013). There is also a need to maintain a cold chain from production to consumption at 4-8°C (Tetra Pak 2016), which is problematic in developing countries that constitute the majority of the global population. These properties of pasteurised milk led to the development of Ultra High Temperature (UHT) treated milk, a shelf-stable product with a long shelf life (6 months or more) which is popular in many developing countries (as no cold chain is required) and developed countries (due to its convenience).

However, some consumers dislike the “cooked” flavour of UHT-treated milk and so further technologies have been developed that utilise thermal treatment temperatures lower than UHT (~135-150°C for 1-10 s), but greater than that of pasteurisation (~72°C for 15 s). These temperatures produce milk that, when the cold chain is maintained, has an extended shelf life (ESL) of 30+ days, and has sensory and nutritional qualities closer to that of pasteurised milk. ESL milk can be produced by a variety of technologies with or without thermal treatment. Hence, ESL milk is commonly loosely defined as ‘milk with an extended shelf life beyond that of pasteurised milk’.

The production of ESL milk does not result in a commercially sterile product and has a long shelf life. Therefore, microbial safety aspects of this food need to be considered throughout the chain from the raw product until end of shelf life. Of particular concern is the potential presence of thermotolerant pathogenic spore-forming organisms such as *Bacillus* and *Clostridium* species. In addition to increased shelf life and associated opportunities for wider geographical distribution, the production of ESL milk also provides milk processors with opportunities to add value by addition of sweeteners, flavours, enzymes or other supplements, and to alter the fat content. However, as with other milk products, these additions or compositional changes may alter the food safety considerations for ESL milk products.

* Unless otherwise stated, all references in this report to milk refer to bovine milk only.

2.2 GLOBAL VIEW OF ESL PRODUCTION AND CONSUMPTION

Publicly available data on ESL milk production and consumption are relatively scarce. The most mature market in Europe is Germany, where ESL milk was first introduced in 1990 (Grabowski et al. 2013a). Since this time, ESL products have largely replaced pasteurised milk due to the growing demand for fluid milk products with a prolonged shelf life, with 2011 data indicating a market share of 20– 25% for ESL milk, 70% UHT milk and 5-10% pasteurised milk (Lorenzen et al. 2011). The most commonly used technique to produce ESL milk in Germany is high heat treatment (~123-127°C for 1-3s), followed by microfiltration (Doll et al. 2017).

ESL milk is also widely consumed in Austria, with an increasing market share of ESL milk consumption from 3.8% in 2003 up to 48.2% in 2015 and a simultaneous decreasing market share of pasteurised milk (Boitz and Mayer 2017).

Data from Tetra Pak (Barnes 2005) indicated that the ESL market share in 2005 was 5% in Italy, 3% in the United Kingdom, and 63% in Japan (non-flavoured milk sales only). The same source also has an indication of ESL milk production in selected countries for 2005 (Figure 1).

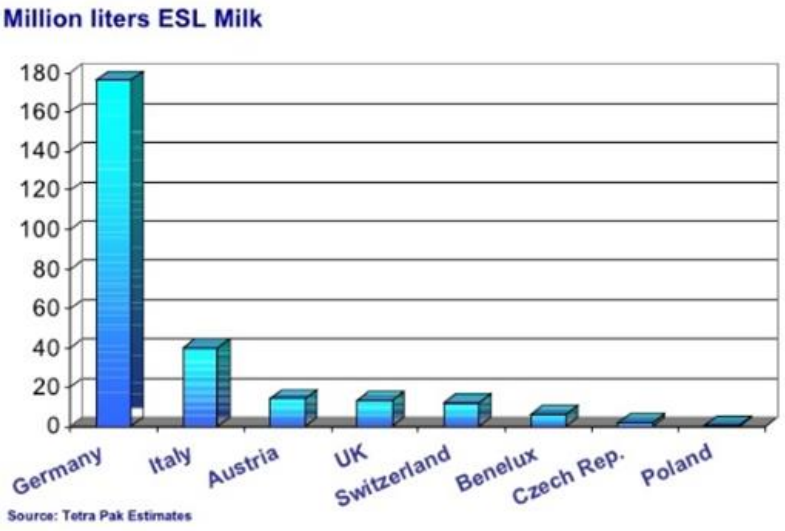

Figure 1. Production of ESL milk in Europe, 2005

2.3 SCOPE OF REVIEW

This report provides a summary of the food safety factors that must be considered for clean-filled, hermetically sealed, ESL refrigerated milk that has been heated to greater than pasteurisation conditions (e.g. 72° for 15 sec), but less than UHT (commercial sterility) conditions.

The report summarizes information from the scientific literature and other resources (e.g. material from government agencies, equipment and packaging manufacturers) that describe:

- The technologies for the production of ESL milk
- Microbial hazards associated with ESL milk
- Reduction of microbial numbers in milk by thermal treatments in the range 90-127°C
- Regulations and standards for ESL milk production

Note that “in container” sterilisation (110-120°C/10-20 min, or 125°C/5 min) used to produce shelf-stable drinking milk is not in the scope of this report.

3 TECHNOLOGIES FOR PRODUCTION OF ESL MILK

Successful processing of ESL milk requires multiple technologies to manage the entire chain, from raw milk production to final delivery to the store shelf and the consumer (Tetra Pak 2016). A summary of the main technological approaches used to achieve this are presented in this section, with raw milk inputs considered separately in Section 4. There is a great diversity of approaches taken to produce ESL products. This is in part due to rapid advancements in equipment and technology, but also due to milk processors adapting existing processing lines used for pasteurised and UHT milk (e.g. by adding bactofugation or hygienic packaging).

3.1 THERMAL AND NON-THERMAL PROCESSING TECHNOLOGIES TO ENSURE MICROBIAL SAFETY AND QUALITY OF ESL MILK

3.1.1 THERMAL PROCESSING

The use of thermal treatment to temperatures above pasteurisation (~72°C / 15 s) but below UHT (~135-150°C / 1-10 s) is one of the most frequently used approaches for the production of ESL milk. Thermal treatment temperatures and times used for ESL milk processing do not result in commercial sterility (Mayr *et al.* 2004a). Therefore, these processes need to be combined with effective hygiene measures to ensure an acceptable shelf life (see section 3.2). Data on the reductions in numbers of bacterial vegetative cells and spores present in raw milk achieved by various thermal time-temperature combinations are discussed in detail in Section 5. In this section, we focus instead on the technology used to reduce numbers of bacterial vegetative and spore cells.

The two main approaches to thermal processing are direct and indirect heating systems (Rysstad and Kolstad 2006). Some of the equipment described in this section can perform both ESL and UHT thermal processing, offering plants greater production flexibility (Tetra Pak 2016, SPX®Flow 2017).

With indirect heating systems, the raw milk flows through hot metal tubes or plates and the milk mass is gradually heated to the desired temperature (e.g. 127°C) where it is held for a few seconds (e.g. 1-3 s). The milk is then gradually cooled to the storage temperature (~4°C) (Buckenhüskes 2015). However, rates of milk heating and cooling in indirect systems are slower than in direct systems. This has the consequence that for the same bactericidal effect, indirect systems cause more chemical changes in milk (including increased cooked flavour) (Deeth 2017).

By contrast, direct heating systems use culinary grade steam to rapidly heat milk by either injection or infusion techniques (Rysstad and Kolstad 2006, Grabowski *et al.* 2013a, Grabowski *et al.* 2013b, Buckenhüskes 2015). The first stage for both steam processes is to preheat the milk to 70-80°C by indirect heating. For steam injection, the steam is then injected in-line into the milk. For steam infusion, the steam and milk are co-introduced into a vessel and the milk is heated as it falls to the bottom of the chamber (SmartBrief Media Services 2013). Both processes then use a vacuum chamber to remove water from the condensed steam and to cool the milk to approximately the same temperature as the preheating (70-80°C), before cooling the milk further to storage temperature (~4°C) (Rysstad and Kolstad 2006, Deeth 2017).

A comparison of typical heating profiles for indirect and direct systems used to produce ESL milk is shown in Figure 2, where it can be seen that a key difference is the shorter treatment time (brief spike on graph) required when using direct heating. It has been speculated that this shorter contact time reduces chemical degradation of the milk, improving sensory quality

compared to indirectly treated milk, however, direct heating requires greater energy and so is more expensive to run (Rysstad and Kolstad 2006).

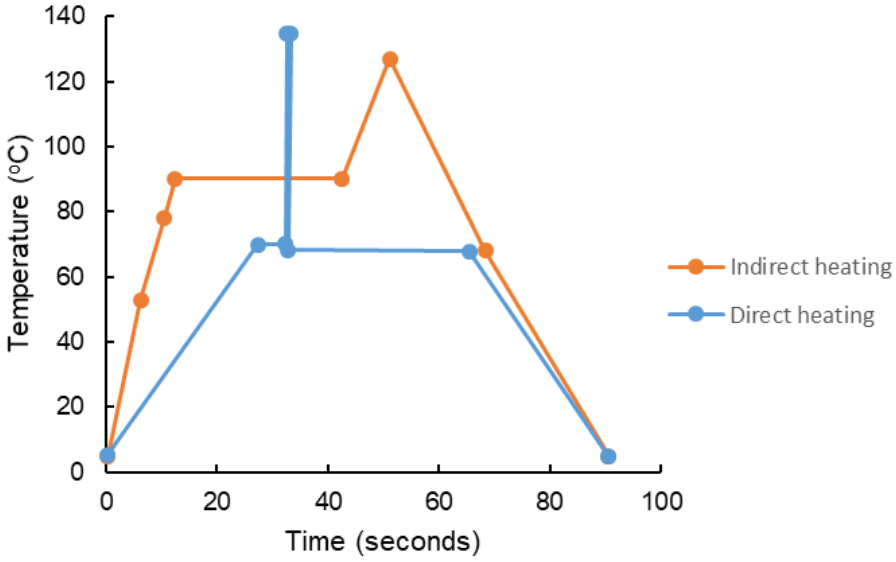

Figure 2. Comparison of typical direct and indirect heating profiles for ESL milk

Figure is adapted from Rysstad and Kolstad (2006)

Whilst there is not a great deal of publicly available information on time-temperature combinations used in commercial practice for the processing of ESL, there are some data from the scientific literature and equipment manufacturers which is summarised in Table 1.

Table 1. Examples of commercial time temperature combinations used for the processing of ESL milk

Country	Heating type	ESL treatment temperature (°C)	ESL treatment time (s)	Reference
Not specified	direct	89-100	not specified	Deeth and Lewis (2017a)
South Africa	not specified	94-100	not specified	Deeth and Lewis (2017a)
US	direct	100-140	4-12	Blake *et al.* (1995)
Germany	direct or indirect	120–127	1-4	Grabowski *et al.* (2013b)
Not specified	not specified	125-140	1-10	IDF (2018)
Germany	direct or indirect	123-127	1-5	Lorenzen *et al.* (2011)
Israel (equipment manufacturer)	indirect	127-130	2-3	MGT Liquid and Process Systems (2013)
Austria	indirect	123-125	2	Boitz and Mayer (2017)
Germany (equipment manufacturer)	indirect	124	~2	http://www.fei-online.com/products/product-news/food-drink-industry/esl-milk-production/index.html
Germany	indirect	125	2	Grabowski *et al.* (2013a)
Germany	indirect	125	2	Kratz (2014)
Germany (pilot plant)	direct	127	5	Brick *et al.* (2017)
Germany	direct	127	~3	Buckenhüskes (2015)
Germany (equipment manufacturer)	direct	127	~3	http://www.fei-online.com/products/product-news/food-drink-industry/esl-milk-production/index.html
Germany	indirect	127	1-3	Buckenhüskes (2015)
Germany	direct (injection)	127	3	Kratz (2014)
Germany	direct (infusion)	127	3	Kratz (2014)
Austria	direct	≤ 127	3	Boitz and Mayer (2017)
Denmark (equipment manufacturer)	direct (infusion)	129	0.5	SPX®Flow (2017)
Canada	direct (infusion)	131-138	2	Henyon (1999)
Finland/Denmark	direct	132	1	Kallioinen and Tossavainein (2008)
US	direct or indirect	138	2	FDA (2017)

3.1.2 COMBINED FILTRATION AND THERMAL PROCESSING

The use of filtration (microfiltration (see 3.1.2.1)) or depth filtration (see 3.1.2.2) in the production of ESL is commonly practised in Europe (Saboyainsta and Maubois 2000, Schmidt *et al.* 2012, Doll *et al.* 2017). In this approach to ESL processing, milk is first centrifugally separated into skimmed milk and cream. The particle size of milk fat globules is similar to microbial cells and spores and so failure to separate these may cause clogging of filters (Rysstad and Kolstad 2006). The skimmed milk portion is then filtered to reduce the counts of microbial vegetative and spore cells, whilst the cream portion (and filter retentate) is subjected to thermal treatment (high-temperature pasteurisation or UHT treatment). The cream and skimmed milk are then homogenised, pasteurised (to eliminate surviving vegetative cells) and cooled prior to packaging (Rysstad and Kolstad 2006, Buckenhüskes 2015).

