New Zealand Food Safety
Haumaru Kai Aotearoa

Review of High Pressure Processes (HPP) applied as an alternative to thermal pasteurisation

New Zealand Food Safety Discussion Paper No: 2019/05

Prepared for New Zealand Food Safety
by Dr Beverley Horn, Dr Isabelle Pattis, Dr Lucia Rivas, Professor Tanya Soboleva & Lisa Olsen

ISBN No: 978-1-98-859460-6 (online)
ISSN No: 2624-0157 (online)

May 2019

New Zealand Government

Ministry for Primary Industries
Manatū Ahu Matua

Disclaimer

While every effort has been made to ensure the information in this publication is accurate, the Ministry for Primary Industries does not accept any responsibility or liability for error of fact, omission, interpretation or opinion that may be present, nor for the consequences of any decisions based on this information.

Requests for further copies should be directed to:

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries website at http://www.mpi.govt.nz/news-and-resources/publications/

Scientific Interpretive Summary

MPI commissioned a review of the scientific literature on high pressure processing (HPP) to address validation expectations for HPP as a control measure for the inactivation of food borne bacterial pathogens. In particular, there is increasing interest in the application of HPP to raw milk as an alternative to pasteurization. To assist food processors, MPI sought to determine whether processing parameters that ensure pathogen inactivation were available and could be applied as default "safe harbours" to reduce the level of validation needed from individual processors.

The review collates and summarises information from the international scientific literature and food safety regulator websites (the current state of knowledge), outlines the impact of product and process variables on pathogen inactivation by HPP, and provides direction on how these should be addressed during validation work.

Unfortunately, the reviewed studies show a high degree of variability and conflict in results (possibly because of variable or inappropriate study design) and often key information is not reported. Although data is not available to support the establishment of default food processing parameters to ensure pathogen inactivation, the study findings provide direction for determining effective operating ranges for various parameters during HPP validation. The kinetics of pathogen inactivation during HPP appears to be complex and data from existing studies should not be extrapolated to scenarios outside the range of HPP parameters used in the studies. The reviewers were unable to recommend pressure resistant strains of pathogens that should be used for inactivation studies nor describe confirmed surrogates that would enable in-process validation studies.

Seventy three scientific publications and a range of regulatory websites were reviewed for parameters specific to HPP of milk. An important finding is that bacteria are more resilient in milk compared to many other matrixes. While, the compiled findings are an excellent resource for processors seeking to use HPP for treatment of milk as an alternative to pasteurisation, the review failed to identify sufficient evidence supporting default parameters.

Generally the research identified the ability of the combined use of high pressure (>550MPa) for at least five minutes and elevated pre-pressure product temperatures (>50^{o} C) to achieve a 5log$_{10}$ (CFU/g) reduction in the concentration of most non-spore forming foodborne bacterial pathogens of concern in New Zealand. Additional studies will, however, be required to identify HPP parameters that are sufficient to produce safe products if the processor is unable to use these parameters for the food type, its intended use and targeted shelf-life.

While the review provides valuable information for processors wishing to use HPP for extended shelf-life or food safety of food products, MPI will still require processors to carry out validation studies for HPP efficacy on a case-by-case basis. The review provides guidelines to processors on parameters to consider when designing validation studies.

Review of High Pressure Processes (HPP) applied as an alternative to thermal pasteurisation

October 2018

Prepared by:

Beverley Horn,
Isabelle Pattis
Lucia Rivas

PREPARED FOR: **Ministry for Primary Industries**

CLIENT REPORT No: **FW18012**

REVIEWED BY: **Peter Cressey**

Manager	Peer reviewer	Author
		
Rob Lake	Peter Cressey	Beverley Horn
Risk, Response and Social Systems Group Manager	Senior Scientist	Senior Scientist

CONTENTS

SUMMARY

Introduction

High-pressure processing (HPP), also described as high-hydrostatic pressure (HHP) or ultra-high pressure processing (UHP), is a methodology that typically subjects liquid and solid foods with or without packaging to elevated pressures.

The use of HPP has been investigated in various food applications, including extending the shelf life of products. This document focuses on the factors that must be considered when validating HPP of foods for the purpose of food safety. That is, the ability of the process to eliminate or reduce any pathogens present in/on the food to acceptable levels for the shelf life of the food.

This report provides a summary of:

- the information from selected review papers and web sites on the effects of HPP on pathogens in food from a food safety perspective,
- examples of international regulations and approvals,
- components of challenge studies that are important to evaluating HPP treatments,
- the HPP facilities in New Zealand suitable for challenge studies or product development, and
- a literature review on the effects of HPP on bacterial pathogens in milk.

Evidence of pathogen inactivation

Collating information from the selected review papers and government websites has illustrated there are multiple factors that may affect the pressure sensitivity of bacterial pathogens during HPP treatments, and therefore the food safety of a ready-to-eat product treated in this way. There is insufficient evidence to provide generic guidelines to ensure specified reduction in cell counts during HPP.

The specific factors that should be taken in to account when using existing studies/literature or conducting a new challenge study to provide evidence of pathogen reduction during HPP include:

- Commercial product formulations should be used. For heterogeneous foods the full size packaged food should be tested.
- Packaging material should be the same as used for the commercial product or have similar behaviour under the HPP treatment.

- The HPP processing factors should be the same as for the commercial equipment (e.g. Pressure and temperature changes over time, holding time at pressure, compression and decompression rates, pre-chilling or heating of the product before pressurisation).
- Methods to enumerate cells post HPP treatment, should be appropriate to detect stressed or injured (but repairable) cells. Challenge studies should sample for the duration of the shelf life of the product as well as immediately after HPP treatment.
- The pathogen strains inoculated onto product should include pressure resistant strains.

The reviews have highlighted that milk can be protective in terms of pathogen reduction during HPP, compared to experiments in broth. A study of the inactivation of New Zealand relevant pathogen strains in milk due to HPP treatment, may be required to establish suitable test strains for validation studies. The literature does not provide a clear indication of which pathogens or pathogen strains should be used for validation studies.

The review papers and the milk specific literature review has identified that the inactivation kinetics of bacteria during HPP does not always follow a log linear relationship with time. If the shape of the inactivation curve cannot be established from existing studies, inactivation should not be interpolated for different holding times between known data points.

Data from existing studies should not be extrapolated to scenarios outside the range of HPP parameters in the existing studies.

HPP and Spore forming bacteria

Compared to vegetative cells, spore-forming microorganisms are highly resistant to HPP when in spore form. Pressure alone at or near ambient temperatures has very limited or no effect on spore destruction.

A combination of high pressure (exceeding 800 MPa) and high temperatures such as in pressure-assisted thermal sterilisation (PATS) processes, or high pressure combined with antibacterial treatments, are required to achieve a significant reduction of bacterial spores in food.

Cyclic pressurisation

The use of cyclic pressurisation can increase inactivation rates for some pathogens, when compared to the same total processing time as a single cycle of pressure treatment.

1. INTRODUCTION

1.1 BACKGROUND

High-pressure processing (HPP), also described as high-hydrostatic pressure (HHP) or ultra-high pressure processing (UHP) is a methodology that typically subjects liquid and solid foods, with or without packaging to elevated pressures. Commercial equipment is commonly capable of elevated pressures up to 600 MPa[1].

HPP has a variety of applications and has been used to:

- Reduce or eliminate pathogens in food, as an alternative to traditional thermal pasteurisation.
- Extend shelf-life by reducing or eliminating spoilage organisms or enzymes.
- Improve the efficiency of other food processes such as extraction, freezing and thawing.
- Intentionally modify the physicochemical properties of functional ingredients.

HPP can be used with or without; heating or chilling, other antimicrobial hurdles or processing technologies, minimising the need for preservatives whilst maintaining natural flavours and the nutritional value of the original food product (Ferreira, 2016).

HPP has been applied to a wide range of foods including fruit juices, fresh-cut fruits and vegetables, raw milk, ready-to-eat meats, guacamole, salad dressings, jams and fruit sauces and oysters, mostly for shelf life extension. Many HPP-treated products are commercially available in different countries. HPP can be performed on unpackaged liquids prior to packaging, but pre-packaging of foods (such as sliced ready-to-eat (RTE) deli meat products and some juices) offers the advantage of preventing recontamination of food after pressurisation.

Other advantages of the technology include that processes can be performed at ambient or low temperatures, which reduces energy consumption associated with heating and subsequent cooling, and it generates little waste.

1.2 REPORT SCOPE

Although the use of HPP has been investigated in various food applications, including extending the shelf life of products, this document focuses on the factors that must be considered when validating HPP of foods for the purpose of microbial safety of food, e.g.

[1] The unit of pressure frequently used associated with food HPP is the Pascal (Pa) or mega Pascal (MPa, 1,000,000 Pa).

the ability of the process to reduce any pathogenic bacteria present in/on the food to acceptable levels for the shelf life of the food.

This report provides a summary of: the information from selected review papers and www sites on the effects of HPP on pathogens in food from a food safety perspective (Section 2), examples of international regulations and approvals (Section 3), components of challenge studies that are important to evaluating HPP treatments (Section 4), the HPP facilities in New Zealand (Section 5) and a literature review on the effects of HPP on bacterial pathogens in milk (Section 6).

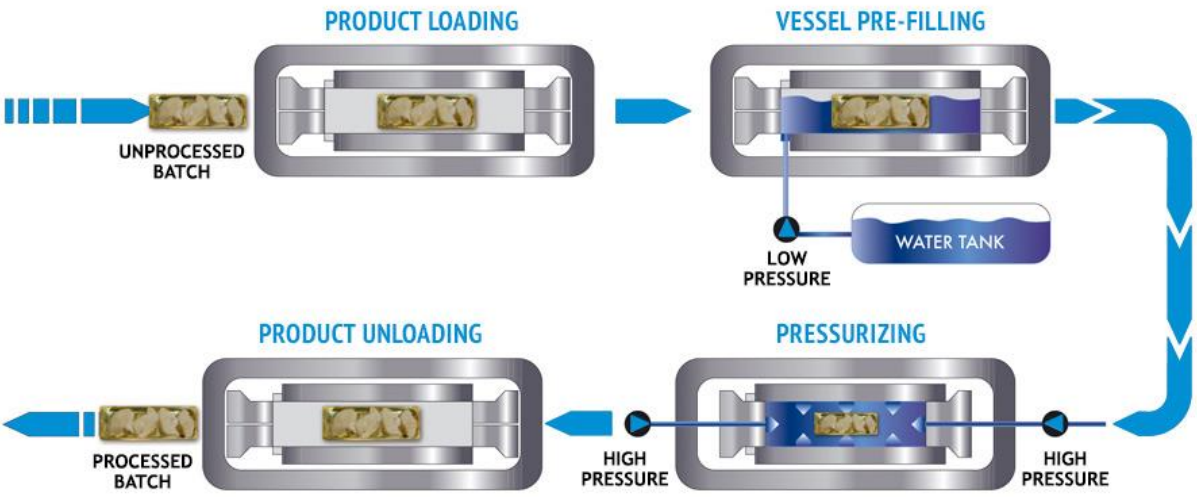
1.3 THE HPP PROCESS

1.3.1 HPP System

A typical modern HPP system consists of a pressure vessel and a pressure-generating device. Components of the HPP system can be arranged to treat unpackaged liquid foods in a semi-continuous manner, while batch configurations are commonly used for packaged foods. The food is loaded and closed into a vessel and the pressure medium, usually water or oil, is pumped into the vessel until the desired pressure is reached and is held at the pressure for the desired amount of time (Figure 1). In some systems, the temperature of the pressure medium and food is controlled before pressurisation.

HPP is governed by the isostatic principle, meaning that pressure is applied uniformly and almost instantly through the food product, regardless of shape or size, therefore foods of different volumes can be processed in the same batch (Huang 2017).

Figure 1: Diagram of the operation of a high pressure processing unit.
(http://www.hiperbaric.com/en/high-pressure)



1.3.2 Temperature and Pressure Changes

The food is exposed to temperature and pressure changes during HPP treatment of food. Figure 2 is an example representation of how temperature (red line) and pressure (blue line) changes through a HPP process. The terms in this figure are used throughout this review.

Before pressurisation, the food and pressure medium may be preheated or cooled to a pre-compression temperature.

During compression, the pressure increases, which causes increases in the temperature in the food, pressure medium and vessel due to adiabatic heating. For homogeneous foods, the adiabatic temperature increase will be uniform throughout the food.

The adiabatic temperature increase is dependent on the pre-compression temperature and the material being pressurised. Table 1 outlines the typical increases in the temperature of various food, pressure medium and materials due to adiabatic heating from a pre-compression temperature of 25°C.

The pressurised temperature of the food is independent of the rate of compression, but can depend on the compressibility and thermal properties of the food, and the pre-compression temperature and the target pressure used for HPP processing (Balasubramaniam et al. 2015). For example, fatty foods have higher compressibility, due to the presence of long-chain unsaturated fatty acids, and lower specific heat capacity, resulting in higher compression heating. Although the heat of compression of water and high moisture-content foods generally increases with increasing initial product temperature, the heat of compression value of fatty materials does not vary as a function of initial temperature (Balasubramaniam et al. 2015; New South Wales Food Authority 2016).

The rate of temperature increase in water based products is usually in phase with the pressure increase, however for fatty products it may take 30-60 seconds longer to reach a maximum temperature after pressurisation (Tao et al. 2014).

After compression, the food is held at the constant target pressure for a period of time, called the holding time. On decompression of the food, the temperature of the food decreases again to a temperature which may be below the pre-compression temperature if heat has dissipated from the food during the holding time.

Any temperature gradients between the food, pressure medium and vessel walls will result in heat transfer from or to the food. This can result in changes to the food temperature during the holding time and non-uniform heat distribution in the food product. The smaller pilot HPP units used for research purposes may exhibit different heat transfer rates than larger, high-throughput commercial systems.

Figure 2: Representation of temperature and pressure profile a food is exposed to during a generic HPP treatment which includes a preheating step before pressurisation (adapted from Balasubramaniam et al. 2015).

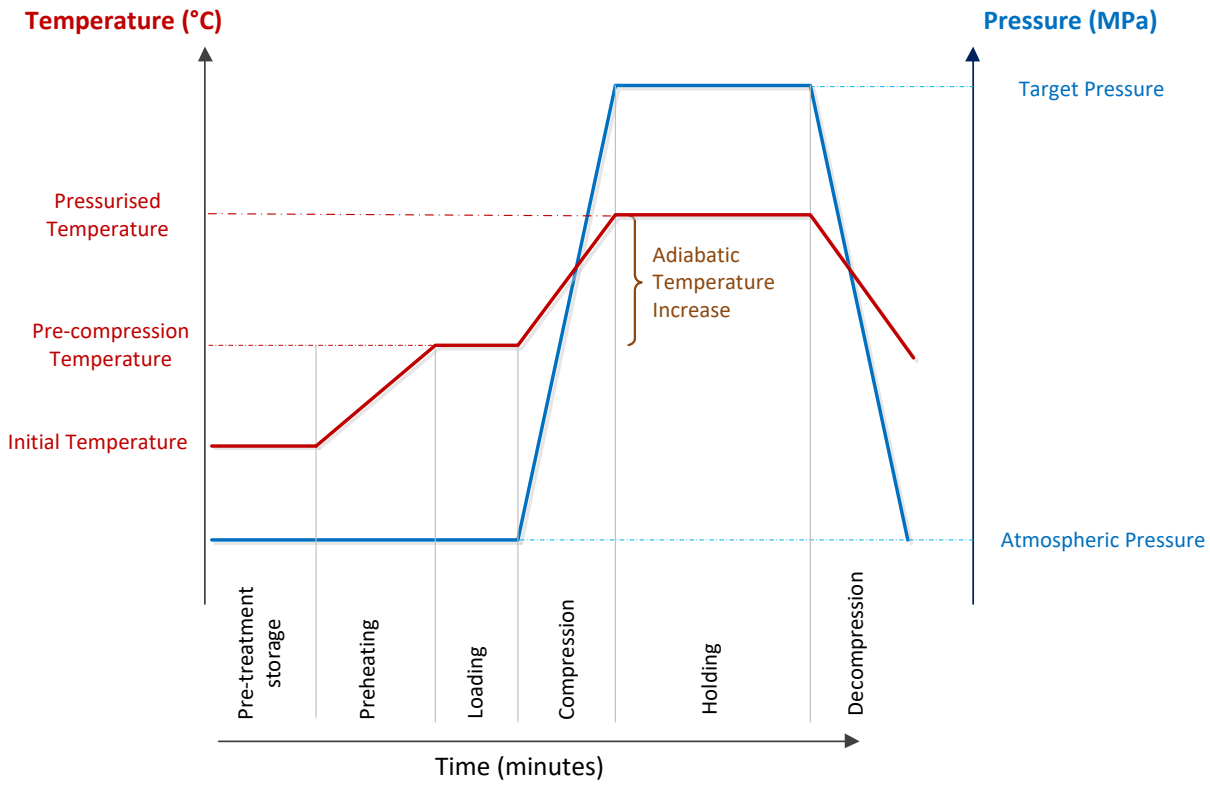


Table 1: Heat of compression values of selected food and materials at 25°C (adapted from Balasubramaniam et al. 2015)

Substance initially at 25°C	Temperature change per 100 MPa
Water, juice, milk (2% fat)	3.0
Egg albumin, Mashed potato	3.0
Tofu, Yoghurt	3.1
Honey, Salmon	3.2
Chicken fat	4.5
Beef fat	6.3
Olive oil	8.7 to 6.3 *(Decreased temperature rise as pressure increased)*
Water / glycol (50/50)	4.8 to 3.7 *(Decreased temperature rise as pressure increased)*
Silicone oil	18.5
Metal	0
Polypropylene polymer	~ 4.0

Some commercial batch pressure vessels have a sensor which can measure the temperature of the pressure medium around the food product being processed, but this is not a feature in all vessels and it does not measure the temperature distribution through the food itself. A more common practice is to monitor the food temperature pre- and post-pressurisation.

One form of HPP is pressure-assisted thermal sterilisation (PATS) which requires a temperature controlled pressure vessel to be used. While traditional HPP has pre-compression temperatures typically below 60°C, PATS processes preheat the product to 75-90°C before pressurisation. The rapid food temperature increase/decrease due to compression/decompression is an advantage of PATS over heating only methods (Balasubramaniam et al. 2015).

1.4 TEMPERATURE DEFINITIONS IN REPORT

The previous section has described how temperature in the pressure medium or food may change during HPP. Small laboratory equipment, as is commonly used by published studies are likely to have different temperature profiles over the holding time than larger commercial systems. It would be preferable to be able to provide information on inactivation rates for different target pressures and food pre-compression temperatures which are easily monitored/controlled on a commercial scale. However, defining a pre-compression temperature and target pressure for a food product does not automatically define the pressured temperature history during the holding time. This will change depending on the transfer rate of heat to or from the vessel, vessel size and whether the vessel is temperature controlled or not.

Review and source papers are not always clear if the temperatures reported are the pre-compression temperature or the pressurised temperature, or if temperature is controlled during the holding time. The temperature of the pressure medium is typically monitored during HPP, as it is not possible to monitor the temperature of the food directly.

In this report, the temperatures will be described as “pre-compression” or “pressurised” temperatures when this is known. When the type of temperature is unknown from the paper, the temperature will be underlined (e.g. 33°C).

In some cases, it may be possible to determine the temperature type by readers locating the source papers listed in the review articles. Evaluating source paper information directly was outside the scope of the review section of this project. Source papers were consulted for the milk literature review in section 6.

2. HPP MICROBIAL INACTIVATION

2.1 INTRODUCTION

The effectiveness of HPP in eliminating or reducing foodborne microorganisms depends on a number of factors intrinsic to the microorganism (e.g. growth phase or cell membrane) and extrinsic factors such as the food composition, food temperature during HPP, and the use of additives and preservatives. A summary of these effects is given in this section.

To date, the majority of research and literature describing the use of HPP for the inactivation of bacterial pathogens has focussed on *Staphylococcus aureus*, *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 in a number of food products. Although not discussed in this document, there is considerably less information available for fungi/yeasts, viruses and parasites. An overview of these topics is available in the publication of Tao *et al.* (2014).

This section primarily summarises information from the following review articles: Baptista et al. 2016; Barba et al. 2017; Ferreira et al. 2016; Food and Drug Association 2000; Huang et al. 2015; Muntean et al. 2016; Syed et al. 2016; Tao et al. 2014; Balasubramaniam et al. 2015; Farkas and Hoover 2011, unless stated otherwise. These reference citations are not repeated further throughout the text and unless cited, the source papers from these reviews have not been consulted.