3.1.2.1 MICROFILTRATION AND THERMAL PROCESSING

When microfiltration is combined with thermal processing, the separated skimmed milk is heated to 50°C and usually flowed across either 1.4 µm or 0.8 µm pore size ceramic filters (Saboyainsta and Maubois 2000, Tetra Pak 2016). The filter size used is based on shelf-life requirements and raw milk input quality. It is reported that 1.4 µm membranes typically achieve a 4 log reduction of thermoduric microorganisms and 0.8 µm membranes a 6 log reduction (Tetra Pak 2016). A review of other filter sizes and their impacts on bacterial removal are described by Fernandez Garcia *et al.* (2013).

Several studies describe the effect of 1.4 µm membrane microfiltration on spores. Hoffman *et al.* (2006) reported a 4-5 log reduction in spores, and Olesen and Jensen (1989) reported 2.3-3.7 log reductions. Malmberg and Holm (1988) demonstrated a 3 log reduction in spores when using 1.4 µm inorganic alumina 1.4 µm filters. Trouve *et al.* noted a 2.25 log reduction in spores with 1.4 µm ceramic filters, although in this instance the membranes may have been acting as depth filters (see 3.1.2.2) rather than screen filters. The work of Holm was later incorporated in a patent for the milk microfiltration process named ‘Bactocatch’ (Holm *et al.* 1989).

In parallel to the microfiltration of the skimmed milk, the filter retentate and separated cream are heat-treated, typically at 90-110 °C for 4-6 s. These are then homogenised with the filtered skimmed milk and pasteurised prior to packaging (Tetra Pak 2016).

An example of the overall effectiveness of this technological approach has been reported by Doll (2017). They studied commercial ESL organic milk production processes and found that the microfiltration (1.4 µm) stage removed on average 2.2 log of psychrotrophic spores. When this process was combined with separation of cream (0.9 log reduction) and pasteurisation (1.2 log reduction) the result was a total 3.7 log reduction of psychrotrophic spores (Doll *et al.* 2017).

A series of experiments by Fernandez Garcia and Rodríguez (2014) explored the effect of different combinations of thermal treatments with microfiltration (Table 2). Whilst these experiments only measured total bacterial counts, it nonetheless provides an indication of the bactericidal effect of these combined technologies.

Table 2. Effect of different heat treatment regimens combined with 1.4 µm filtration on milk total bacterial counts

Heat treatment (combined with 1.4 µm microfiltration)	Treatment temperature (°C)	Log reduction (total bacterial count)
Indirect	75	1.8
Indirect	80	2.0
Indirect	90	2.7
Indirect	115	4.8
Direct	115	4.6
Indirect	120	4.9
Direct	120	6.3
Indirect	125	5.3
Direct	125	6.5
Indirect	130	6.4
Direct	130	9.1

Table modified from Fernandez Garcia and Rodríguez (2014)

3.1.2.2 DEPTH FILTRATION AND THERMAL PROCESSING

The depth filtration method for processing ESL milk begins in a similar manner to that used for microfiltration, whereby the milk is separated into skimmed and cream fractions. The skimmed milk is then passed through a series of polypropylene candle filters with pore sizes of 0.3 µm (pre-filter) and 0.2 µm (main filter) (Buckenhüskes 2015). This technology differs from that of microfiltration, as the microbes are not size-excluded from the pores but rather accumulate within the pores of the filters. A greater than 2 log reduction in total microbial counts is achieved using this filtration approach, with further reductions achieved when the skimmed milk and (ultra) heat-treated cream streams are homogenised and pasteurised (Buckenhüskes 2015). The advantages of depth filtration over microfiltration are that depth filtration is carried out at the separation temperature, no additional pumps are needed due to the low-pressure requirements, and no retentate is produced (http://www.fei-online.com/products/product-news/food-drink-industry/esl-milk-production/index.html).

3.1.3 COMBINED CENTRIFUGATION AND THERMAL PROCESSING

The use of centrifugation, often referred to as bactofugation due to Tetra Pak equipment (Bactofuge™), is another well-established technology for ESL milk processing. The principle is similar to that for microfiltration, as the centrifugation is used to reduce the concentration of spores and vegetative cells. Centrifugation is effective in reducing vegetative microbial and spore numbers as these have a higher density (1.07-1.12 g ml^{-1} and 1.13 g ml^{-1}, respectively) than raw milk (1.01-1.03 g ml^{-1}) and so can be removed by sedimentation (Gésan-Guiziou 2010, Frahm and Meyer 2013).

There are two main types of centrifuges used. One-phase centrifuges have a single outlet for bacteria-reduced milk, while the particles including spores and bacteria are collected as a sludge in the bowl and discharged continuously or in intervals through a port in the bowl. Alternatively, in some types of one-phase centrifuges a continuous portion (~3%) of milk is

recycled into the bowl and the bacteria-enriched portion is ejected periodically (every ~15-20 min) (Gésan-Guiziou 2010).

With two-phase centrifuges, there are two outlets; one for the bacteria-reduced milk and one for the continuous discharge of the centrifugate. In these types of centrifuges, the milk enters the bowl along the central axis and flows over stacks of conical discs where the centrifugal force pushes the bacteria and spores towards the outside of the bowl and the bacteria-reduced milk moves towards the central axis (Gésan-Guiziou 2010, Frahm and Meyer 2013). Both one- and two-phase centrifuges have similar bacteria reduction capabilities but have different characteristics for milk fat and protein separation, which likely guides their usage.

The centrifuge (no matter which type) is placed downstream from the skimmed milk separator and skimmed milk enters the centrifuge at 50-68°C with a typical transit time of less than 1s through the centrifuge. Centrifugation is always followed by pasteurisation of the permeate to kill any remaining vegetative cells (Gésan-Guiziou 2010, Frahm and Meyer 2013, Tetra Pak 2016).

It has been reported that use of a single centrifuge combined with pasteurisation usually achieves 1-2 log reductions in psychrotrophic spore counts ((Rysstad and Kolstad 2006, Tetra Pak 2016, Mugadza and Buys 2018)[1,2]). Reported reductions were 97.4-98.7% for anaerobic spores such as *Clostridium* spp. and 94.1-97.7% for aerobic spores such as *Bacillus* spp. (Te Giffel and van der Horst 2004, Gésan-Guiziou 2010). Similar reductions were also observed for thermoduric vegetative microorganisms using centrifugation technology (Tetra Pak 2016, Mugadza and Buys 2018).

Compared with a single centrifuge, the use of two centrifuges in series is reported to achieve a higher (2-3 log vs. 1-2 log) reduction in spore counts (Tetra Pak 2016). However, in experiments by Torres-Anjel and Hedrick (1971) utilising a single commercial centrifuge, the use of a second centrifugation produced quite variable reductions in spore numbers (an additional 0-48% decrease compared to single centrifugation). Their work also indicated that centrifugation efficacy for reducing spore counts did not appear to be significantly influenced by temperature (71 or 82°C), milk flow rate (1800 or 5400 kg h^{-1}), *Bacillus* species present (*B. cereus, B. subtilis, B. stearothermophilus*), or concentrations of spores in raw milk (% removal unchanged) with one or two centrifugations (Torres-Anjel and Hedrick 1971).

3.1.4 NON-THERMAL PROCESSING

In addition to the thermal treatments, new processing technologies for producing pasteurised and ESL milk have also been developed, but these technologies are not yet in widespread use. They are included in this section to present an overall summation of potential technologies that could be used for ESL processing but are not considered elsewhere in the report.

3.1.4.1 ULTRAVIOLET LIGHT TECHNOLOGY

Ultraviolet (UV) light technologies work by flowing milk over UV-C-producing fluorescent tubes (200-280 nm), which are enclosed in clear transmittable materials. Upon exposure to the UV-C irradiation, DNA damage occurs in microbes that render them non-viable. In these systems, it is important that the flow of milk is turbulent to ensure all microbes have sufficient contact time with the light (Rossitto *et al.* 2012, Cappozzo *et al.* 2015).

The 'SurePure' UV-C system[3] for milk claims reductions of *E. coli* and coliforms of >5 log CFU KJ^{-1} UVC exposure, and reductions of 2-3 log CFU KJ^{-1} UVC exposure for *Klebsiella*,

[1] https://www.gea.com/en/products/bacterial-clarifiers-milk-whey.jsp

[2] https://www.agriculture.ny.gov/DI/dpo20180710/Dr%20Moraru%20Cornell%20University%20Project%20Reports.pdf

[3] http://surepure.net/sure_pure_applications_dairy_general.html

Enterococcus and psychrotrophic bacteria. *Bacillus sporothermodurans* reductions were also measured at up to 2 log CFU J L^{-1} UVC exposure.

Studies by Crook *et al.* (2015) and Rossitto *et al.* (2012) confirm up to 5 log reductions in pathogenic and non-pathogenic microflora can be achieved in milk using UV treatment systems. Generally, more energy is required to inactivate spore-forming microorganisms compared with non-spore-forming microorganisms. For instance, the decimal reduction value (D-value) was 350 J L^{-1} milk for *Listeria monocytogenes* and 1250 J L^{-1} milk for *B. cereus* (Rossitto *et al.* 2012).

When UV treatment is used in conjunction with pasteurisation, the shelf life of milk can be increased by at least 30% (Koutchma and Barnes 2013). The technology has been used in the United Kingdom (UK) to reduce post-pasteurisation contamination, extending milk shelf life from 12 days to 21 days (Koutchma and Barnes 2013).

In addition to the static UV treatment technology described here, several researchers have described the use of pulsed UV light systems, but these appear less developed. The use of pulsed power sources has the potential to deliver greater energy than conventional power sources, thus potentially producing a greater germicidal effect. Smith *et al.* (2002) achieved a 2 log reduction of *Serratia marcescens* in raw milk with a pulsed UV laser and Innocente *et al.* (2014) reported a 3.2 log reduction of total bacterial counts in raw milk using a pulsed-light xenon lamp.

3.1.4.2 PULSED ELECTRIC FIELDS

Pulsed electric field (PEF) technology was originally developed in the 1960s for use on liquid foods such as milk (Kempkes *et al.* 2016). However, a number of factors, including a lack of standardised equipment and operating parameters, have held the technology back from widespread commercial use until recently (discussed in Kempkes *et al.* (2016)). In PEF, the milk is pumped through a small chamber where a high voltage (35-50 kV cm^{-1}) is applied for a short time (0.01-20 µs). This high voltage kills microorganisms by disrupting their cell membranes (Gaudreau *et al.* 2006).

McAuley *et al.* (2016) have reported an extension in milk shelf life of 3-4 days when comparing a 63°C, 15 s thermal treatment to a PEF treatment (63°C, 22 µs, 35 kV cm^{-1}) when milk is stored at 4°C, and 3 days shelf life extension when milk is stored at 8°C. However, there was no significant difference in shelf life compared with 72°C, 15 s pasteurised milk. Similarly, work by Sepulveda *et al.* (2009) demonstrated an equivalent shelf life to standard pasteurisation when testing PEF treatments (5 pulses at 35 kV cm^{-1} for 2.3 µs) at 65°C on raw milk.

One aspect currently limiting the utility of PEF technology to extend the shelf life of milk is its relatively limited efficacy against spores. The use of PEF treatment (30-40 kV cm^{-1}, 2.5 µs) alone was found to have no significant effect on *B. cereus* spores (Bermúdez-Aguirre *et al.* 2012). Whilst a combination of mild heating (40°C) with PEF actually increased spore germination and vegetative cell growth (Bermúdez-Aguirre *et al.* 2012). At high spore concentrations (10^8 ml^{-1}), up to 6.6 log reductions were noted for milk that was heated to 65°C in combination with PEF. However, at more realistic lower concentrations of spores (10^4 ml^{-1}) the reduction was around 2.5 log. Addition of the antimicrobial nisin (50 IU ml^{-1}) further enhanced spore reductions by ~1 log (Bermúdez-Aguirre *et al.* 2012).

3.1.4.3 HIGH PRESSURE PROCESSING

The use of high-pressure processing (HPP) with foods, including for fluid milk, is the subject of a recent comprehensive review (Horn et al. 2018), so is described here only briefly. HPP is a current commercial technology where high pressures (100-600 MPa) are used to inactivate microorganisms in liquid or solid foods and can increase milk shelf life (Evelyn and Silva 2015). Most pathogens routinely found in milk are susceptible to HPP inactivation, though the

treatment times and pressure required vary (see Horn *et al.* (2018) for more detail). However, inactivation of spores in milk cannot be reliably achieved using HPP alone (Sarker *et al.* 2015).

Combinations of HPP with a mild thermal treatment (45-100°C) have been reported to be effective against spores in milk. Experiments undertaken in New Zealand (Evelyn and Silva 2015) using spores of *B. cereus* in skimmed milk showed that at a constant temperature (70°C) inactivation of spores increased in a pressure-dependent manner (from 200-600 MPa). Furthermore, at constant pressure (600 MPa) inactivation increased in a temperature-dependent manner (from 38°-70°C). The Weibull distribution was found to be the best fit to describe inactivation of psychrotrophic spores via the pressure-thermal process. Maximum reductions of *B. cereus* spores in skimmed milk were obtained using 600 MPa pressure at 70°C. In these conditions, a 4 log reduction in spores was achieved after 20 min treatment and a 5 log reduction after 40 min treatment (Evelyn and Silva 2015).

The combination of HPP with pasteurisation has shown promise as a tool to increase the shelf life of donkey milk in Italy (Giacometti *et al.* 2016). Milk was pasteurised for 30 min at 65°C and then pouches of milk were HPP treated for 3 min at 600 MPa at 4-6°C. A shelf life extension of up to 30 days was observed by combining these treatments.

3.2 HYGIENE CONSIDERATIONS FOR ESL MILK PROCESSING

ESL milk processing does not destroy all microorganisms present in raw milk and so the safety and shelf life of the product will be determined by the quality of the raw milk inputs, the effectiveness of bacterial reduction technologies (see Section 3.1) and by control of post-processing microbial contaminants (Schmidt *et al.* 2012, Doll *et al.* 2017).

The raw milk quality used for ESL processing is recommended to be ≤10^5 CFU ml^{-1} total viable bacteria to ensure the effectiveness of microbial reduction procedures (Tetra Pak 2016, Deeth 2017). Levels of viable bacteria in New Zealand milk are often required to be lower than this (e.g. Fonterra requires ≤5x10^4 CFU ml^{-1} APC equivalents from its suppliers, with high-quality milk suppliers generally producing <3x10^3 CFU ml^{-1} (New Zealand Farm Source Fonterra (2017)). Microorganisms present in processed ESL milk will include spore-forming bacteria whose spores are not destroyed by the thermal (or equivalent) process, as well as spore-forming and non-spore-forming bacteria contaminating the milk after the thermal process step (Deeth 2017). The types of microorganisms known to be responsible for post-processing contamination of ESL milk are described in detail in Section 4.

A diagram depicting an ESL milk processing line and highlighting potential areas of post-thermal processing contamination is shown in Figure 3. ESL milk processing line highlighting potential post-thermal treatment contamination pointsFigure 3. For reference, a short video of a working ESL milk packaging line is also available on the SMF Germany website[4]. Levels of hygiene for milk processing can be categorised into three basic designs: high hygiene, ultra-high hygiene and aseptic hygiene (Tetra Pak 2016). Each of these categories has different requirements for tank design, ventilation, valve design and cleaning (Table 3). For ESL milk, an ultra-high hygiene design is frequently implemented, with aseptic packaging not usually used (Deeth 2017). In practice, the whole ESL processing system, including hygienic design, should be considered and optimized to achieve the targeted shelf-life for the ESL milk product (Buckenhüskes 2015).

[4] http://www.smfgmbh.com/en/offer/turn-key/milk-bottling-line

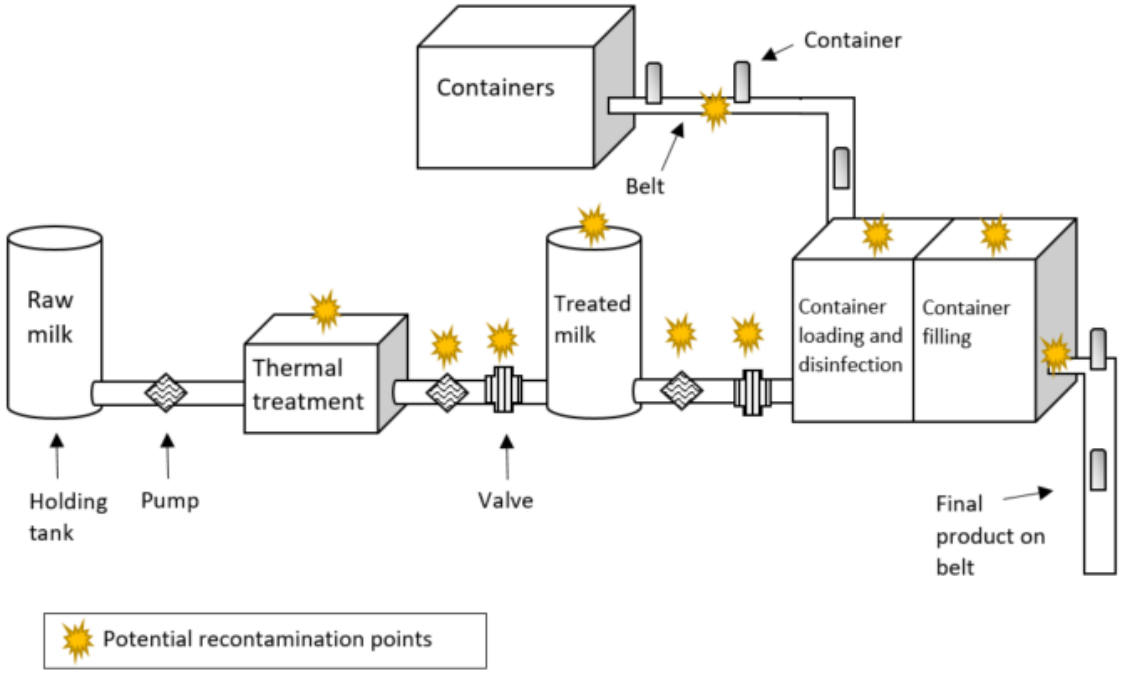

Figure 3. ESL milk processing line highlighting potential post-thermal treatment contamination points

Table 3. Levels of hygienic design for ESL milk production

Hygienic design	Targeted shelf life (days)	Tank configuration	Tank ventilation	Valve design	Cleaning in place regimen
High	10-15	Closed. Open ventilation during filling, emptying and cleaning.	Filtered air (50 kPa overpressure)	Mix proof	Hot water ≥95°C
Ultra-high	15-24	Closed. If the tank is not designed for vacuum, careful control of the tanks during cooling must be ensured.	HEPA filter (200 kPa overpressure)	Mix proof / spiral clean	Superheated water >100°C
Aseptic	≥24	Closed, aseptic, designed for vacuum.	HEPA filter (200 kPa overpressure)	Aseptic	Steam 100-150 kPa

Table adapted from Tetra Pak (2016)

In packaging materials, contamination from Gram-positive microorganisms is reported to be the most problematic (Rysstad and Kolstad 2006). Choice of packaging material for ESL milk can be driven by consideration of multiple factors including unit cost, distribution and marketing requirements, and fit into the production process (Rysstad and Kolstad 2006). Common packaging formats include PET and HDPE plastic bottles[5], laminated fibre cartons[6] and plastic pouches[7]. No data could be found on the comparative microbiological safety profiles of these packaging types for ESL milk.

Packaging containers may be shipped into the plant complete or assembled in line by blow moulding (plastic bottles) or folding (fibre-based cartons). Preformed PET bottles can be delivered to the plant presterilised[5] eliminating the need for post-moulding treatments. The two most widespread methods for inline decontamination of packaging materials are either hydrogen peroxide (~35%) combined with heat, or hydrogen peroxide (~1%) combined with UV-C light and blown HEPA filtered air (Rysstad and Kolstad 2006, Tetra Pak 2016). The latter method is reported to result in a ~5 log reduction in *B. subtilis* spores (Rysstad and Kolstad 2006), but each system will require independent validation. Finally, the packaging must be effectively sealed and have adequate barrier properties to prevent light and oxygen diffusion, in order to maintain the sensory properties of the milk during its shelf life (Tetra Pak 2016).

The hygienic design of the filler and packaging machine will have a high correlation with the shelf life of ESL milk. Several studies have noted an increase in the numbers of spore-forming microorganisms following packaging. With the key source of contamination identified as the packaging filler nozzles where organisms such as psychrotrophic *Bacillus* and *Paenibacillus* species can contaminate ESL milk and proliferate during low-temperature storage of the packaged product (Rysstad and Kolstad 2006, Mugadza 2015, Khoza 2016, Mugadza and Buys 2018, Mugadza *et al.* 2018). Gram-negative spoilage microorganisms in milk processing are also known to be present in the air in and around filling machines (Rysstad and Kolstad 2006). To reduce the risks of post-processing contamination of ESL milk, filler machines must be cleaned in place rather than dismantled, and the filling operation performed under positive-pressure HEPA-filtered air. However, despite these measures it is reported that the filling operation is still very sensitive to environmental factors and must be carefully monitored, and preferably undertaken at a milk temperature of 4°C or lower (Tetra Pak 2016).

Some technical engineering aspects of hygienic design for high-temperature thermal processing of ESL milk can be found in documentation[8] prepared by the Canadian Food Inspection Agency (CFIA 2019).

3.3 EXPECTED SHELF LIFE OF ESL MILK PRODUCED BY THERMAL PROCESSES

The shelf life of food is generally defined as the period of time for which it remains safe and suitable for consumption, provided the food has been stored correctly (MPI 2016). The shelf life of foods is usually limited by microbiological, physical, biochemical and chemical stability; the most important of which is the microbiological effect. Non-microbiological defects limiting the shelf life of ESL milk are reported to be rare (Tetra Pak 2016).

For ESL milk, thermoduric psychrotrophic microorganisms and psychrotrophic spores originating from raw milk, and post-processing microbial contaminants, are the main contributors to determining shelf life (Schmidt *et al.* 2012, Doll *et al.* 2017). Concentrations of these microorganisms and spores in ESL milk products will be affected by:

[5] https://www.dairyfoods.com/events/836-esl-filling-technology-the-right-solution-for-dairy-pet-and-hdpe-bottle-filling
[6] https://www.tetrapak.com/packaging/materials
[7] https://www.thimonnier.com/procede/pillow-pouch/2/
[8] http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/preventive-controls-food-businesses/dairy-products/hhst-esl/eng/1539632249860/1539711207517

(1) the quality of raw milk inputs

(2) the efficiency of microbial reduction technologies

(3) post-processing hygienic design

(4) the maintenance of the cold chain during distribution and domestic storage

Due to these factors, the range of technologies used, and the lack of specific standards or regulatory guidelines relating to ESL milk in most countries (see section 6), it is challenging to provide a consensus on the expected shelf life of ESL milk. However, there are equipment manufacturer guidelines, notably from Elopak and Tetra Pak, which outline the expected shelf life of ESL milk processed using their equipment. A summary of this information is in Table 4, which demonstrates that lower range thermal treatments combined with additional technologies to reduce microbial numbers can be effective in increasing the shelf life beyond that of pasteurisation alone. Higher range thermal treatments can extend ESL milk shelf life beyond a month if stored according to the manufacturer's guidance (≤ 8°C).

Table 4. Predicted shelf life of common ESL milk processing technologies

Thermal milk treatment process	Estimated[1] shelf life at 6°C (days)	Estimated[2] shelf life at 8°C (days)	Estimated[1] shelf life at 10°C (days)
Pasteurisation alone (baseline)	10-12	7-10	3-4
Pasteurisation with single centrifugation	14	10-12	4-5
Pasteurisation with double centrifugation	-	12-15	-
Pasteurisation with microfiltration (1.4 µm)	30	18-21	6-7
Pasteurisation with microfiltration (0.8 µm)	-	27-30	-
High thermal ESL treatment (127°C / 2 s, 130°C / 1 s, 135°C / 0.5 s)	>45	>30	≤ 45

Adapted from [1]Elopak (Rysstad and Kolstad 2006) and [2]Tetra Pak (Tetra Pak 2016).
Dash indicates no data available.