2.2 MECHANISMS OF CELLULAR CHANGE

Two principles underlie the impact of high pressure on microorganisms. Le Chatelier's principle, according to which any phenomenon (phase transition, chemical reactivity, change in molecular configuration and/or chemical reaction) accompanied by a decrease in volume will be enhanced by pressure. Secondly, pressure is instantaneously and uniformly transmitted independent of the size and the geometry of the food. This is known as isostatic pressure.

Le Chatelier's principle drives cellular and molecular changes in response to increased pressure. Cell death may be due to a combination of damage to different parts of the cell.

At the cellular level, the cellular membrane undergoes changes resulting in a phase transition from the physiological, liquid-crystalline phase to the gel phase and also results in changes to membrane permeability. The cell membranes can separate from the cell wall and in some cases the cell wall will rupture.

At a molecular level, enzymes, proteins and ribosomes may be denatured by loss of their three-dimensional structure, reducing their volume. This occurs primarily through the disruption of electrostatic intra-molecular interactions, but HPP is not able to disrupt the shorter covalent bonds. Consequently, the smaller molecules responsible for organoleptic

characteristics of foods are unaffected. Denaturation of enzymes and ribosomes disrupts metabolic processes such as maintenance of cell pH and inhibits protein synthesis.

The difference in chemical composition and structural properties of the cell membrane of Gram-positive (e.g. *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus* and *Clostridium* spp.) and Gram-negative (e.g., *E. coli*, *Salmonella* spp. and *Campylobacter* spp.) bacteria result in differences in resistance to HPP. The review articles state Gram-positive bacteria are generally more resistant than Gram-negative bacteria, however there is considerable overlap in resistance to pressure and the within strain variation discussed in the next section is greater than the variation between the two types of bacteria.

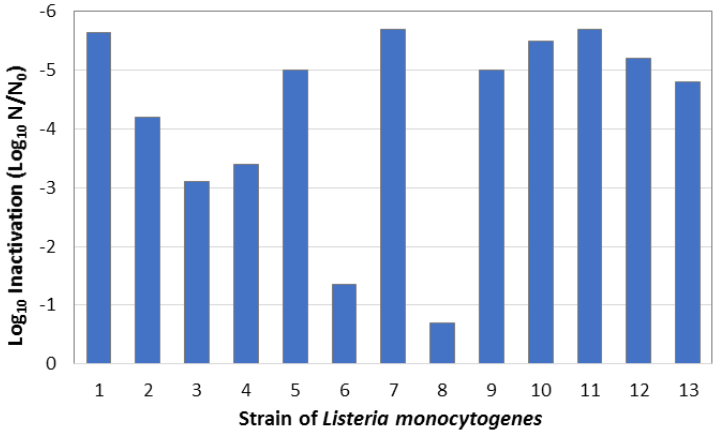
2.3 STRAIN VARIATION IN CELL INACTIVATION

Significant bacterial reduction can be obtained through HPP treatments, however, microorganisms, including pathogens, can vary significantly in their response to high pressure. This variation exists not only between different species but also between strains of the same species. Examples of strain variation in inactivation rates are:

- A recent study (Tamber 2018) conducted in phosphate-buffered saline (PBS) investigated the response of 99 *Salmonella enterica* strains to HPP treatment (600 MPa, 3 min). Reductions in bacterial concentrations ranged between 0.9 and 6 $\log_{10}$ cfu/ml.

- Patterson et al. (2011) subjected cooked chicken inoculated individually with 13 strains of *L. monocytogenes* to HPP (600MPa/20°C[2]/2 minutes). The $\log_{10}$ inactivation of *L. monocytogenes* cfu/g ranged from no significant reduction to over 5 $\log_{10}$, as shown in Figure 3.

Figure 3: Average pressure inactivation (600MPa/20°C/2 minutes) of *L. monocytogenes* strains on cooked chicken. Adapted from Patterson et al (2011). N_0 is the initial cell concentration (cfu/g) and N is the concentration following treatment.



[2] The pre-compression temperature was 20°C, however the vessel was temperature controlled. The temperature profile during the holding time is not known.

- Thirty-nine Shiga toxin producing *E. coli* (STEC) strains inoculated individually into 80% lean ground beef, were treated at 350MPa/4°C. The D values (the time taken to achieve a 1 $\log_{10}$ reduction) for this pressure/temperature combination ranged from 0.89 to 25.7 minutes (Sheen et al. 2015).

The review paper by Baptista et al. (2016) also describes differences in pressure resistance between strains of *S. aureus*. It is suggested that the difference may be due to the strains having different carotenoid content or the σ^B factor. The ability of the strain to produce staphylococcal enterotoxins is also identified as making a strain more susceptible to inactivation by pressure, however this relationship seems to be based on results from a limited number of strains. The protective effect of the σ^B factor has also been identified in *L. monocytogenes.*

2.4 SPORE FORMING BACTERIA

Spore-forming pathogens of food safety concern mainly come from the *Bacillus* and *Clostridium* genera, including the species *Bacillus cereus*, *Clostridium botulinum* and *Clostridium perfringens*. The sporulation process is initiated in response to harsh environmental conditions. The structure and thickness of the bacterial spore coat enables the spore to withstand or resist various stresses, including heat, pressure, chemicals and desiccation.

Compared to vegetative cells, spore-forming microorganisms are highly resistant to HPP when in spore form. HPP applied at room (pre-compression) temperature, has very limited or no effect on spore destruction. At room temperatures pressure cannot be used alone to ensure food safety. For example, spores have shown tolerance to pressures above 1000 MPa at room temperature (Syed et al 2016).

As a result, a combination of high pressure treatment at pressures exceeding 800 MPa and at temperatures above 80°C, such as PATS processes, or increased pressure in combination with other antibacterial treatments are required to achieve a significant reduction of bacterial spores in food.

At moderate pressures (up to 400 MPa), it is possible for spores to germinate into vegetative cells which may then multiply during product storage. Pressure treatment can trigger germination by activating nutrient germinant receptors (Balasubramaniam et al. 2015). Pressure induced germination has been investigated as a component of cyclic treatments, where the spores are exposed to alternating low and high pressures. At lower pressures, the spores germinate, and the resultant cells are subsequently killed during the higher pressure section of the treatment.

A good review paper on the effects of HPP on spore germination and inactivation has been written by Reineke et al. (2013).

2.5 TOXINS

Staphylococcal enterotoxins (SE) produced by *S. aureus* are very pressure tolerant. HPP treatments up to 800 MPa/4 or 20°C had no effect on the reactivity of SE A or SE E tested by enzyme immunoassays. At 80°C and 800 MPa, a decrease in toxin activity was observed (Babtista et al 2016). A combination of high pressure and temperature are required to denature the toxin to a safe state.

2.6 GROWTH PHASE PRE-PRESSURISATION

Vegetative bacterial cells in the exponential growth phase (where cells are growing at their highest rate) are normally more sensitive to HPP than cells in stationary phase (slow or no growth of cells). Stress resistance proteins produced by some bacteria in the stationary phase may contribute to this difference.

It is unlikely that processors will know the physiological state of any pathogens on the food. Therefore, validation studies should aim to the use cells in stationary phase.

2.7 SUB-LETHAL INACTIVATION AND RECOVERY

Following HPP treatment, vegetative bacterial cells may either be;

- unaffected, healthy and are able to grow post treatment.
- stressed or injured, which given favourable environmental conditions and time can become functionally normal again.
- inactivated without the ability to recover.

Immediately after HPP treatment, the population of bacterial cells in/on the food is likely to be made up of a mixture of the above three states. Healthy cells will be able to grow and can therefore be cultured and enumerated on selective and non-selective media. However, stressed cells will only grow on non-selective media.

Given favourable conditions, both stressed and injured cells can continue with normal growth after a period of time. This has been observed in a number of HPP studies. For example, in Queso fresco cheese, an immediate loss of viability of *L. monocytogenes* was observed following HPP, but growth recommenced after one-week storage at 4°C (Ferreira et al. 2016). In another study, freshly ground mince was HPP treated (200 to 450 MPa/20°C/20 minutes) and then stored at 3°C in air or under vacuum. Microbial growth was delayed 2 to 6 days following HPP.

The product matrix will affect the rate of recovery of stressed or injured cells during storage. For example, the recovery of *E. coli* varied in Tris buffer, skimmed milk and orange juice, following treatment of 600 MPa/ 3 minutes (temperature not given). After 24 hours at 4°C, *E. coli* was recovered at levels of 1.19 and 0.79 cfu/ml in buffer and milk, respectively, while no

E. coli was recovered from in the orange juice. However, the lower pH of the orange juice compared to milk or buffer would have hindered the ability of the stressed *E. coli* to recover in such conditions (Syed et al. 2016).

Sub-lethal inactivation of bacterial cells should be taken into account when validating a HPP treatment for a particular food product. It is important to ensure that following HPP processing that injured or stressed bacterial cells are not able to recover and grow during the shelf life of the product. For the purpose of validating a HPP process, it is important to include a culture isolation procedure that will detect stressed or injured bacterial cells during the shelf life of the product.

2.8 FOOD MATRIX

A number of extrinsic or food-related factors, including water activity (a_w), pH, fat content and food structure must be considered for efficient HPP processing. The composition of the food matrix can affect the level of microbial inactivation by HPP. When optimising processing conditions for specific foods, it is important to consider both the effects of the food substrate on microbial inactivation kinetics and the effect of the HPP on the properties of the food.

Some food components, such as proteins, lipids, carbohydrates, amino acids, vitamins and cations, can have a protective effect on microbial cells (Patterson et al. 1995). Microorganisms generally show a higher resistance to pressure in food systems compared to buffers or broths. For example, components within dairy products, including calcium ions, fat or proteins can bind and protect bacterial cells so that they are less susceptible to the effects of pressure (Ferreira et al. 2016). As a result, inactivation data obtained in studies performed in buffers or broth studies cannot be directly applied to real food situations. HPP process conditions have to be validated in the food of interest rather than extrapolating data from other food matrices (Dogan and Erkmen 2003).

2.8.1 Water activity (a_w)

Lowering the water activity of food appears to protect microbes against inactivation by HPP, it has been suggested that lower a_w stabilises proteins, helping to prevent protein denaturation during HPP. However, it is expected the recovery of microbes sub-lethally injured by pressure can be inhibited by low a_w. Therefore, the net effect of a_w on microbial inactivation by HPP may be difficult to predict (Tao et al. 2014).

An example is given in Baptista et al. (2016), where a cocktail of three *S. aureus* strains was inoculated onto three sliced meat products and then HP processed at 600 MPa/31°C/6 minutes. The following $\log_{10}$ inactivation in *S. aureus* levels for each product type was determined after two days of storage (4°C) post-HPP:

- marinated beef loin (a_w: 0.987, pH: 6.26): 2.7 Log cfu/g
- cooked ham (a_w: 0.982, pH: 6.11): 1.1 Log cfu/g, and
- dry cured ham (a_w: 0.918, pH: 5.88): 0.5 Log cfu/g.

No inactivation of *S. aureus* was observed in matched meat products that did not undergo HPP treatment. In this example, the inactivation of *S. aureus* during HPP decreases with decreasing a_w. However, given these particular meat products are produced in different ways, it is not possible to say that the difference is entirely due to the difference in a_w.

Syed et al (2016) observed a decrease in efficacy of HPP to inactivate *B. cereus* in foods with a_w below 0.9. Other guidance on relevant a_w values for effective HPP inactivation of microbes is not provided in the review papers.

Increasing the a_w of a food product may enhance the HPP inactivation of bacteria. One study has shown that wetting and soaking Jalapeno and Serrano peppers prior to HPP treatment, increases the inactivation of *Salmonella* during HPP compared to non-soaked product. No inactivation values are provided in the review paper (Barba et al. 2017).

2.8.2 **pH**

In general, microorganisms are more sensitive to pressure in lower pH (acidic) environments and the survival and repair of pressure-damaged cells is also reduced in more acidic environments.

A study using citric and lactic acid matrices show a continual increase in cell sensitivity to pressure damage with increasing acidity for two strains of *S. aureus*. A HPP treatment of 345 MPa/35°C/5 minutes resulted in a 1.5 to 2.4 $\log_{10}$ cfu/g inactivation when pH was 6.5 compared to a 3.5 to 5 $\log_{10}$ cfu/g inactivation when the pH was 4.5.

The review by Farkas and Hoover (2000) reported that a HPP treatment of 580 MPa/3 minutes inactivates 6 $\log_{10}$ cfu/g of *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp. or *Staphylococcus* spp. in salsa and apple juice with pH less than 4 and a_w close to 1 (temperature not given).

No further guidance considering the effect of pH on HPP inactivation of microorganisms in foods is given in the review papers.

The pH of a food can be a hurdle to prevent the re-growth of microorganisms following HPP treatment as introduced in section 2.7. Table 2 lists the minimum pH values for growth under conditions that are otherwise optimal for growth (ICMSF 1996). Cells which have been injured during HPP treatment are likely to need higher pH conditions to allow repair and subsequent growth to occur. It is not clear from the review papers what the minimum pH values are to support repair of injured cells.

Table 2: Minimum pH values for growth under otherwise optimal conditions (MPI Pathogen Data Sheets)[3]

Micro-organism	*B. cereus*	*Campylobacter spp.*	*C. botulinum*	*C. perfringens*	*Pathogenic E. Coli*	*L. monocytogenes*	*Salmonellae spp.*	*Shigella spp.*	*S. aureus*	*V. parahaemolyticus*	*Y. enterocolitica*
pH	4.5	4.9	4.6	5.1	4.4	4.4	3.8	4.8	4.2	4.8	4.2

Compression of foods may shift the pH of the food, either temporarily or permanently, as a function of the imposed pressure. It is not possible to measure pH during HPP processing and the minimum pH supporting growth post HPP treatment will be affected by the food matrix. Therefore, it is important to use commercial formulations of food products during validation studies.

2.8.3 Heterogeneous foods

HPP is governed by the isostatic principle, meaning that pressure is applied uniformly and almost instantly through the food product, regardless of shape or size. However, care is still required for complex heterogeneous foods such as meat with bones where pressure gradients can form, resulting in non-uniform pressure and temperature distributions. Given the temperature and pressure are controlling factors for microbial inactivation, this may result in different inactivation rates spatially across the food product. Therefore, it is important for validation studies of heterogeneous foods, that the complete packaged food product is tested, rather than a smaller subsample of the food.

2.9 ADDITIVES

The ability of HPP to inactivate microorganisms during processing or to inhibit their growth during post- HPP storage, can in some cases be increased by including an antimicrobial hurdle. The efficiency of antimicrobial agents can be specific to different microbial species, so validation studies or data collection evaluating the use of an antimicrobial hurdle will need to consider the pathogens/microbes of concern for the particular food product

The review papers provide examples of studies where a combination of HPP and an antimicrobial have been studied in a food matrix. The reviews do not give any indication of the amount of the antimicrobial that has been considered or full test and storage conditions. Table 3 gives a summary of these examples and which review paper they came from.

Increases in pressure can result in increased membrane permeability. This may explain why bactericidal compounds, such as nisin, are more effective in combination with the HPP; the

[3] https://www.mpi.govt.nz/food-safety/food-safety-and-suitability-research/food-risk-assessment/hazard-data-sheets/data-sheets/ (Accessed 19 October 2018)

increased membrane permeability permitting greater influx of the bactericide. Bacteriophages inoculated in or onto food before HPP may also be more effective following HPP due to sub-lethal cell injury, which makes cells easier to infect (Baptista 2016).

Table 3: Examples of food and additive combinations that have been studied with HPP and inactivation compared to HPP alone.

Food matrix	HPP[1]	Additive	Test pathogen	Inactivation (Log_{10} cfu/g or ml)	Review paper
Rice pudding	500 MPa/22°C/5 minutes	*No Additive* Enterocin[3] AS-48 Nisin[3] Cinnamon oil Clove oil	*S. aureus*	*2.9* 3.3-3.5 3.8 4.2[2] 4.7[2]	Baptista (2016)
Skimmed milk	250 MPa/25°C/30 minutes	*No Additive* Lacticin 3147	*S. aureus*	*2.2* >6.0	
Pasteurised milk	345 MPa/50°C/5 minutes	*No Additive* Nisin+Pediocin[3] AH	*S. aureus*	*5.5* 8.3	
Fuet (fermented sausage)	400 MPa/17°C/10 minutes	Enterocin AS-48 Enterocin A & B	*S. aureus*	Failed to enhance inactivation over HPP alone	
Cured beef carpaccio	450 MPa/5 minutes	Lactoperoxide Activated lactoferrin	*S. enteritidis &* *E. coli* O157:H7	Increased inactivation over HPP alone	Barba (2017)
Sliced dry cured ham	450 MPa/10 minutes	Lactoperoxide Lactoferrin	*L. monocytogenes*	Failed to enhance inactivation over HPP alone	
Yoghurt	600 MPa/Room Temperature /5 minutes	Mint essential oil	*L. monocytogenes*	Increased inactivation over HPP alone	Ferreira (2016)
Semi-skimmed milk		Carvacrol (component of oregano essential oil)	*L. monocytogenes*	Increased inactivation over HPP alone	
Cooked chicken (25g samples, initial inoculum)	600 MPa/20°C/2 minutes	*No additive* 2% sodium lactate	*L. monocytogenes*	*positive samples* *3/3[4]* 0/3	Patterson (2011)

1: It is not clear if these are pre-compression or pressurised temperatures

2: The values in the Baptista (2016) Table 3 are incorrect, this table has the correct values

3: Nisin (*Lactococcus lactis*), Pediocin (*Pediococcus acidilactici*, *Pediococcus pentosaceus*), Enterocin (*Enterococcus*)

4: 3/3 means three out of the three samples were positive for *L.monocytogenes*.

2.10 PRESSURE

2.10.1 Inactivation as a function of target pressure and holding time

HPP is similar to thermal processing in that there is a threshold value (specific to each microorganism) below which no inactivation occurs. Above the threshold, the lethal effect of the process tends to increase as the pressure and/or temperature increases. For example, for a single strain of *S. aureus* in peptone water, HPP treatment for 5 minutes at 40°C and target pressures over 300MPa, resulted in changes to the cell surface and shape, but pressures up to 250 MPa showed no significant changes (Baptista et al 2016).

Optimum levels of pressure and temperature need to be established to determine the most efficient and consistent kill rates for bacteria in foods (Food and Drug Association 2000).

However, unlike thermal process experiments, it is not possible to take samples during the HPP treatments. Samples can only be enumerated for microorganisms following decompression of the sample. Therefore, study results incorporate any inactivation due to compression, holding time and decompression.

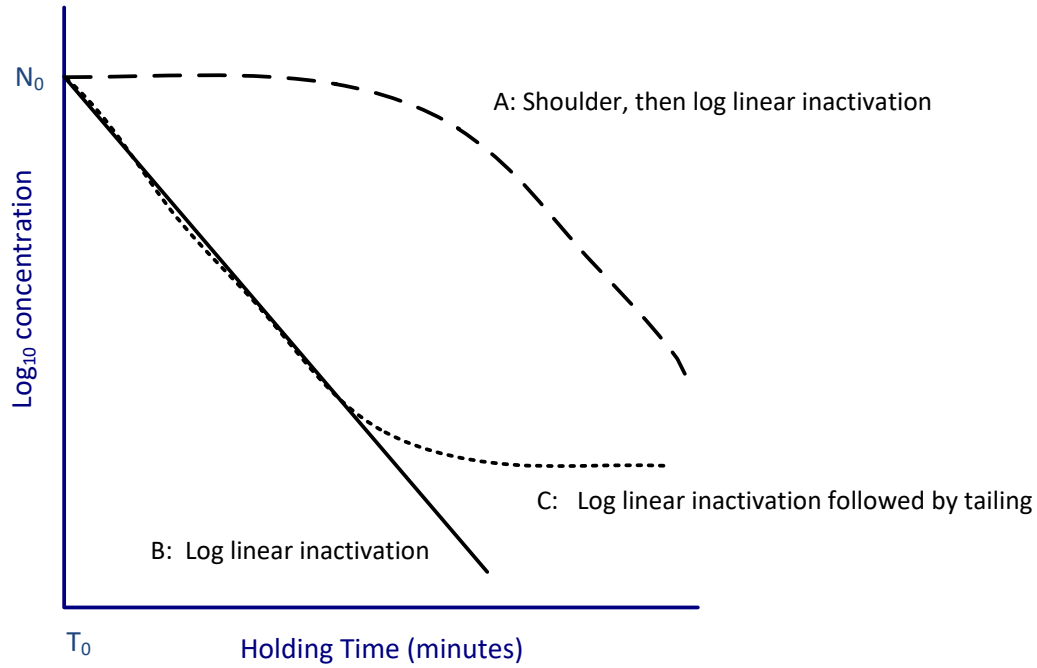
Figure 4 shows some possible shapes of the relationship between the logarithm of the microorganism concentration following decompression and the holding time:

- Curve A illustrates a shoulder effect where the cells can survive HPP for shorter holding times, but for longer holding times the cells become lethally injured by the process.
- Curve B illustrates a log linear inactivation (first order kinetics) which can be modelled using the D value approach, commonly used for thermal inactivation.
- Curve C illustrates a tailing effect, where it is possible a small population of the cells are more pressure resistant or protected by the food matrix and are able to survive the HPP treatment.