Pilot plant experiments by Fernández García and Riera Rodríguez (2014) also provide insights into ESL milk shelf life for a range of thermal and thermal-microfiltration processing techniques (Figure 4 & Figure 5). With lower range heat treatments (80°C and 90°C), the microbial quality was unacceptable at 10 days. However, when these treatments were combined with microfiltration (1.4 µm) the shelf life was extended to 31 days. For higher heat treatments (115°C-125°C), the shelf life was more than 35 days. The use of microfiltration with these higher thermal treatments further extended shelf life by 5-14 days. However, this comparison should be treated with caution as the high heat-treated samples were stored at 20-22°C and the low heat-treated samples at 4-6°C, and so the differences observed may reflect the growth of different groups of microorganisms (i.e. psychrotrophic vs. mesophilic).

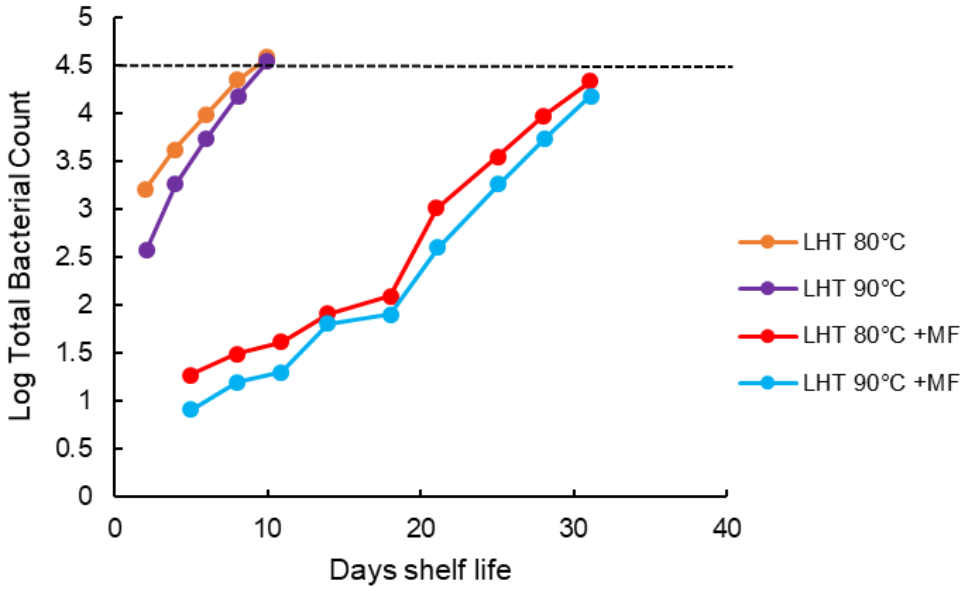

Figure 4. Effect of low heat treatment with or without microfiltration on ESL milk shelf life

Adapted from Fernández García and Riera Rodríguez (Fernández García and Riera Rodríguez 2014). Treatment time 15 s, aseptically packed and stored at 4-6°C, LHT (low heat treatment). MF (microfiltration – 1.4 µm), dotted line is microbial acceptability limit for study.

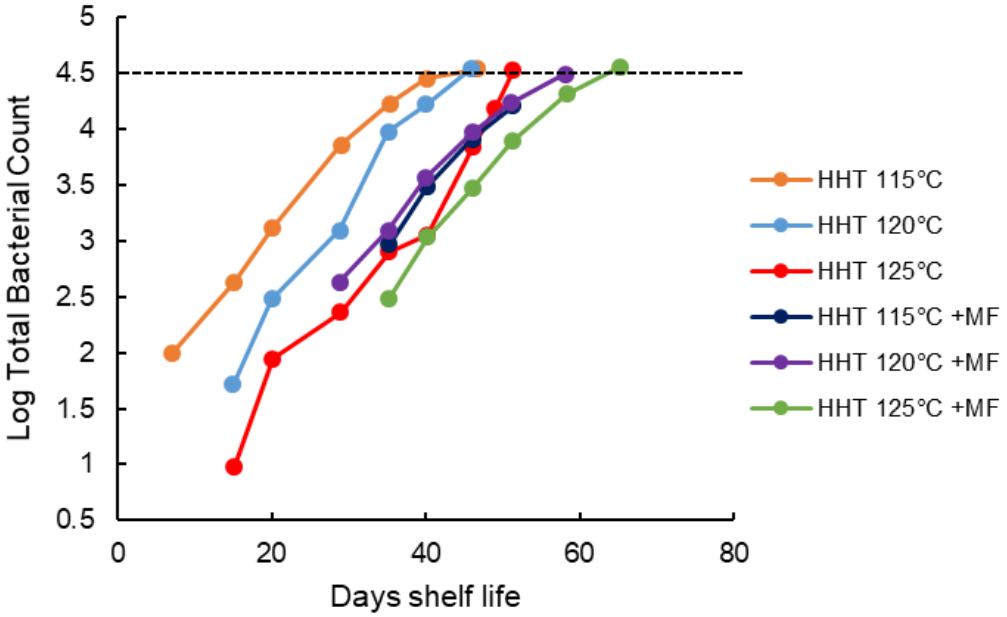

Figure 5. Effect of high heat treatment with or without microfiltration on ESL milk shelf life

Adapted from Fernández García and Riera Rodríguez (Fernández García and Riera Rodríguez 2014). Treatment time 2 s, aseptically packed and stored at 20-22°C, HHT (high heat treatment). MF (microfiltration – 1.4 µm), dotted line is microbial acceptability limit for study.

Further data relating to the practical commercial shelf life of ESL milk can be found in the scientific literature and are presented in Table 5. These data reveal that commercially produced ESL milk (mostly in Europe) has a typical shelf life of three to four weeks when refrigerated (≤ 8°C). This is generally less than the theoretical maximum shelf life calculated for the technology (see Table 4 & Figure 5), which is most likely due to processors adding a quality buffer to ensure a consistent consumer experience.

Table 5. Reports of ESL milk shelf life limits from literature

Recommended ESL shelf life limit (days)	ESL treatment process	Country	Reference
16-27	Direct heated, indirect heated, or microfiltered / heat treated	Germany	Lorenzen *et al.* (2011)
21	Microfiltration / Pasteurisation	Austria	Boitz and Mayer (2017)
21	Microfiltration / Pasteurisation	South Africa	Mugadza and Buys (2018)
22	Microfiltration / UHT	Germany	Schmidt *et al.* (2012)
24	Microfiltration / Pasteurisation	Germany	Doll *et al.* (2017)
27	Thermal (123 - 127°C)	Austria	Boitz and Mayer (2017)

Limited regulatory guidance relating to the shelf life of ESL milk is also available (see section 6). Most notably, the Austrian Food Book recommends that ESL milk labelled "longer fresh" has a best before date of 25-27 days post heat treatment and ESL milk labelled "long shelf life" has a best before date of fewer than 45 days post heat treatment[9].

Mayr *et al.* (2004a) have examined the shelf life of more than 250 1L high thermal treated (127°C, 5s) ESL milk packs beyond the recommended expiry date in the German market. The packs were all from the same dairy processor, purchased on different days over 6 weeks at a local supermarket, and on the day of purchase had an expiry date of 11 ± 2 days remaining. All packs purchased on the same day had the same expiry date. Packs were examined at the expiry date and then twice weekly until classified as spoiled (total plate count $>10^6$ CFU ml^{-1}). The results (Figure 6) show that a low number of ESL milk packs were spoiled on the expiry date (day 0) indicating shelf life was overestimated. Few packs were spoiled up to day 11, many packs did not spoil for several more weeks, and some packs (number not stated in article) were not spoiled at the completion of the study (40 days post their expiry date). ESL milk packs stored at 8°C may potentially have delayed spoilage compared to those stored at 10°C, but this was not analysed in the study. The results show that the ESL milk was not a sterile product at purchase and that slow-growing microorganisms, spore-formers and post-production contaminants, caused unpredictable spoilage over time due to the large variety of microorganisms present (Mayr *et al.* 2004a).

[9] http://www.lebensmittelbuch.at/milch-und-milchprodukte/konsummilch-und-rahm/beschreibung/konsummilch-waermebehandelte-konsummilch/

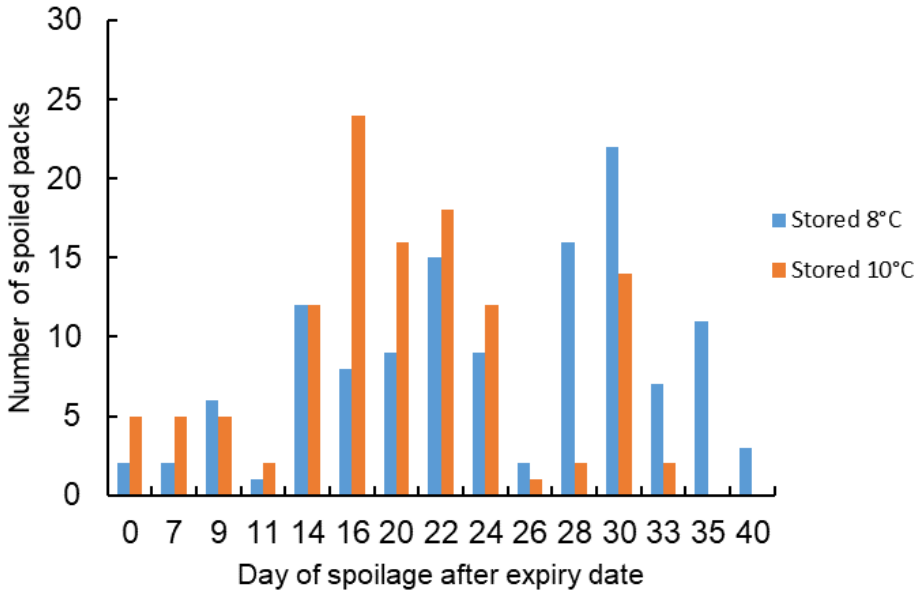

Figure 6. Spoilage of German ESL milk after expiry date

Figure adapted from Mayr *et al.* (2004a)

Finally, as with all pasteurised and UHT milks, once the sealed package of ESL milk is opened the useable shelf life of the product with refrigeration (≤ 8°C) is 3-4 days (Bayerisches Staatsministerium für Umwelt und Verbraucherschutz 2017).

Key Points

- The most frequently applied parameters for commercially producing ESL milk by heat alone are in the range 123-127°C for 1-3s.
- High thermal treatments (e.g. 123-127°C for 1-3s) produce ESL milk with a longer shelf life than pasteurisation treatments combined with either microfiltration or centrifugation.
- Pilot plant experiments indicate that intermediate temperature (80°-120°C) treatment combined with microfiltration may produce milk with a shelf life close to that of high thermal treatment (e.g. 123-127°C for 1-3s).
- Manufacturers recommended shelf life of ESL milk is in the range of 21-27 days with refrigeration.
- The time until spoilage occurs for ESL milk batches may be unpredictable due to the diversity of contaminating microorganisms.
- Non-thermal treatments, such as UV light, PEF and HPP, are potential alternative technologies to extend milk shelf life but these are not currently in widespread commercial use.
- ESL milk is not usually packaged aseptically, so vigilance is required in filling and packaging operations to achieve an acceptable shelf life.

4 MICROBIAL HAZARDS ASSOCIATED WITH ESL MILK

4.1 OCCURRENCE OF MICROBIAL HAZARDS FOR HUMANS IN RAW MILK

Significant microbial hazards for humans likely to occur in raw milk, including those in New Zealand, have been reviewed previously (Hudson *et al.* 2003, MPI 2014b, Cressey *et al.* 2016). As such, only brief details are described here, with an emphasis on those pathogens likely to survive thermal treatments in excess of pasteurisation, but less than UHT. Outlined in Table 6 are microbial hazards likely to occur in raw milk and, where data are available, the prevalence of these in New Zealand raw milk.