From Figure 4, it can be seen that increased holding time does not always lead to increased inactivation, when shoulders (A) and/or tails (C) in the inactivation rates are present.

If the HPP inactivation of cells does not follow first-order kinetics, then non-linear models such as the Weibull model must be used to explain inactivation curves. Other studies have suggested statistical based models that do not express the physical meaning of the HPP process, but are able to correlate high pressure-related processing parameters with the determined responses and provide robust predictive results (Tao et al. 2014).

Figure 4: Inactivation kinetics



If the inactivation has been shown to be log linear with holding time (Figure 4, line B), the D value approach[4] can be applied. The proposed commercial holding time must be within the holding times of the experimental/validation data. If D values are calculated from studies with shorter holding times, but tailing of inactivation occurs after this time (Figure 4, line C), the D value approach will over-estimate the inactivation.

A study considering the pressure inactivation of *E. coli* MG1655 in fresh carrot juice found D values were appropriate to be applied for pressures in the range 150 to 600 MPa, with temperatures in the range 5 to 49.5°C (Syed et al 2016). The same review paper suggests D values are also appropriate for *S. aureus* in cow's milk at 20°C, however the inactivation against time plot provided, suggests that for pressures of 300 to 350 MPa, a D value approach may be valid, but at 200 MPa some shouldering may be present.

Tailing has also been observed in studies for *Salmonella* and *Yersinia* strains inoculated in ultra-heat treated (UHT) milk, and processed at target pressures of 350-600 MPa. It is unclear from the review paper (Trujillo et al. 2016), the length of holding time at the target pressure after which tailing of inactivation rates was observed.

It is important when evaluating published study results for validation purposes, to be aware that only specific holding times are reported on, and it is not possible to automatically extrapolate between these holding times or outside the holding times given by the data.

[4] D value is the time taken to achieve a one $\log_{10}$ reduction in the cell concentration.

2.10.2 Compression and decompression rates

The few studies that have reported the effects of compression and decompression rates on the inactivation kinetics of microorganisms report some contradictory results and conclusions. However, some of the differences in these results may be explained by differences in experimental factors other than compression and decompression rates, such as temperature at the target pressure and the microorganism used.

It is not clear from the review papers what quantitative range of compression/ decompression rates are considered to be high or low.

Studies have suggested:

- A low compression rate induces a stress response from microbial cells, consequently leading to a less effective process, i.e. lower microbial inactivation.
- A high compression rate will lead to a rapid temperature increase, which is unlikely to dissipate during the compression phase, but may dissipate during the holding time depending on the HPP equipment. For lower compression rates, depending on the heat transfer abilities of the equipment, it is possible heat will dissipate during the compression phase, reducing the temperature reached at the start of the holding time.
- A low decompression rate will potentially increase the time at higher pressures and temperatures closer to the pressurised temperature.
- A high decompression rate may induce a fast adiabatic expansion of water generating an impulsive force which is more likely to inactivate or damage cells.

One study suggested that if the temperature at the target pressure is the same, the inactivation of *L. innocua* was similar for compression: 100 MPa/min with decompression: 500 MPa/min, and vice versa. Another study looking at HPP inactivation of *Bacillus* spores at 600 MPa/60-70°C, suggested the combination of fast compression and slow decompression “resulted in the highest injured/germinated population” (Syed et al. 2016).

It is unclear from the review papers which compression and decompression rates would optimise microbial inactivation, and how much the choice of rates effects efficiency of inactivation. Therefore, it is important to use the similar compression and decompression rates in validation studies as will be applied during commercial processing.

2.10.3 Cyclic Pressure

A number of studies have considered applying a series of pressurisation cycles, fully decompressing between each cycle, sometimes referred to as pulsed HPP treatment. In some cases, the pulsed approach can achieve greater inactivation, than a single treatment with the same combined holding time. As discussed in section 2.4, a series of pressurisation cycles may assist in the inactivation of spore forming bacteria.

An example is given in the review by Baptista et al. (2016), where the inactivation of a single strain of *S. aureus* in broth resulted in an inactivation of 1.9 log cfu/ml after a single holding time of 15 minutes, compared to 4.15 log cfu/ml following 5 cycles of 3 minutes holding time. The target pressure is not given in the review paper.

The *Listeria* review paper (Ferreira et al, 2015) states that studies have shown pulsed applications have been more effective than a single holding time for reducing *Listeria* in kiwi fruit juice, but not in pineapple juice, whole milk or raw milk cheese. However, no HPP parameters (pressure, holding times, number of cycles, temperature) are given in the review.

2.11 TEMPERATURE

The mechanisms of bacterial inactivation due to pressurisation is different to thermal inactivation. Combinations of pressure and temperature can be chosen to enhance the inactivation rates of microorganisms during HPP.

2.11.1 Pressurised temperature

The review papers suggest there is a phase transition of membrane lipids at temperatures above 35°C (Baptisita et al. 2016) and at temperatures above 45°C, hydrogen bonds and hydrophobic bonds may be weakened (Vachon et al. 2002), making bacterial membranes less resistant to pressure.

There is insufficient data in the review papers to provide threshold values for temperature and pressure combinations to ensure a given inactivation of microorganisms.

One review paper described a study which gave examples of the pressurised temperature and target pressure combinations required to produce a 5 log inactivation from a 5 minute holding time (Syed et al. 2016). The test matrices were not given. The results suggested there was a pressurised temperature range (10 and 40°C) for which bacteria was most pressure resistant.

Moving away from this temperature range the pressure resistance decreases. At pressurised temperatures above 40°C for the *L. monocytogenes* and *C. jejuni* strains, and above 50°C for the *E. coli* strain, the required target pressure decreased rapidly with increased temperature (Table 4). For temperatures between 0 and 10°C the pressure resistance slightly decreases with decreasing temperature.

Any differences in inactivation due to different pressurised temperature may be influenced by holding time. One study using UHT whole milk, considered the inactivation of a single strain of *S. aureus* at 600MPa and 4, 21 or 45°C. No difference in the inactivation was observed between the different temperatures after a holding time of 4 minutes (~4 log cfu/ml). At a holding time of 8 minutes, the temperature effect became visible, however it is not clear from the review paper what or how big these effects were.

Table 4: Exemplar pressures required to achieve a 5 log inactivation after 5 minutes of holding time at given pressurised temperatures (Numbers approximated from Figure 2 of Syed et al (2016), source publication by Buckow and Heinz (2008)).

	Required target pressure (MPa) at pressurised temperatures of			
	5°C	**10-40°C**	**50°C**	**60°C**
L. monocytogenes strain	450	In range between 480 and 520	430	270
C. jejuni strain	330	In range between 330 and 370	250	50
E. coli strain	310	In range between 320 and 330	280	150

While some commercial HPP units have temperature sensors to measure the temperature of the pressure medium during HPP, it is not possible to directly measure the temperature of the food, or establish if there are temperature gradients throughout heterogeneous foods. It is also challenging to simulate the temperature variation in pressure processed products and to predict the local temperature profiles during processing using mathematical tools. Knoerzer and Chapman (2011) provide an example of how computational fluid dynamics models may be useful to model temperature profiles and changes in the vessel and food during HPP.

It is important when evaluating published study results for validation purposes, to be aware that only specific temperatures are reported on, and it is not possible to automatically extrapolate between these temperatures or outside the temperatures given by the data.

2.11.2 Cell growth temperatures prior to HPP

The efficiency of inactivation by HPP can be significantly affected by the temperature at which microorganisms are growing prior to HPP treatment. Inactivation of *L. monocytogenes* was less efficient when the bacterium was grown at temperatures near 43°C (for 12-14 hours) compared to lower temperatures,10-25°C, for longer time periods (Ferreira et al. 2016). This can have practical implications because it is possible that bacteria from warm animal sources, may be more pressure resistant than cooler environmental sources of contamination such as equipment surfaces.

Baptista et al (2016) state that “bacterial cells previously subjected to other stress conditions such as sub-lethal heat or cold shock (due to the increase on the percentage of polyunsaturated fatty acids in cell membranes) become more resistant to pressure.” This statement is based on a study on *E. coli*, but no further details are provided in the review paper.

2.11.3 Equipment dependant temperature

Small laboratory equipment, as is commonly used by published studies are likely to have different temperature profiles over time than larger commercial systems. Small systems without some external temperature control of the vessel will lose or gain heat faster than larger systems during HPP, due to the larger surface area to volume ratio.

This means it is important to know the expected temperature profile in the commercial equipment. If smaller HPP equipment is used for a validation study, the time-temperature profile should be comparable. As has been discussed above, pressure sensitivity is temperature dependant. Depending on the temperature range, a higher temperature in the commercial equipment compared to smaller scale equipment may either be protective to pathogen cells or make them more sensitive to injury or inactivation.

2.12 PACKAGING

In a batch processing system, food products need to be packed in a flexible container prior to HPP, to compensate for the potential volume reductions in the food inside the package as well as the collapse of the head space[5]. The head space should be minimised; to assist in pressure transmission to the food, to help minimise the deformation of packaging and make the most use of the space inside the pressure vessel.

A number of factors require consideration when selecting appropriate packaging types for HPP, including:

- The flexibility of the packaging to ensure it is structurally sound during volume reduction and during the return of the product to the original volume.
- The barrier properties of the packaging such as the permeability (water, oxygen or carbon dioxide) and the potential migration of packaging material to the food should not change due to HPP.
- The packaging material should not delaminate under pressure. For example, metalised polymeric film may undergo changes in structure and barrier properties due to the different compressibility of metal and polymer layers (Tao et al 2014).
- The seal or lid integrity must be sufficient to be maintained throughout HPP.

In general, most synthetic materials used for food packaging can withstand HPP treatment without changes to their structural and functional properties, but must have a compressibility of at least 15% for HPP. For batch in-container processing, plastic packaging materials are the best suited for HPP applications, because of their reversible response to compression, flexibility and resilience. Glass, metal and paper are not suitable. The most common

[5] Head space refers to the void space between the top of the food material and the packaging material

packaging materials used for high pressure processed food are polypropylene (PP), polyester tubes, polyethylene (PE) pouches, and nylon cast polypropylene pouches.

Active packaging can be used in combination HPP to reduce microorganism concentrations. For example, the addition of coriander essential oil to active packaging combined with HPP (500 MPa/1 minute), resulted in a reduction in *Listeria* spp. counts on RTE chicken breast to below the limit of quantification during the 60 days of storage at 4°C. (Barba et al. 2017).

2.13 CONCLUSIONS

This section has illustrated there are multiple factors which have the potential to effect the pressure sensitivity of bacterial pathogens during HPP treatments, and therefore the food safety of a RTE product treated in this way.

Collating information given in the review papers and from searches of government websites does not provide sufficient evidence to provide generic guidelines to ensure sufficient inactivation of cells during HPP. Inactivation data specific to the product formulation, packaging and HPP parameters needs to be sourced to validate a commercial process.

While specific guidelines are not able to be determined, general observations are identified in the review papers and these are summarised in Table 5.

Table 5: Factors influencing microbial sensitivity to high pressure processing

	Factor	Effect
EXTRINSIC PROPERTIES	Food composition	Food ingredients or elements such as proteins, fats, sugars, salts and minerals can provide a protective effect and therefore increase the microbial resistant to pressure.
	pH	As pH is lowered, most microbes become more susceptible to HPP inactivation and sub-lethal injured cells fail to repair.
	Water activity (a_w)	Reducing a_w tends to protect microbes against inactivation by HPP. Foods with low water activity (e.g. flour) are not appropriate for HPP. Recovery of injured cells can be inhibited by low a_w.
	Pressurised Temperature	The pressurised temperature effects the sensitivity of bacterial cells to pressure. Temperatures in the range 10-40°C are likely to correspond to the highest pressure resistance of bacterial cells, while temperatures above 50°C rapidly decrease the pressure resistance with increasing temperature. Pressurised temperatures below 10°C can also produce a decrease in pressure resistance with decreasing temperature.
	Cell temperature prior to HPP	The temperature cells are growing prior to HPP may affect their sensitivity to damage by pressure.
	Antimicrobials	The combination of HPP and antimicrobial compounds can promote the elimination of pressure-resistant microorganisms, decrease the temperature necessary to inactivate microbes and help prevent the repair of sub-lethal injured cells during storage.
INTRINSIC PROPERTIES	Growth phase	Microorganisms in the exponential phase of growth (a period of time where the cell numbers are doubling and growth is at it fastest) are less resistant to pressure than in the stationary phase of growth (a period of time following the exponential phase in which the growth rate and death rate are equal).
	Spore-formers	Spores are highly resistant to pressure. A combination of pressure (>800 MPa) and heat (>80°C) such as PATS processes or high pressure in combination with other antimicrobial treatments are required to achieve a significant reduction of spores in foods Spores have been observed to germinate during pressure treatments up to 400MPa.
	Sub-lethal injured cells	Sub-lethal inactivation by HPP can lead to stressed or injured cells that can recover under certain conditions and present a risk of re-growth of the microorganism during the shelf life of a food. If pathogens are not permanently inactivated by HPP, robust food formulations or storage conditions that inhibit the growth of injured cells after processing required.

3. INTERNATIONAL REGULATION / APPROVALS

3.1 AUSTRALIA AND NEW ZEALAND

The Advisory Committee on Novel Foods (ACNF) determines whether a food is subject to the requirements of Standard 1.5.1 – Novel Foods. Novel food is a food that is classed as a non-traditional food and the food requires an assessment of the public health and safety considerations. A commonly consumed food can be considered non-traditional if prepared by a process not previously applied to the food.

The ACNF determined HPP as a well-established food processing technology which is not considered a novel process. “The use of HPP for traditional foods that do not have a prescribed pasteurisation step (or where HPP is used in addition to pasteurisation) is not considered to make a food non-traditional”.[6]

In New Zealand, HPP is not considered a novel food processing technology. HPP foods are required meet the requirements of the food standards in terms of microbiological limits (Standard 1.6.1) and schedule 27 of Food Standards Code[7]. Validation of these requirements is required. Section 7 of the Further Processing Code of Practice[8] gives general guidance on HPP and validation of HPP processes.

No specific guidelines for acceptable HPP processes were found on Australian government www sites. New South Wales has produced generic guidelines[9] which provide an introduction to aspects of HPP food safety.

3.2 EUROPE

In July 2001 the European Commission concluded that HPP was no longer to be considered a novel process requiring extra risk assessment (Cholewińska, 2010). However, some states argued that HPP foods should be assessed on a case by case basis and the approach taken by member states may vary. No generic or specific guidelines for acceptable HPP processes were found on the European Food Safety Authority www site.

The thesis (Annex IV) by Cholewińska (2010) provided one detailed process approval in 2001 for producing fruit based preparations treated with HPP as an equivalent to a heat pasteurisation process. The process parameters were 800MPa / 20°C / 6 minutes. Fruit had

[6] http://www.foodstandards.govt.nz/industry/novel/novelrecs/Documents/Novel%20Foods%20-%20Record%20of%20views%20Mar%202018%20Update%20%28002%29.pdf (Accessed 20 August 2018)
[7] https://www.mpi.govt.nz/law-and-policy/legal-overviews/food-safety/australia-new-zealand-co-operation/food-standards-australia-new-zealand-fsanz/ (Accessed 19 October 2018)
[8] https://www.mpi.govt.nz/dmsdocument/1365/loggedIn (Accessed 19 October 2018)
[9] http://www.foodauthority.nsw.gov.au/news/newsletterarticles/foodwise/2016/high-pressure-processing (Accessed 20 August 2018)

to be stored for a minimum of 15 days at -20°C before pressurisation, 40% to 60% of the fruit added to product, product pH between 3.2 and 4.2 and a_w of < 0.95 (assured by sugar content). Final storage was for a maximum of 60 days at 5 °C.

3.3 CANADA

From, December 2016 in Canada, food products treated with HPP were no longer considered to be novel foods[10]. HPP products must comply with Food and Drugs Act and Regulations.

In the Canadian Guidance on Food Products Treated with High Pressure Processing[11] the following guidance is given:

- Where HPP is used as a critical control process for pathogen reduction, there is a requirement for consultation and validation with the Canadian Food Inspection Agency (CFIA).
- HPP treatment of RTE meat and poultry products for 3 minutes at 600 MPa has been validated to achieve a minimum 3 log reduction in *L. monocytogenes*. No other parameters or source references for the validation are given. However, this could be based on the novel food validation listed below.
- When HPP is used as a post-lethality treatment, 3 minutes (to a maximum of 27 minutes) at 600 MPa may be used to move the product to a lower relative risk level for sampling purposes, but does not move the product to a lower risk RTE product category.

There are a number of evaluations for HPP products that were completed by the Novel Foods Section of Health Canada[12]. These included the HPP of fruit purees, RTE meat and poultry and raw ground beef.

In 2005, Health Canada concluded there were no human food safety concerns associated with apple sauce and apple sauce fruit blends packaged in single serve flexible tube packaging, after being treated for 1 minute at 550 MPa (pre-compression temperature, product pH and a_w not listed). The product was to be stored at room temperature post HPP. This ruling was based on validation trails using raw product spiked with separate cocktails (5 strains but not listed) of *Salmonella* spp., *E. coli* O157:H5, *L. monocytogenes* spp. and *Cryptosporidium parvum*. A reduction in *Salmonella* spp., *E. coli* O157:H5, *L.*

[10] https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/position-high-pressure-processing-no-longer-novel-process-treated-food-products-treated-food-products-2013.html (Accessed 20 August 2018)

[11] http://www.inspection.gc.ca/food/safe-food-production-systems/technical-references/high-pressure-processing/eng/1498504011314/1498504256677 (Accessed 20 August 2018)

[12] https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products.html (Accessed 20 August 2018)

monocytogenes spp. of more than 7 log units were obtained after HPP and growth was not observed on testing at 2 months following treatment. *Cryptosporidium parvum* initially inoculated at 4.25 log oocycts/g puree was not detected after treatment.

Validation trials were also assessed in 2007 to establish the efficacy of *inactivating L. monocytogenes* in RTE meat and poultry products, including those with a_w above 0.9 (ham and sliced turkey) and a_w below 0.9 (sliced prosciutto and salami). The trials suggested that a treatment of 600 MPa for three minutes was sufficient to achieve a 4.5 log reduction in *L. monocytogenes* (cocktail of five strains) inoculated onto prosciutto (whole and sliced), Genoa salami (sliced), porchetta (sliced) and cooked sliced hams. A treatment of 600MPa for two minutes was sufficient to achieve a 4 log reduction in *L. monocytogenes* in sliced cooked turkey. In both trials no survivors were detected by direct counts 10 days post treatment, but the limit of detection is not given. No product pH or pressurised temperature is given in the summary.

In a later submission (2013), an assessment was made for HPP applied to raw ground beef (protein fat ratio of 73%:19%) for both extending shelf life and to inactivate *E. coli* O157:H7. This validation trial used a cocktail of 5 *E. coli* O157:H7 strains inoculated onto meat and treated at 600MPa for 1 or 3 minutes. The study suggested a greater than 5 log cfu/g reduction was achieved using a 1 minute holding time.

The Bureau of Microbial Hazards assessors said it was possible to state that 600 MPa for 1 minute reduced the concentration of *E. coli* O157:H7. However, it would not issue an opinion regarding the specific efficacy of inactivation, because, no information was provided regarding the resistance to pressure of the trial strains compared to the variability known to exist between *E. coli* strains.

The earlier submission assessments did not comment on the pressure resistance of the strains chosen for the validation studies.

3.4 USA

HPP is not considered a novel process in the USA. When a processor uses HPP as an anti-microbial treatment, USDA Inspection Program Personnel (IPP) are required to verify the hazard analysis supports the use of HPP treatment in controlling pathogens in the product.

FSIS directive 6120.2 (2012) provides general guidance to IPP on the validation of HPP processes, but does not give any examples of critical operating parameters for any food types. It identifies the following as critical to the evaluation of supporting evidence that the HPP process can address identified hazards in commercial processes:

- Operating parameters - target pressure, holding time, pre-compression and pressurised temperatures, compression and decompression times and the absence or presence of added CO_2 within packaging[13].

- Food specific factors such as pH, water activity, composition and preservatives.