Table 6. Significant microbial hazards in raw milk

Microbiological hazard	**Prevalence in New Zealand raw milk surveys* (% samples tested)**	**Reference**	**Thermoduric (Hudson *et al.* 2003)**
Bacillus spp.	4% (*B. cereus* 0.07%)	Cressey *et al.* (2016)	Yes, spore-former
Brucella spp.	Organism not present in New Zealand	MPI (2014a)	No
Campylobacter	0.34-0.58%	MPI (2014a)	No
Clostridium botulinum	No data available	-	Yes, spore-former
Coxiella burnetii	Organism not present in New Zealand	MPI (2014a)	No
Cryptosporidium	No data available	-	No
Escherichia coli O157:H7	0.00-0.28%	MPI (2014a)	No
Listeria monocytogenes	0.68-4.09%	MPI (2014a)	No
Pathogenic streptococci	48% (blood aesculin-positive data only)	Howard (2006)	No
Salmonella spp.	0.00%	MPI (2014a)	No
Staphylococcus aureus	74.1%	MPI (2014a)	No
Yersinia enterocolitica	0.0-1.4%	McIntyre *et al.* (2009)	No

Table adapted from Hudson *et al.* 2003
*679 samples tested in two independent surveys

4.2 OCCURRENCE OF SPORE-FORMING MICROORGANISMS IN NEW ZEALAND DAIRY FARMING SYSTEMS

Dairy farm effluent is frequently used in irrigation of pasture on New Zealand dairy farms (Gupta and Brightwell 2017). A study of spore-forming bacteria in New Zealand dairy farm effluent has demonstrated the presence of *Bacillus* and *Clostridium* species (Gupta and Brightwell 2017). *Bacillus* spp. (19 isolates) and *Paenibacillus* spp. (5 isolates) were isolated by aerobic culture. The most commonly isolated *Bacillus* species, determined by 16S rRNA sequence, were *B. licheniformis*, *B. altitudinis, B. pumilus*, *B. megaterium* and *B. cereus*. The types of *Bacillus* species present are similar to that reported in overseas farming systems, where *B. cereus* is the predominant psychrotrophic species (Sutherland and Murdoch 1994, Lukasova *et al.* 2001, Gupta and Brightwell 2017).

Anaerobic culture of the dairy farm effluent resulted in 17 Clostridia sequence types being isolated, with the most commonly isolated types being *C. bifermentans, C. perfringens*, *C. botulinum* Group I and *C. botulinum* Group II. Of the isolates which clustered to *C. botulinum* Group I, 4 were most similar to *C. sporogenes*, 1 to *C. sporogenes* subsp. *tusciae* and 9 to *C. botulinum* B1 (strain Okra). For the isolates which clustered to *C. botulinum* Group II, 5 were most similar to *C. butyricum* E4, 2 to *C. butyricum* E3, 2 to *C. botulinum* E3 (strain Alaska) and 5 to *C. botulinum* B (strain Eklund) and *C. botulinum* E1 (strain Beluga) (Gupta and Brightwell 2017). Further identification of these *C. botulinum*-like isolates, including the presence or absence of botulinum neurotoxin toxin genes, was not reported. *Clostridium* species are ubiquitous in the environment and have been reported on dairy farms and in raw milk overseas including in Australia and Italy (Huss 1980, Feligini *et al.* 2014, McAuley *et al.* 2014).

A survey of sulphite-reducing *Clostridium* spp. (SRC) in New Zealand bulk raw tank milk revealed a very low level (prevalence: vegetative cells 0.67% (<10 CFU ml^{-1}), spores 3.3% (=1 spore ml^{-1})). SRC are abundant in the farm environment, but most dairy associated *Clostridium* species are not pathogenic for humans as only a few strains carry toxin genes (MPI 2014b). SRC are used by the dairy industry and trade bodies in some countries as hygiene indicators, however, it has been concluded that the levels of SRC in New Zealand are too low to use for this purpose (MPI 2014b). Work by the International Commission on Microbiological Specifications for Foods (ICMSF 2014) has determined that there is no direct mathematical correlation between levels of spores of SRC and those of *C. botulinum.*

4.3 OCCURRENCE OF MICROORGANISMS IN ESL MILK

There are a number of studies that describe the microflora present in ESL milk produced by pasteurisation with microfiltration (e.g. Schmidt *et al.* (2012), Doll *et al.* (2017)) or bactofugation (e.g. Mugadza and Buys (2017), Mugadza and Buys (2018), Mugadza *et al.* (2018)), but these are considered out of scope and are not reported here, except where they are compared to thermal processing alone.

The presence of aerobic spore-forming microorganisms in ESL milk has been tested after thermal treatment (127°C, 5s), but before packaging, at a German dairy plant (Mayr *et al.* 2004b). Samples of ESL milk were taken from the plant and incubated at 10°C, 30°C and 60°C. Samples incubated at 10°C showed no detectable growth of spore-formers (by most probable number (MPN) tests) after 23 weeks, but the transfer of these samples to 30°C induced the growth of spore-formers within 10 days. For samples incubated at 30°C for 5 weeks, 13-130 MPN L^{-1} were detected. For samples incubated at 60°C for 5 weeks, 1.7-5 MPN L^{-1} were reported.

Microorganisms were isolated from MPN tests undertaken on ESL milk samples incubated at 30°C, with all 645 isolates confirmed as spore-formers by microscopy. The majority of isolates (90%) could be further identified by FT-IR spectroscopy and *B. licheniformis* was the predominant species (73% of isolates), followed by *B. subtilis* (5%), *B. cereus* (4%) and *B. brevis* (3%). Other *Bacillus* spp., *Paenibacillus* spp. and *Aneurinibacillus* spp. comprised the

remainder of those isolates identified. Of 320 isolates tested for cold tolerance (growth at ≤21°C), ~12% could grow at 11°C and further assays demonstrated 50 of 84 isolates tested could grow at 8°C. *B. cereus* was the most cold-tolerant species and *B. licheniformis* the least tolerant (Mayr *et al.* 2004b).

ESL milks (directly heated / indirectly heated/microfiltered) produced by German dairies were tested at the end of the declared shelf life (17-26 days stored at 8°C) and compared with pasteurised and UHT milks (Lorenzen *et al.* 2011). Total viable counts (TVC) performed on directly heated ESL milk did not show growth at 30°C or 6.5°C in six out of eight samples, however growth occurred in all samples (n = 6) of microfiltered ESL milk (but not in all cartons from each sample - 5 cartons per composite sample tested). Statistical analysis (ANOVA, $P<0.01$) showed that TVC in directly heated and indirectly heated ESL milk were significantly lower than in pasteurised milk samples. There was no significant difference in counts between pasteurised and microfiltered ESL milk. *Enterococci*, *Enterobacteriaceae* and *Listeria* spp. were not found in any milk samples. *Bacillus* spp. were detected in most cartons of the pasteurised milk ($1.8x10^1$-$2.2x10^5$ CFU ml^{-1}), but were not observed in any cartons of ESL milk.

German high heat-treated (127°C, 5s) retail ESL milk at the day of purchase (11 ± 2 days before expiry date) and during storage at 8-10°C (up to 40 days post expiry date) was investigated to determine numbers and types of microflora present (Mayr *et al.* 2004a). MPN counts of total viable bacteria at the day of purchase showed only low numbers (~2-10 MPN L^{-1}) when the milk was incubated at 8°C or 10°C; while incubation of the milk at 30°C resulted in approximately 1log greater MPN counts. Representative colonies derived from MPN-positive cultures were also tested by microscopy to determine total spore counts (TSC). TSC were generally equal to, or less than, those recorded for viable bacteria when incubated at 8°C or 10°C, and less than viable bacterial counts at 30°C incubation (a maximum of 31 spores L^{-1} reported).

These ESL milk samples (Mayr *et al.* 2004a) were also analysed for microorganisms by microscopy and Fourier-transform infrared spectroscopy (Gram-positive samples only) when spoilage occurred (0-40 days post-expiry date). Of those packs spoiled (191 total), 76 contained aerobic spore-formers, 31 had Gram-negative bacteria and 78 had Gram-positive non-spore-formers; some packs contained multiple species. Gram-positive species identified by FT-IR included *Rhodococcus* (in 11 packs), *Anguinibacter* (7 packs), *Arthrobacter* (4 packs), *Microbacterium* (4 packs), *Enterococcus* (23 packs), *Staphylococcus* (20 packs) and *Micrococcus* (3 packs). Clearly, the presence of non-spore-forming microorganisms in the ESL product indicates that these are a result of post-thermal treatment recontamination. The authors conclude that “non-sterile filling and extended shelf life of fluid milk products may be incompatible with food safety” and that there is a “need for a fast method to determine the presence of slow-growing, mainly Gram-positive recontaminants”.

Another study of German retail ESL milk produced by thermal processing (125–127°C, 2-4 s) revealed only very low counts (1-2 CFU ml^{-1} in 2 of 5 packs) of total bacteria at the start of shelf life and no significant difference in counts at the end of shelf life (12-40 days) (Grabowski *et al.* 2013b). No *Enterobacteriaceae* were detected in any ESL milk packs.

Key Points

- In common with other countries, microbial hazards are present in the New Zealand raw milk supply.
- The majority of microbial hazards likely to be present in New Zealand raw milk are not thermoduric.
- *Bacillus* species have been detected in New Zealand raw milk. Some strains of *Bacillus* are hazardous and *Bacillus* spores can survive ESL milk production processes.
- The levels of sulphite-reducing *Clostridium* spores in New Zealand raw milk are very low.
- Isolates with genetic similarity to *Bacillus* and *Clostridium* species have been found in New Zealand dairy farm effluent.
- Analysis of overseas retail ESL milk produced by thermal treatments (125-127°C, 2-5s) shows that *Bacillus* species are the main thermoduric contaminant, including *B. cereus,* which is a foodborne hazard.
- The majority of *Bacillus* strains isolated from overseas retail ESL milk did not grow when the temperature of the package was maintained at refrigeration temperatures (≤8°C).
- *Bacillus* and *Clostridium* species are potentially thermoduric spore-forming microorganisms that should be considered in risk analysis before implementing thermal ESL processing.
- The majority of recontaminants detected in overseas retail thermally treated ESL milk were slow-growing non-spore-forming Gram-positive isolates, with some Gram-negative strains also detected, indicating post-thermal processing contamination.
- To reduce post-processing contamination, overseas researchers have recommended that the production of ESL milk should not be undertaken using non-sterile filling.

5 REDUCTION OF MICROBIAL NUMBERS IN MILK BY THERMAL TREATMENTS IN RANGE 90-127°C

5.1 MEASURES OF MICROBIAL REDUCTION

Thermal treatment is used to inactivate microorganisms to extend the shelf life of food products and reduce the risk of pathogen exposure to consumers. The effects of time and temperature on microorganisms can be tested empirically to give D-values and z-values and produce thermal inactivation curves (van Asselt and Zwietering 2006). It is assumed that combinations of time and temperature will result in a log-linear inactivation of any given microorganism (van Asselt and Zwietering 2006).

5.1.1 D-VALUE

The D-value is used in relation to the effectiveness of any thermal process, particularly in the food industry. The decimal reduction time/dose is the time needed at a certain temperature (or condition) to reduce the microbial or spore count by 1 log (i.e. to kill 90% of the microbial/spore load). D-values for microorganisms of concern should be determined for the specific process being used (van Asselt and Zwietering 2006).

5.1.2 z-VALUE

The z-value is the number of degrees increase in temperature that is needed to reduce the D-value tenfold.

5.1.3 B*

B* denotes a bactericidal effect, such as the inactivation of thermophilic spores. A process or treatment that produces a 9 log reduction in spores has a B*=1. It is proposed that the heat treatment to produce ESL milk should have a B* of >0.3 (Deeth 2017).

5.2 INACTIVATION OF MICROBIAL FLORA IN RAW MILK

There is no correlation between total microorganism count, spore count or thermoduric count in raw milk (Fredsted *et al.* 1996). Therefore, these groups need to be considered separately in ESL processing. There are data on the effectiveness of ESL thermal processing on counts of total flora in raw milk. Direct steam injection was used to heat raw milk containing a high initial bacterial load ($4x10^8$ CFU ml^{-1}) to temperatures in the range of 100-140°C for 4-12 s (Blake *et al.* 1995). Coliforms were not isolated immediately post-heating at 100°C for 4s, but they survived and grew to high numbers (10^7 CFU ml^{-1}) by 15 days when stored at 7°C. High total and psychrotrophic counts (10^7 and 10^6 CFU ml^{-1}, respectively) were found by day 15 in milk heated at 100-110°C for 4s and stored at 7°C. No microorganisms were isolated from milk heated at temperatures ≥134°C.

Measures of the reduction of total numbers of bacteria in raw milk have also been calculated for both indirect or direct heat treatments in the range 80°-130°C (Fernández García and Riera Rodríguez (2014), **Error! Reference source not found.**). Total bacterial counts in the milk prior to thermal treatment ranged between 5×10^4 and 2×10^5 CFU ml^{-1}.

Table 7. Logarithmic reductions of total bacterial counts in milk at selected time-temperature treatments

Heat treatment method	Temperature (°C)	Time (seconds)	Logarithmic reduction of total bacterial counts
Indirect	80	2	2.0
Indirect	90	2	2.7
Indirect	115	2	3.3
Direct	115	6	4.0
Indirect	120	2	4.6
Direct	120	6	5.3
Indirect	125	2	5.2
Direct	125	6	5.6
Indirect	130	2	6.5
Direct	130	6	7.8

Table adapted from Fernández García and Riera Rodríguez (2014)

Thermal ESL processing (127°C, 15s) has been applied to New Zealand sheep milk in a pilot plant and an approximate 4 log reduction ($1x10^7$ to ~$2\text{-}3x10^3$) in aerobic plate-count bacteria was achieved (Li Day, Pers. Comm.).