One application to the US FDA in 2017 for HPP treatment of juice was rejected, partially due to the challenge studies their application was based on[14]. Each study was based on a single HPP process run with multiple samples, so there was no replication of the HPP process to understand process variability. They also stated that insufficient data was given about the juice properties to conclude the HPP processing conditions could be applied to all juices with a pH equal or less than the juices studied as the processor was suggesting. They state the "FDA is not aware of any broad HPP validation study that covers juice products with varying compositions and characteristics".

[13] Farkas and Hoover (2000) discuss the use of CO_2 combined with the HPP process as a preservative method. Most of the listed papers used very low pressures e.g. < 15 MPa in combination with CO_2. The holding times listed were for 20 minutes to multiple hours, which would questionably be too long for most commercial applications. The efficiency of the inactivation was dependent on the food matrix.

[14] https://www.fda.gov/iceci/enforcementactions/warningletters/2017/ucm564197.html (Accessed 24 July 2018)

4. HPP FOOD SAFETY VALIDATION

4.1 INTRODUCTION

The earlier sections of this report have highlighted the need for careful consideration of a range of factors when validating a HPP as a critical control step for food safety.

Processors wishing to use HPP will generally look to apply published processing conditions for a food that closely matches their product to provide evidence that HPP can be used as a critical control step. While there has been an increase in the scientific literature available in this field in recent years, most studies are focussed on extending shelf life of a product. In most cases there are insufficient detail in the published studies about the product composition or HPP parameters/equipment to allow a direct application to commercial processes.

Potential sources of information include published scientific studies, challenge studies, in-plant data or other types of scientific support from an expert research organisation or from the equipment manufacturer. Where challenge studies are required then a processor would usually require the services of a HPP expert and a microbiological laboratory with experience in conducting challenge trials (New South Wales Food Authority 2016).

4.2 VALIDATION FACTORS

This section summarises the factors that should be considered either in conducting a challenge study or in evaluating evidence from third party studies. Table 6 lists the factors that have been identified in sections 2 and 3 as important in providing robust evidence in respect to HPP treatments. Factors generic to all food safety challenge studies are not listed in the table.

Table 6: Critical factors relevant to HPP validation studies

Factor	How	Why
Test microorganisms (section 2.3)	For **each** pathogen of concern the inoculum should be a cocktail of strains including some pressure resistant strains.	There is a wide variation in resistance to pressure between strains of the same bacteria
Inoculum (section 2.6)	The inoculum should be in a stationary growth phase	A processor is unlikely to know the physiological state of pathogen cells prior to HPP Cells in the stationary growth phase are more resistant to pressure treatment than actively growing cells

Factor	How	Why
Spore forming bacteria (section 2.4)	If spore forming bacteria are a pathogen of concern, these must be included as one of the microorganisms in challenge study. An inoculum of spore forming bacteria should include cells insporolated form.	High pressure at commercial HPP target pressures will not inactivate spores. HPP combined with other hurdles and/or cyclic HPP are required to ensure inactivation of spores Pressure treatments up to 400 MPa may help trigger germination of spores
Enumeration methods (section 2.7)	The procedure to enumerate the number of surviving cells following treatment should be appropriate to detect stressed or injured (but repairable) cells.	It is important to identify if sub-lethally injured cells may be able to recover and grow during the shelf life of the product
Sampling time points (section 2.7)	The product should be tested (i) before start of HPP treatment, (ii) following decompression and (iii) at times suitable for testing during the shelf life of the product and beyond.	It is important to identify both the immediate effect of the HPP treatment and also if sub-lethally injured cells are able to recover and grow during the shelf life of the product.
Food product (section 2.8-2.9)	The same product formulation should be used as for the commercial product. Different batches of the product should be tested For heterogeneous foods, the complete packaged food should be tested.	Product composition and properties have been shown to have an effect on the pressure resistance of cells. Current knowledge does not allow extrapolation of results between different product formulations. To capture process variability, due to seasonal or between batch differences in ingredients. It is possible that non-uniform pressure and temperature gradients may result in heterogeneous foods. These are not currently able to be modelled or measured, so the commercial product should be tested.
Packaging and sample size (section 2.12)	Packaging should be the same material as used in the commercial HPP. If the package is fully flexible with minimum head space, and the product is not heterogeneous, it may be possible to test a smaller size sample in same packaging material.	Packaging may affect the temperature and pressure of the product during HPP.

Factor	How	Why
Pressure and temperature parameters during HPP (sections 2.10-11)	Equipment used for the challenge study, should provide comparable pressure and temperature against time profiles as the commercial equipment that will used, including any storage, pre heating or chilling stages prior to pressurisation. Post HPP storage temperatures should be comparable to commercial/domestic storage.	Cell inactivation rates have been shown to be effected by: • Compression and decompression rates • Target pressure • Pre-compression and Pressurised temperatures • Post HPP storage temperature
Holding time during HPP (section 2.10)	Holding time should be the same as for the commercial product. Inactivation data from studies should not be extrapolated between or beyond the holding times given, unless sufficient evidence of the shape of the inactivation curve is given.	The inactivation kinetics of pathogens is non-linear for some HPP treatments. It is not clear when linear relationships can be assumed.

Sample analysis performed for validation purposes must be done using methods that permit the accurate and reproducible recovery of the target microorganisms. In particular, care must be taken with experiments involving spore-formers as recovering and enumerating the number of spores following HPP treatment can be difficult and at times not reproducible and can cause false-negative results (Dr Alvin Lee, Director of Illinois Institute of Technology, personal communication). Therefore, it is recommended that the experimental design and the microbiological testing for spore-formers and vegetative cells is comprehensive and includes a number of experimental repetitions to provide confidence in the inactivation results.

A change in; product ingredients, product formulation, pre-HPP processing, packaging or the HPP process may require further validation studies to be completed.

4.3 CHOICE OF TEST MICROORGANISMS

4.3.1 NACMCF Guidelines

The choice of bacterial species for lethality or survival studies relevant for HPP depends on the selection of resistant strains relative to the process and technology as well as the compliance with applicable regulations for the food. NACMCF guidelines (NACMCF 2010) for conducting inhibition and inactivation challenge studies on pathogens does not specify any bacterial strains for the purpose of validating HPP processes. In general the choice of the organisms for inactivation studies should be based on the likelihood of pathogen association with the specific food and pathogen resistance to inactivation, as well as the public health objective of the process and the intended use of the product (NACMCF 2010).

Many studies investigating the use of HPP to inactivate microorganisms in foods have used bacterial isolates from in-house collections, which were primarily selected because they were originally associated with the food product of interest (whether isolated from the food or processing environment or from clinical cases). This is an important criterion when selecting isolates for the purpose of validation as noted in the NACMCF guidelines.

In addition, the guidelines recommend using an inoculum composed of multiple strains (i.e. a cocktail) of a given pathogen in order to encompass the variability among organisms and may also reduce the number of tests required. An alternative approach is also to screen several strains in a food matrix under investigation and determine which strain has the greatest resistance and conduct subsequent experiments using that single strain.

4.3.2 Surrogate organisms

The NACMCF guidelines recommend the use of surrogates, which are non-pathogenic species and strains that have demonstrated an equivalent response to HPP treatment than the equivalent pathogenic species and strain. Surrogates are useful as they allow validation of a HPP treatment without introducing pathogens into a processing area.

The choice of surrogate for HPP validation purposes needs to be justified. If no direct relevant published comparison data are available, then studies need to be conducted to establish the validity of using a particular surrogate-pathogen-process combination. Care must be taken as a surrogate that works well to predict the target response for one type of process may not be appropriate for a different type of process; for example the heat resistance of various strains of *C. botulinum* spores do not correlate with their resistance to HPP (Margosch et al. 2004).

B. amyloliquefaciens TMW 2.479 has been proposed as a surrogate for *C. botulinum* in high-pressure and high-temperature processing of low acid foods, as it is non-pathogenic and non-toxigenic and exhibits a higher resistance to combined heat and pressure treatments than spores from *C. botulinum* (Margosch et al. 2004). *L. innocua* has been used as a surrogate for *L. monocytogenes* in the HPP literature.

Sheen et al. (2015) have suggested the use of a non-pathogenic *E. coli* strain, *E. coli* O128 B-59801, as a surrogate for pathogenic *E. coli* strains. This strain had resistance to inactivation by HPP (measured by *D* value) at 350MPa and pre-compression temperature of 4°C, was similar to the mean D value of 39 Shiga toxin producing strains of *E. coli*.

4.3.3 Microorganism strains identified more resistant to HPP or suitable for challenge studies

Table 7 gives suggested microbial cultures for HPP process development, challenge work and process validation, as reproduced from the review of Farkas and Hoover (2000). Pathogens are indicated with (P) whilst a surrogate organism is indicated by (S), followed by the relevant food type. However, the review does not state the justification as to why these bacterial isolates are recommended.

Table 7: Suggested micro-organisms for HPP process development listed in Farkas and Hoover (2000), no justification provided for recommendations.

Microorganism	Strain	Food Matrix
L. monocytogenes (P)	Scott A (NCTC 11994)	Dairy, meat, seafood, vegetables
E. coli O157:H7 (P)	NCTC 12079	Meat
S. aureus (P)	NCTC 10652	Poultry products
S. Typhimurium (P)	DT104	Poultry products
C. botulinum (P)	62A, 17B or Beluga	Meat, seafood
C. sporogenes (S)	PA3679	Meat

A heat-resistant beef isolate of *E. coli* AW1.7 has been identified in the literature as one of the most pressure resistant vegetative bacterial isolates characterised to date (Hauben et al. 1997; Liu, Betti, and Ganzle 2012). *E. coli* AW1.7 was found to have comparable resistance to a pressure resistant mutant *E. coli* isolate (LMM1030) in poultry meat (Liu, Betti, and Ganzle 2012). Further work would be required to establish if this could be a worst case surrogate for all vegetative pathogen inactivation.

In the literature reviewed in section 6 on the HPP treatment of milk, a few strains have been identified as being more pressure resistant than other strains the researchers have tested. These are summarised in Table 8.

Table 8: Examples of pressure resistant pathogen strains identified in the Section 6 literature.