5.3 INACTIVATION OF SPORE-FORMING BACTERIA FOUND IN ESL MILK

Spore-forming and thermoduric microorganisms can survive pasteurisation of milk (Fredsted *et al.* 1996). The thermal conditions used to prepare ESL milk, including heating in the range 90°-127°C, should be sufficient to destroy non-spore-forming pathogens and all but the most heat-resistant spore-formers. However, germination of spores can be activated at these temperatures (Blake *et al.* 1995) and the extended shelf life of ESL milk may permit surviving psychrotrophic spore-forming microorganisms to grow. For ESL milk, it has been recommended to have temperature and time conditions that provide at least a 6 log reduction in the psychrotrophic spore count (Fredsted *et al.* 1996, Deeth 2017). While this is the ideal, most ESL milk thermal processes do not achieve this goal (Fredsted *et al.* 1996, Deeth 2017).

The levels and types of bacterial spores can vary notably across different samples of raw milk and are dependent on the animals' environment. Spores are transferred to the milk from the teat and udder surfaces during milking (Deeth 2017). Raw milk from cows that are grazed, such as in New Zealand, generally contains low levels of bacterial spores (≤10^2 CFU ml^{-1}) and rarely exceeds spore counts of 10^3 CFU ml^{-1} (Deeth and Lewis 2017b).

In a study, raw milk was heated at 120-140°C for 4s using direct steam injection, packaged in 120 ml containers, and assessed for different types of spores (Blake *et al.* 1995).

Psychrotrophic, thermotrophic and mesophilic spores were found in milk heated to 120°C. Psychrotrophic spores were not detected in milk treated to ≥128°C. Thermotrophic spores were not detected in milk treated at ≥132°C. No spores were found in samples heated to ≥134°C (Blake *et al.* 1995).

Total numbers of surviving aerobic spores were tested after heat treatment of raw milk at 90-130°C for 30s (Te Giffel *et al.* (2002), Figure 7). From these data, it can be seen that substantial reductions (~5 log) in spores can be achieved by heating raw milk to 120°C for this time. It is also apparent that low levels of spores survive in milk when heated to 130°C. These results are broadly comparable with those of Blake *et al.* (1995) above, although the exposure times were longer than typically used for ESL milk processing (30s vs.1-4s).

Different types of milk product (skimmed, whole, cream, concentrated skimmed) may also result in different levels of spore inactivation at a given temperature, and data from various studies are so far inconclusive (Mazas *et al.* 1999). Inactivation of spores from the same species can be variable as well (e.g. spores from different *B. cereus* strains give different D-values) (Mazas *et al.* 1999).

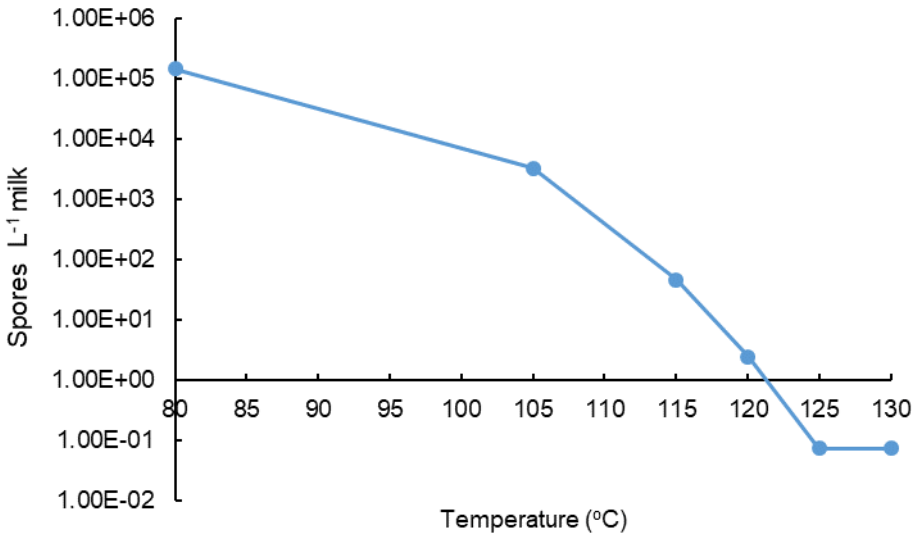

Figure 7. Effect of 30s heat treatment on survival of aerobic spores in milk

Adapted from Te Giffel *et al.* (2002)

5.3.1 BACILLUS

Members of the genus *Bacillus* are common aerobic spore-forming bacterial contaminants of raw and processed milk, and dairy products. ESL milk may be particularly susceptible to the effects of *Bacillus* spp. due to the longer shelf life which provides sufficient time for high counts to be reached (Deeth and Lewis 2017b). *Bacillus cereus* is of particular relevance due to its ability to cause food poisoning. Its optimal growth temperature is 30-37°C but some *B. cereus* strains are able to grow at 7°C (psychrotrophic) and as well as up to 50°C which is of concern to ESL milk producers. Psychrotolerant *Bacillus* able to grow at 4°C, but not at 43°C, have been described and this group have been classified as *B. weihenstephanenesis* (Deeth and Lewis 2017b). There is also a threat from emerging species, such as *B. thermoamylovorans* which produces highly heat-resistant spores and has been associated with thermally processed milk spoilage (Flint *et al.* 2017).

Studies have investigated the effects of ESL milk temperature-time conditions on *Bacillus spp.* In one study, raw milk was heated by direct steam injection to temperatures between 100 and 145°C for 4-12s, packaged in 120 ml plastic containers and stored at 7°C, with sub-samples tested every 15 days out to 60 days (Blake *et al.* 1995). *Bacillus* species including *B. insolitus*, *B. cereus, B. thuringiensis*, *B. coagulans* and *B. licheniformis* were detected in containers stored for 30 days at 7°C from milk heated to 120-132°C, but not in containers where the milk was heated to134°C.

A study by Te Giffel *et al.* (2002) involved indirectly heating raw milk samples (150 L) for 30s at various temperatures between 90 and 130°C using a plate heat exchanger. *Bacillus cereus* were recovered from milk after heat treatment at both 90 and 105°C, *B. licheniformis* was recovered at temperatures up to 125°C, and *B. subtilis* at temperatures up to 130°C.

In a Brazilian study, the effects of milk superpasteurisation (96°C for 13s) was compared to standard pasteurisation (74°C for 15s) and ultrapasteurisation (138°C for 2s) (Aires *et al.* 2009). Milk was heated and then stored at 4±2°C for up to 6 weeks. Psychrotrophic *B. cereus* was not detected (i.e. <10 CFU ml^{-1}) in any sample following any of the heat treatments. Mesophilic *B. cereus* was consistently detected after ≥7 days storage in pasteurised milk but was only found in one of three samples of superpasteurised milk at weeks 2 and 6 of storage (Aires *et al.* 2009).

Studies with *B. cereus* spores show that there is a large variation in D- and z-values suggesting that spores from different strains have a wide range of resistance to heat (Bergere and Cerf 1992, Deeth 2017). Therefore determining temperature-time conditions that will consistently inactivate all spores becomes difficult (Deeth 2017). The heat resistance of *B. cereus* spores can also be affected if the *Bacillus* strains are within a biofilm or areas of the milk processing equipment that are difficult to heat-treat (Deeth 2017). A meta-analysis of 465 data points from 12 different publications resulted in a mean D_{120}-value of 0.041 minutes for *B. cereus* spores (van Asselt and Zwietering 2006). While these data were from *B. cereus* isolates from a variety of sources, they provide useful reference points applicable to ESL milk thermal processes (van Asselt and Zwietering 2006, Deeth 2017).

A number of studies have reported the range of D- and z-values determined for spores from *B. cereus* strains isolated from milk and these are summarised in Table 8 (Bergere and Cerf 1992, Deeth 2017). It has been shown that non-toxic and toxic (diarrhoeic or emetic) strains of *B. cereus* have similar characteristics with respect to heat resistance (Bergere and Cerf 1992). Therefore, non-toxic strains could potentially be used as surrogates when determining the effectiveness of thermal processing conditions.

Table 8. Heat resistance of *Bacillus cereus* spores

Number of *B. cereus* strains tested	Heating medium	Temperature (°C)	D-value (min)	z-value (°C)
9	Milk	95	0.5-3.48	6.7-9.4
43	Phosphate buffer	100	0.9-6.9	8-11
4	Milk	100	0.3-11	8-12
4	Milk	121	0.03-2.35	7.9-9.9

Information summarised from Bergere and Cerf 1992.

Spores from some strains of *B. cereus* have been reported to be extremely heat resistant (e.g. resist 135°C for 4h), but these instances appear to be relatively rare (Deeth 2017). The heat inactivation curves for spores from *Bacillus* spp. are not always linear, with some exhibiting shoulders and others tails (Deeth and Lewis 2017b). Non-linear curves can result from extremely heat-resistant spores. The heat-resistant nature of *Bacillus sporothermodurans* spores was compared to *Geobacillus stearothermophilus* spores in skimmed milk at 110-140°C (Huemer *et al.* 1998). The heat resistance of *B. sporothermodurans* spores between 110 and 120°C was similar to *G. stearothermophilus* and results published for *B. subtilis* (Huemer *et al.* 1998). The spores of *B. sporothermodurans* were very heat resistant to treatment temperatures above 120°C, with a D_{140} of 3.4–7.9s compared to a D_{140} 0f 0.9s for *G. stearothermophilus* (Huemer *et al.* 1998). The z-values *for B. sporothermodurans* and *G. stearothermophilus* were 13.1-14.2°C and 9.1°C respectively.

5.3.2 PAENIBACILLUS

The *Paenibacillus* genus used to comprise part of the *Bacillus* genus and isolates have been associated with animal feed and silage, particularly in the US (Deeth and Lewis 2017b). The genus grows in a broad range of temperatures from about 5°C to 55°C, with an optimum of 28-42°C (Deeth and Lewis 2017b). It is difficult to separate *Paenibacillus* spp. from *Bacillus* spp. using traditional microbiological methods and so advanced molecular methods are used (Ranieri and Boor 2010). Like *Bacillus* spp., *Paenibacillus* spp. have the potential to grow at low temperatures and spoil refrigerated milk, but are reported to be only opportunistic pathogens of humans (Grady *et al.* 2016).

While a common contaminant of raw milk at low levels (Scheldeman *et al.* 2004), few data are available on temperature-time parameters for the inactivation of *Paenibacillus* species in milk. However, *Paenibacillus* has been reported to spoil UHT milk packs (Scheldeman *et al.* 2004) and their spores can survive heat treatment in raw milk at up to 130°C (Te Giffel *et al.* 2002). Ranieri and Boor (2010) showed that by day 14, *Paenibacillus* spp. began to dominate *Bacillus* spp. in pasteurised milk stored at 6°C. If *Paenibacillus* spp. survive the ESL heating process, they may also overtake *Bacillus* as the dominant species during the shelf life of ESL milk, but this is yet to be proven.

5.3.3 CLOSTRIDIUM

Clostridium species are anaerobic spore-formers which can be found in raw milk. *Clostridium botulinum* is the main public health concern as botulinum intoxications have (very infrequently) been associated with consumption of milk and dairy products overseas (Lindstrom *et al.* 2010, Doyle *et al.* 2015). The prevalence of Clostridia in raw milk appears to be low, although there are few studies reported (Lindstrom *et al.* 2010, Deeth and Lewis 2017b). Factors in botulism outbreaks associated with commercial dairy products include insufficient heat processing, post-processing contamination, formulation issues and mild-to-moderate temperature abuse (Lindstrom *et al.* 2010).

Clostridium botulinum strains can produce potent neurotoxins during vegetative growth. Depending on the type of neurotoxin secreted (toxin type A–G), *C. botulinum* can be separated into one of four groups. Group I and Group II *C. botulinum* strains are the main concerns for human health. Group I *C. botulinum* are proteolytic and can utilise casein in milk for nutrition, have heat-resistant spores (though less so than *Bacillus*), and are the most common group that has been associated with dairy products. The minimum growth temperature for Group I *C. botulinum* is likely to be in the range 10-12°C (Lindstrom *et al.* 2010). As such, any surviving microorganisms are unlikely to proliferate and produce toxin during refrigerated storage of heat-treated milk, provided temperature abuse does not occur.

To inactivate botulinum neurotoxin, temperatures of 80°C for 20 min or 85°C for 5 min are required. Therefore, pasteurisation (72-74°C, 15s) is unlikely to inactivate the toxin if present in raw milk, but higher temperatures (115-135°C) used for longer shelf life milk treatment such as in-container sterilisation, ESL or UHT would likely do so (Lindstrom *et al.* 2010).

The heat resistance of Group I *C. botulinum* spores have been determined in various buffers and milk (reviewed in Lindstrom *et al.* (2010)). For Group I type A and B *C. botulinum* strains, the $D_{104\text{-}105°C}$ values in buffer were 3.6-18 minutes, the $D_{110°C}$ was 1.4-4.4 minutes and the $D_{121\text{-}122°C}$ was 0.07-0.2 minutes. For the same types, the $D_{121\text{-}122°C}$ in milk was calculated to be 0.03-0.003 min. Experiments conducted with milk inoculated with *C. botulinum* spores demonstrated 10^3 type A and B spores could be inactivated by heat treatment at 125°C for 5s, but treatment at 122°C for 4s permitted toxin production in milk when incubated at 20°C for 2 weeks (reviewed in Lindstrom *et al.* (2010)).

Group II *C. botulinum* strains are psychrotrophs with the ability to grow and produce neurotoxin at temperatures as low as 3°C, and within the range of ESL milk storage at 4-8°C. However, the spores of Group II *C. botulinum* are only moderately heat-resistant and are inactivated at temperatures below 100°C (generally 75-95°C), so would not survive ESL thermal treatments. Therefore, the only potential contamination route of ESL milk for Group II *C. botulinum* would be via post-processing contamination. Group II strains are non-proteolytic and utilise carbohydrates as a key carbon source so may be capable of growing in milk if anaerobic conditions exist. However, such conditions appear to be uncommon in ESL milk (though there are no definitive data) and there have been no reports of Group II *C. botulinum* being associated with any type of dairy product (Lindstrom *et al.* 2010, Deeth and Lewis 2017b).

As indicated, vegetative *C. botulinum* cells, spores, and botulinum toxin may be inactivated by appropriate thermal treatment (Lindstrom *et al.* 2010, Deeth and Lewis 2017b). When using heat treatments below these optima, growth of remaining *C. botulinum* vegetative cells, outgrowth of spores, and subsequent production of neurotoxin, will likely be influenced by the physicochemical properties of ESL milk, packaged environment, and storage conditions. No data were located on the specific behaviour of *C. botulinum* and/or botulinum toxin in ESL milk.

5.4 IMPACT OF HEAT TREATMENT ON GERMINATION OF SPORES

Germination of spores typically occurs in response to particular nutrients, including sugars, nucleosides and/or amino acids. The nutrients enter the cell and interact with germinant receptor (Ger) complexes (Krawczyk *et al.* 2017). In addition, spores from some species need to be activated before they can germinate into vegetative cells. Heat treatment can be an activating trigger for germination or may increase the proportion or rate of sporulation. Germination of spores is known to be induced at temperatures between 85°C and 120°C, and at >120°C for the times used for producing ESL milk (Mayr *et al.* 2004a).

Bacillus cereus spores do not require heat for germination, but can be activated in milk heated for various times between the temperatures of 65°C and 95°C (Deeth 2017). Fast-germinating and slow-germinating spores have been described for *B. cereus* and they are differentially activated by temperature-time conditions. Fast-germinating spores are reported to be less heat-resistant than slow-germinating spores of *B. cereus* (Deeth 2017).

Heat treatment is thought to inactivate the germination system of spores of non-proteolytic *C. botulinum*. For a Group II *C. botulinum*, heat treatment at 80°C reduced the outgrowth of spores by 93%, and extended the lag time and increased the lag variability of the surviving spores (Stringer *et al.* 2009). Lowering the incubation temperature of treated spores extended the lag rate, mostly by extending the time for outgrowth. The main effect of the heat treatment was on germination time, with the median germination time being extended 16-fold, from 0.9 to 15.2 h (Stringer *et al.* 2009).

Different strains of *B. subtilis* show variation in heat activation of spore germination. Recent studies suggest that some of this variation is associated with the number of copies of a specific genetic element each strain carries (Krawczyk *et al.* 2017). Food isolates had a higher copy number than laboratory or environmental isolates which correlated with differences in spore germination patterns. Heat activation of germination (70°C to 100°C for 30 min) was also

affected by the type of germinant used (L-alanine or AGFK[10]) suggesting that the receptors played a role (Krawczyk *et al.* 2017). Although ESL milk isolates were not tested in this study, the research highlights the risk of using laboratory or culture collection strains for determining factors which control or affect the germination of spores *in situ*.

The complexity of strain differences, nutrient signals and activating conditions (such as heat) on the germination of spores makes it difficult to find generalisations that can be applied to ESL milk. Therefore, each ESL process would need to be assessed to determine whether conditions support the germination of any contaminant spores.

5.5 IMPACT OF MILK ADDITIVES AND FLAVOURINGS ON MICROBIAL INACTIVATION

The effect of the addition to whole milk, skimmed milk, cream and concentrated skimmed milk of the stabilisers disodium phosphate, sodium citrate and monopotassium on D-values at various temperatures for spores from three species of *B. cereus* was investigated (Mazas *et al.* 1999). The only effect observed was increased D-values in skimmed milk containing disodium phosphate and sodium citrate for spores of one strain of *B. cereus* (Mazas *et al.* 1999). It is thought that these differences may be due to the effects of the stabilisers on the pH of the skimmed milk rather than an effect of the stabilisers themselves on the spores. The fat content of the milk products had no impact on the effect of different temperatures on different *B. cereus* spores (Mazas *et al.* 1999).

Flavourings, such as cocoa for chocolate milk, can be independent sources of heat-resistant spores or microorganisms (Lima *et al.* 2012). Cocoa beans and nibs have a natural microbiota that includes mesophilic and thermophilic aerobic microbes, including bacteria, fungi and spore-formers. Additional microorganisms may contaminate nibs during processing. Alkalizing, drying, and roasting of nibs to make cocoa powder was generally found to destroy the majority of microorganisms except for spore-formers, particularly *Bacillus* species including heat-resistant *B. subtilis* complex and *B. licheniformis* (Lima *et al.* 2012).

In a separate study, heat-resistant (100°C for 30 min) aerobic spore-formers were isolated from a variety of milk products and cocoa powder, with spores from these species being tested for their heat-resistance at 110, 120 or 125°C for 30 min. Isolates of *Geobacillus pallidus* from cocoa powder were the most-heat resistant overall, with spores surviving treatment at 125°C for 30 min. Spores from *B. subtilis* strain 244, isolated from ESL chocolate milk, showed the lowest log reduction among all spores heated at 110°C or 120°C for 30 min (Witthuhn *et al.* 2011). While *G. pallidus* may be a common thermotolerant contaminant of cocoa powder, it has not generally been associated with spoilage, while *B. subtilis* is noted for the production of spoilage-related enzymes (Lucking *et al.* 2013).

[10] Germinant mixture AGFK = L-asparagine, D-glucose, D-fructose, potassium ions (Krawczyk *et al*. 2017)

Key points

- The temperature-time conditions used to prepare ESL milk should be sufficient to destroy non-spore-forming pathogens and all but the most heat resistant psychrotrophic spore-formers.
- It has been recommended in the scientific literature that the heat treatment used to produce ESL milk should have a B* of >0.3.
- It is also recommended in the literature that temperature and time conditions be used that give at least a 6 log reduction in the psychrotrophic spore count. But this is rarely achieved in practice.
- Isolates of *B. cereus* spores can survive heat treatment in milk up to 135°C, though this is rare.
- For *B. cereus* there is a large variation in reported D-values and z-values, therefore each ESL milk process should be validated with appropriate isolates.
- Heat treatment can be an activating trigger for spore germination or may increase the proportion or rate of sporulation. However, germination is dependent on a complex interplay of physical and biological factors, so each ESL milk process should be assessed to determine if conditions support the germination of spores.
- *Paenibacillus* spores can survive heat treatment up to 130°C in raw milk and can spoil heat-treated milk.
- Vegetative *C. botulinum* cells, spores, and botulinum toxin may be inactivated by appropriate thermal treatment.
- Growth of any surviving post-heat processing *C. botulinum* vegetative cells, outgrowth of spores and production of neurotoxin, will likely be influenced by the physicochemical properties of ESL milk, packaged environment, and storage conditions.
- It is important to note that data on the behaviour of *C. botulinum* and/or botulinum toxin in ESL milk, including growth and toxin production, have not been reported.
- Milk stabilisers appear to have only minor effects (increases) on D-values for spores of *B. cereus*.
- Milk flavourings such as cocoa can naturally harbour heat resistant spores (such as *Bacillus* spp.) and may also be cross-contaminated with spores during processing.

6 REGULATION AND STANDARDS FOR ESL PRODUCTION

This section summarises available information on any regulations or standards relating to ESL milk processing in countries with high consumption of ESL milks, or those that have similar dairy production systems to New Zealand. These regulations or standards may not be the same as import requirements for those countries.

6.1 AUSTRALIA

The primary production and dairy processing standards for milk in Australia are described in Standard 4.2.4 of the Food Standards Code (FSANZ 2017).

There are no specific regulations for the production of ESL milk within these regulations.

It should be noted that:

- Milk must be pasteurised by (a) heating to no less than 72°C for no less than 15 seconds; or (b) heating using any other time and temperature combination of equivalent or greater lethal effect on any pathogenic microorganisms in the milk; or (c) using any other process that provides an equivalent or greater lethal effect on any pathogenic microorganisms; unless an applicable law of a State or Territory otherwise expressly provides.
- In the case of (c), this process needs to be validated by the business and verified by the authority.
- The provision for States and Territories are currently being reviewed.
- The accompanying guide (FSANZ 2009) to the standard recommends UHT treatments be performed at a minimum of 132°C “to ensure commercial sterility”.

6.2 AUSTRIA

The Austrian Food Codex (Codex Alimentarius Austriacus) provides information on names, definitions, methods of examination and assessment, and directives on the marketing of goods including those related to ESL milk. Legally, the Austrian Food Book is an "objectified expert opinion". It is not strictly a legal provision as Austria falls under EU regulations but nevertheless provides instructive information.

The Codex is available online and the information below is derived from Chapter B 32: Milk and milk products (http://www.lebensmittelbuch.at/milch-und-milchprodukte/konsummilch-und-rahm/beschreibung/konsummilch-waermebehandelte-konsummilch/ (accessed 29/01/2019)). Note that comments in this section have been translated from German using Google Translate and checked by a native German speaker.

Summary of key information relating to ESL milk:

- ESL milk refers to drinking milk that has a longer shelf life than fresh drinking milk.
- The extent of the heat treatment is lower than that of the UHT milk and higher than that of pasteurised milk.
- High-temperature treatments, such as those used for the production of ESL milk, requires a few seconds at least at 85°C or a time/temperature combination with the same effect.

- Highly heated or similarly labelled drinking milk is peroxidase-negative.
- In addition to heating, in the context of manufacturing heat-treated drinking milk, physical methods such as centrifugation and/or centrifugal sterilisation, filter sterilisation and homogenisation can be used.
- For ESL drinking milk labelled "longer fresh", the time between the recovery of raw milk and the heat treatment is to be no more than 72 hours.
- The best before date for "longer fresh" ESL milk may not exceed 25 days after the heat treatment day (except when working on a day before a weekend or a holiday, when a maximum of 27 days after the heat treatment day can be used).
- For ESL milk labelled "longer shelf life", the best before date may not exceed 45 days after the heat treatment.
- For ESL milk, the type of technology for preservation (e.g. high heat, filtered) needs to be indicated on the packaging.

6.3 CANADA

Canada does not appear to have any specific regulations regarding the processing of ESL milk. However, The Canadian Food Inspection Agency does provide recommended practices for performing higher heat shorter time processing to extend the shelf life of dairy products (CFIA 2019).

In these recommendations:

- Higher heat shorter time (HHST) treatment of fluid milk and milk products is the application of heat to a continuously flowing product using high temperatures, generally above 100°C, for such time to extend the shelf life of the product under refrigerated conditions. This type of heat process can be used to produce dairy products with ESL.
- ESL means the ability to extend the shelf life of a product beyond its traditional life by reducing the major sources of re-infection and maintaining the quality of the product all the way to the consumer.
- ESL products are not considered to be commercially sterile products and, as such, must be cooled immediately after pasteurisation to a temperature of 4°C or less and stored continuously under refrigeration at a temperature of 4°C or less.
- The HHST system, although similar to a high-temperature short time (HTST) pasteuriser, operates at higher temperatures (above 100°C) and pressures. It also uses a pasteurisation or sterilisation cycle to pasteurise or sterilise the entire system prior to commencing production.
- To achieve the required pasteurisation of ESL products in HHST systems, the generally accepted best practice is to design the scheduled ESL process to provide thermal destruction of the target microorganism equivalent to that achieved by a process with a minimum lethality value F0=0.1. (Note: F0 is associated with commercially sterile products targeting a 12 log reduction in *Clostridium botulinum* spores; nevertheless, F0 was chosen in this case because it is the preferred method used by process authorities for calculating process kill).

6.4 CHINA

New Zealand's overseas market access requirements for China indicate that **"**China does not have a GB standard for extended shelf-life milk" (MPI 2018).

6.5 EUROPEAN UNION

For countries that are members of the European Union the specifications for the thermal treatment of milk are outlined in Chapter XI of Annex II to Regulation EC No 852/2004 (Anon 2004) and amended per EC No 2074/2005 (Anon 2018).

There are no specific regulations for the production of ESL milk within these regulations.

However, it is noted that:

- Pasteurisation is achieved by: (i) high temperature for a short time (≥72°C for 15s) or (ii) low temperature for a long time (≥63°C for 30 min) or (iii) "any other combination of time-temperature conditions to obtain an equivalent effect such that the products show, where applicable, a negative reaction to an alkaline phosphatase test immediately after such treatment".
- UHT is achieved by: (i) a continuous flow of heat at a high temperature for a short time (≥135°C in combination with a suitable holding time) "such that there are no viable micro-organisms or spores capable of growing in the treated product when kept in an aseptic closed container at ambient temperature"; and (ii) "sufficient to ensure that the products remain microbiologically stable after incubating for 15 days at 30°C in closed containers or for 7 days at 55°C in closed containers or after any other method demonstrating that the appropriate heat treatment has been applied".
- Mayr *et al.* (2004a, 2004b) noted that for high heated ESL milk, EEC regulation 92/46/EEC (repealed 31/12/2005) "demands temperatures between 85°C and 127°C but does not stipulate a certain holding time". However, we were unable to locate these data in the referenced EEC document.

6.6 FRANCE

Up to 97.5% of the milk sold in France is long-shelf-life milk produced by ultra-high thermal treatment at 140°C for 2 s (Syndilait 2018). This treatment meets the definition of UHT milk under EU regulations (see Section 6.5), therefore is not considered to be ESL milk.

6.7 RUSSIA / EURASIAN CUSTOMS UNION

There are no specific regulations relating to ESL milk production in the Eurasian Customs Union technical regulations on the safety of milk and dairy products (Eurasian Economic Commission 2013) or in the preceding (and more descriptive) Russian Federal Law technical regulations for milk and milk products (The Russian Federation Federal Law 2008).

However, there are references to high-temperature pasteurisation:

- Pasteurised milk, sterilised milk, ultra-pasteurised (ultra-high temperature) milk are defined as fluid milk that has undergone heat treatment to comply with the applicable microbiological safety requirements.
- Pasteurisation to be carried out in conditions (temperature, time) at 63°C to 120°C long enough to reduce the number of any pathogenic microorganisms in raw milk, or its processing products, to a level at which the microorganisms will not significantly harm human health.

- Low-temperature pasteurisation to be carried out at a temperature of not more than 76°C and will inactivate the alkaline phosphatase.
- High-temperature pasteurisation to be carried out in conditions (temperature, time) at 77° to 120°C and will inactivate both phosphatase and peroxidase.

6.8 UNITED STATES OF AMERICA

Standards for drinking milk in the United States of America (US) are guided by the Grade “A” Pasteurised Milk Ordinance (PMO) (FDA 2017). In this document, the only reference to extended shelf-life milk is as ultra-pasteurised milk, whereby the “milk and/or milk product shall have been thermally processed at or above 138°C (280°F) for at least two (2) seconds, either before or after packaging, so as to produce a milk and/or milk product, which has an extended shelf life under refrigerated conditions”.

Also, to be noted from the same document:

- There are guidelines to ensure the safety of aseptic processing and packaging of pasteurised milk
- Milk products for pasteurisation “may be processed by micro-filtration systems prior to pasteurisation for the sole purpose of the removal of micro-organisms”. The pore-size of microfiltration devices are not specified therefore this process may not remove spores.

6.9 JAPAN

Guidelines for the heat treatment of milk are given in the Japan Food Sanitation Act (JETRO 2011).

There are no specific regulations for the production of ESL milk within this document.

However:

- For drinking milk, cow’s milk is to be heated at 63°C for 30 minutes by holder pasteurisation or by an equivalent or more effective method
- For milk drinks, conditions are the same as cow’s drinking milk, except when packed in a container for storage and these are pasteurised by heating at 120°C for 4 minutes or heating to have at least an equal pasteurising effect.

Key points

- No regulations were found in the countries investigated that provide definitions on the requirements for thermal processing of ESL milk at <135°C.
- The US FDA PMO standard does describe the thermal processing of ESL milk, though at 138°C for ≥2 s this would be considered as a UHT treatment in other countries.
- The Austrian Food Codex provides some guidance on ESL milk processing; however, this is not legally binding. This guidance provides for treatment of a few seconds at least at 85°C or a time/temperature combination with the same effect.
- The Canadian Food Inspection Agency suggests that high heat ESL processing generally occurs above 100°C and that the ESL process should be designed to achieve a minimum lethality value of F0=0.1.

7 CONCLUSIONS

Currently, the most frequent temperature and time combinations used for commercially producing ESL milk by heat alone are in the range of 123-127°C for 1-3s. These temperatures produce ESL milk with a longer shelf life than the other most frequently used technologies for the production of ESL milk (i.e. pasteurisation treatments combined with either microfiltration or centrifugation). However, pilot plant experiments indicate that a combination of intermediate milk treatment temperatures (80°-120°C) with microfiltration has the potential to produce ESL milk with similar shelf life to thermal-only treatments. Information on the commercial use of thermal treatments below 100°C for processing of ESL milk is scarce and non-thermal technologies for production of ESL milk (e.g. UV light, high-pressure processing) are not currently widely used commercially. The recommended shelf life of ESL milk available in overseas markets using these technologies is in the range 21-27 days with refrigeration, but the time until spoilage occurs may be unpredictable due to the diversity of contaminating microorganisms. Once the package is opened, ESL milk has the same durability as other types of milk (i.e. ~3-4 days with refrigeration).

Analysis of overseas retail ESL milk produced by thermal treatments (125-127°C, 2-5s) shows that *Bacillus* species are the main thermoduric contaminant originating from raw milk, including *B. cereus* which is a foodborne hazard. The majority of *Bacillus* strains isolated from overseas retail ESL milk did not grow when the temperature of the package was maintained at refrigeration temperatures (≤8°C) highlighting the need to control the cold chain during ESL milk storage and distribution. The majority of non-thermoduric contaminants detected in overseas retail heat-only treated ESL milk were slow-growing non-spore-forming Gram-positive isolates, with some Gram-negative strains also detected, indicating post-heat treatment recontamination. Whilst most ESL milk is not packaged aseptically, the use of HEPA-filtered air and vigilance in cleaning operations is recommended for filling and packaging operations to achieve an acceptable shelf life. Time until spoilage for ESL milk batches may be unpredictable due to the diversity of contaminating microorganisms. ESL milk produced in a pilot plant using lower range ESL thermal treatment temperatures (100-110°C) resulted in substantial growth of both total viable bacteria and psychrotrophic *Bacillus* species after 15 days refrigerated storage, and so this temperature regime is not recommended.

Experimental data show psychrotrophic *Bacillus* spp. are able to survive at temperatures that could be used for ESL milk processing (90-132°C) and their spores show a large variation in heat resistance, even within strains. For the processing of ESL milk, it has been recommended by Fredsted *et al.* (1996) to use temperature and time combinations to give at least a 6 log reduction in the psychrotrophic spore count to maintain the safety and quality of the product.

Paenibacillus spores can survive heat treatment up to 130°C in raw milk and can spoil heat-treated (UHT) milk. They can predominate over *Bacillus* species after 14 days in stored pasteurised refrigerated milk, but data for ESL thermal treatments are lacking. *Paenibacillus* are also thought to be only opportunistic pathogens of humans, but substantial data are lacking.

Spores from Clostridia could potentially survive thermal milk treatments, with *C. botulinum* presenting the main public health concern. However, suitable anaerobic conditions for *Clostridium* growth in milk appear to be uncommon and numbers of Clostridia spores in raw milk are thought to be low (but there are few data). Group I *C. botulinum* are proteolytic and can utilise casein in milk for nutrition, have heat resistant spores, and are the most common group that has been associated with dairy products. However, the minimum growth temperature for Group I *C. botulinum* is likely in the range 10-12°C and so surviving microorganisms are unlikely to proliferate during refrigerated storage of heat-treated milk, provided temperature abuse does not occur. Some strains of Group II *C. botulinum* are

psychrotrophic, but they have not been associated with dairy products and their spores are only moderately heat resistant (<100°C). The higher temperature ranges used for milk treatment such as in-container sterilisation (115°C) or UHT (135°C) may also inactivate botulinum neurotoxin.

Heat treatment can be an activating trigger for spore germination or may increase the proportion or rate of sporulation. However, spore germination is dependent on a complex interplay of physical and biological factors, so each ESL process would need to be individually assessed to determine if conditions support the germination of spores. In particular, the concentrations of dissolved oxygen levels in ESL milk during storage are not well defined. There may be an increased risk of *C. botulinum* in ESL milk during storage if there is an absence of competing microflora and a reduced oxygen environment occurs.

The addition of milk stabilisers appears to have only minor effects (increases) on inactivation rates for thermotolerant spores (*B. cereus*) and so are unlikely to significantly affect the safety profile of ESL milks at a given processing temperature. However, milk flavourings such as cocoa can naturally harbour heat resistant spores, such as *Bacillus* spp., and cocoa may be cross-contaminated with spores during processing. These factors should also be considered when designing ESL thermal processes.

Regulations that provide definitions on the requirements for thermal processing of ESL milk at <135°C were unable to be located in any of the countries investigated. The US FDA PMO standard does describe the thermal processing of ESL milk, although at 138°C for ≥2 s this would be considered as a UHT treatment in other countries. The Austrian Food Codex provides guidance on ESL milk processing, suggesting the use of treatment of a few seconds at least at 85°C or a time/temperature combination with the same effect. The Canadian Food Inspection Agency suggests high heat ESL processing generally occurs above 100°C and that the ESL process should be designed to achieve a minimum lethality value of F0=0.1.

In common with other countries, microbial hazards are present in the New Zealand raw milk supply. The majority of microbial hazards likely to be present in New Zealand raw milk are not thermoduric and so would be destroyed by thermal ESL processing, but *Bacillus* species have been detected. Additionally, isolates with genetic similarity to a diversity of *Bacillus* and *Clostridium* species have been found in New Zealand dairy farms, as with other countries. However, a detailed understanding of the types and subtypes of *Bacillus* and *Clostridium* present on farms, their toxicological status, and the potential for contamination of raw milk is yet to be established. Until these data are established it would be prudent to take a conservative approach to the design of ESL milk processing systems (i.e. use conditions likely to result in the greatest reductions in numbers of these organisms). *Bacillus* and *Clostridium* are thermoduric spore-forming microorganisms and some members of these genera are recognized foodborne hazards, therefore these microorganisms will need to be considered in risk analysis prior to use of thermal only ESL processing in New Zealand.

ESL milk appears to have had a long history of safe consumption overseas, particularly in Europe. However, there are data gaps regarding the behaviour of some pathogens (notably *C. botulinum*), spores, and toxins in ESL milk during processing, packaging and storage. Therefore, this places an onus on ESL milk processors to validate their systems to ensure microbial hazards are adequately controlled.

8 GLOSSARY

EEC	European Economic Community
ESL	Extended Shelf Life
EU	European Union
FDA	Food and Drug Administration
FSANZ	Food Safety Australia New Zealand
HEPA	High-Efficiency Particulate Air
HHST	High Heat Short Time
HHT	High Heat Treatment
HPP	High-Pressure Processing
HTST	High-Temperature Short Time
JETRO	Japan External Trade Relations Organisation
LHT	Low Heat Treatment
MF	Microfiltration
MPN	Most Probable Number
PEF	Pulsed Electric Field
PMO	Pasteurized Milk Ordinance
SRC	Sulphite Reducing *Clostridium*
TVC	Total Viable Count
UHT	Ultra High Temperature
UK	United Kingdom
US	United States of America
UV	Ultraviolet

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