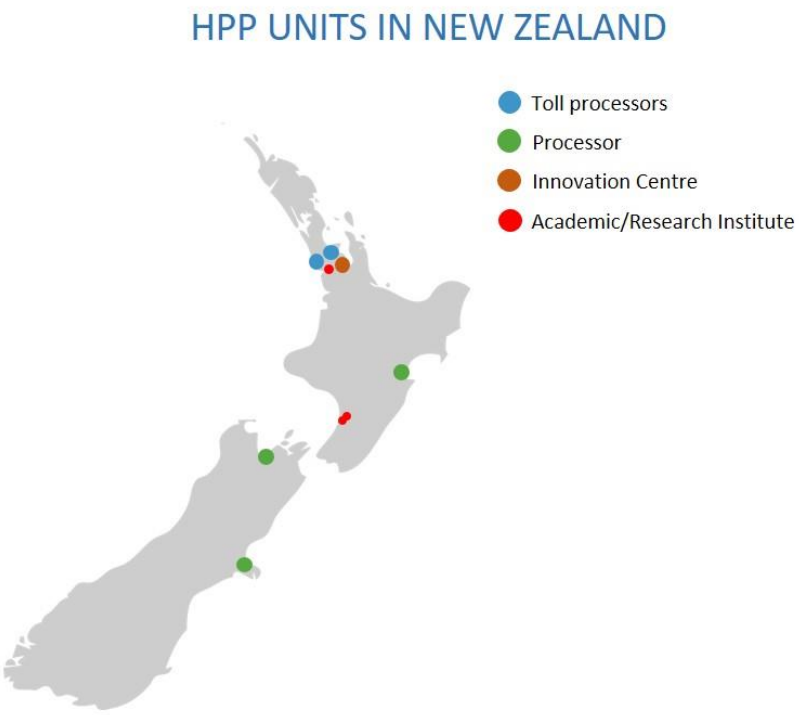
Bacteria	Strain	Reference
Campylobacter spp.	*C. jejuni* NCTC 11351 *C. lari* NCTC 11457 *C. lari* NCTC 11352 Note: *C. fetus* strains tested were found to be less pressure resistant than the *C. jejuni*, *C. lari* and *C. coli* strains tested.	Martínez-Rodriguez, 2005
C. sakazakii	CECT 858	Arroyo, 2011
E. coli O157:H7	933 C7927	Alpas, 1999
L. monocytogenes	CA Scott A V7 35091 ATCC 19117	Alpas, 1999 Koseki, 2008
S. aureus	315 485 565 743 765 778 ATCC 6538	Alpas, 1999 Baptista, 2015

5. NEW ZEALAND HPP EQUIPMENT AND EXPERTISE

Four main HPP units have been identified within New Zealand academic/research institutes and innovation centres that could potentially be used for research or validation purposes (Figure 5).

There are also a number of processors or toll processors (processors that undertake HPP for other companies) that have HPP units in NZ, but these would not be available for research or validation studies.

Figure 5: High-pressure units identified in New Zealand (map credit: Kris Tong from the New Zealand Food Innovation Network)



1) The NZ Food Innovation Network (NZFIN) is an accessible, national network of science and technology resources created to support the growth and development of NZ food and beverage businesses of all sizes, by providing facilities and the expertise needed to develop new products and processes. Within this network, the FOODBOWL, situated in Auckland, has a HPP unit. The unit has a 55L capacity which normally runs at ambient temperatures, but cannot undertake cyclic processes. (http://foodinnovationnetwork.co.nz /high-pressure-processing).

The pressure medium can be chilled to as low 4-5°C or heated above room temperature to a maximum of 38°C, however the machine supplier does not recommend the pressure medium being heated above 30°C. Currently this unit cannot be used to carry out challenge trials on products inoculated with pathogens, and the assessment for the use of surrogates would require further discussion.

Al Baxter who is the Business Development Manager (al.baxter@nzfin.co.nz, Cell: 027 839 6768) is the initial contact for enquiries.

2) Plant and Food Research (PFR), Auckland has a HPP unit with a 300 ml chamber that can process ~200 ml samples at a time. The unit is currently not situated in a PC2 facility but there are protocols in place to work with vegetative organisms, but not spore-formers.

Dr Graham Fletcher (Graham.Fletcher@plantandfood.co.nz) is the main contact person for the HPP unit at PFR. Staff at PFR have undertaken research work on the use of HPP to inactivate *Listeria* in shellfish and mussel meat (Fletcher, Youssef, and Gupta 2008; Gupta et al. 2015; Stollewerk et al. 2017), as well as spore reduction in vanilla bean pulp. PFR has an extensive collection of Class II pathogens, used for HPP and other research.

3) Auckland University (AU), Department of Chemical and Materials Engineering has a unit with a 2L chamber[15]. They are able to test non-pathogenic bacteria and enzymes and conduct experiments focussing on food quality. The Avure 2L unit can be operated at pressures up to 600MPa and a temperature range of 4 to 70°C.

Prof. Mohammed Farid (m.farid @auckland.ac.nz) is the main contact person for HPP research. HPP research has been undertaken on honey, fruit purees and juice and shellfish (Akhmazillah et al. 2012, Fauzi et al. 2017, Gupta et al. 2015). PATS processing of baby food has also been investigated (Wang et al. 2017)

Both Prof. Farid and Dr Fletcher are able to upscale (non-pathogen) trials to the Auckland NZFIN facilities if needed.

4) Massey University (MU), Palmerston North campus has a HPP unit with a 3L chamber, but practically processes 2 x 200 ml samples at a time. It can process up to a temperature of 80°C and has the ability to perform cyclic/dynamic processes if required. The unit is currently within the commercial Food Pilot facility which is a part of the NZFIN, and is operating under a risk management programme. As a result, strict precautions and established procedures to process products containing pathogens would be required and approved by MPI prior to any experimental work being undertaken. Relocation of the unit into a PC2 facility is under consideration.

[15] http://www.uniservices.auckland.ac.nz/Portals/0/All%20One%20Pagers/High%20Pressure%20Processing%20of%20Food.pdf?_ga=2.73036496.2135980545.1532903422-350804013.1525740978

Dr Jon Palmer (J.S.Palmer@massey.ac.nz) is the main contact person for the HPP unit at Massey University and has expertise in HPP for food safety and has undertaken experimental and validation work for commercial clients.

6. APPLICATION OF HPP IN MILK

6.1 INTRODUCTION

Recently there has been a growing interest in the processing of raw milk using HPP as an alternative to thermal pasteurisation.

This section summarises the findings of a literature review of pathogen inactivation HPP experiments performed in milk.

6.2 MPI RECOMMENDATIONS

MPI has provided guidance on the expected microbiological inactivation to be achieved in milk for sale on the domestic and export markets (Table 9). The two categories of inactivation relate to the $\log_{10}$ reduction to be achieved to make safe product (column 1, domestic market), and to achieve an equivalent outcome to a thermal pasteurisation process of 72° for 15 seconds (column 2, export).

Table 9: Minimum $\log_{10}$ reduction of specified microorganisms in milk for domestic and export markets.

Pathogen	Log_{10} reduction for safe product	Log_{10} reduction to be equivalent to thermal pasteurisation
Campylobacter spp.	5	>7
Listeria monocytogenes	5	>7
Shiga toxin-producing *Escherichia coli* (STEC)	5	>7
Salmonella spp.	5	>7
Staphylococcus aureus	5	>7
Mycobacterium avium sub spp *paratuberculosis* (MAP) (surrogate for M. bovis) [1]	6 [2]	Estimate >7, may need data on *M. bovis*
Bacillus cereus	5	No data available

1. The inclusion of MAP is as a surrogate for *M. bovis*. The requirement to reduce the levels of *M. bovis* is based on the current NZ TB status
2. The addition of a 1 log buffer to recognise that these bacteria may not have identical inactivation profiles.

Technical specifications and regulatory requirement for raw milk produced in New Zealand are described in Animal Products Notice: Raw Milk for Sale to Consumers. HPP treated milk is not classed as raw as it undergoes treatment to achieve inactivation of microorganisms.

6.3 LITERATURE REVIEW

A systematic literature review on the effect of HPP on pathogens potentially present in New Zealand raw milk was conducted using the databases Pub Med and Web of Science. Experimental data and predictive models where no heat pasteurisation was applied prior to the high pressure treatment were included. Pressurised temperatures of up to 50°C and target pressures ≤ 600 MPa were taken into consideration as these were considered to be within the scope of future commercial milk HPP treatments. Details on the search strategy and inclusion/exclusion criteria are provided in Appendix B.1.

Additionally, websites from government, commercial and research organisations were searched, a list of which is available in Appendix B.2.

Although the main aim of this literature review was to identify data on HPP treatment of raw milk, other milk types were included for the following reasons:

- Most studies were done in heat-treated milk, i.e. for MAP, *Campylobacter* spp., *B. cereus*, *S. aureus*, *Yersinia* spp. and *Cronobacter sakazakii* all available studies were carried out in UHT or pasteurised milk. For *Listeria* spp., *E. coli* and *Salmonella* spp. studies in raw milk and UHT milk are available.

- No direct comparison of inactivation rates in raw and UHT treated milk (for *Listeria* and *E. coli)* was possible because studies were conducted under different process conditions. In raw milk studies, lower pressures were used compared to studies in heat treated milk.

The following microorganisms, including the most relevant pathogens for New Zealand's raw milk, were included in the scope of the literature review:

- *Bacillus cereus* (*B. cereus*)
- *Campylobacter spp.*
- *Clostridium* spp.
- *Cronobacter* spp.
- *Listeria monocytogenes* (*L. monocytogenes*) and *L. innocua* as surrogate for *L. monocytogenes*
- *Mycobacterium avium sub spp paratuberculosis (MAP) (surrogate for M. bovis)*[16]
- *Salmonella* spp.
- *Staphylococcus aureus* (*S. aureus*)
- STEC and other *E. coli*
- *Yersinia* spp.

[16] The inclusion of MAP is as a surrogate test for *M. bovis*. The requirement to include the elimination of *M. bovis* is based on the current NZ TB status.

6.4 ADIABATIC TEMPERATURE INCREASE IN MILK

In the milk studies described in section 6.6, temperature increases as a result of the adiabatic heating effect (section 1.3.2) have been found to range from 1 to 3.8°C per 100 MPa of increasing pressure (Table 10).

The adiabatic temperature increase is handled differently in different research settings. Some of the microorganism specific studies described in section 6.6 adjust the temperature of the HPP pressure chamber, pressure fluid and sample to a target (pressurised) temperature and record an increase in temperature due to adiabatic heat increase for a short period of time. After a peak at about 30 seconds, the temperature was found to drop to target temperatures after a few minutes, due to heat transfer from the milk/pressure medium to the stainless steel of the vessel (Chen and Hoover 2003; Guan, Chen, and Hoover 2005).

Other studies take the amount of expected heat increase into account and adjust the pre-pressurisation temperature (isothermal-endpoint procedure), avoiding an increase in sample temperature over the treatment (pressurised) temperature (Erkmen 2011; Guan et al. 2006).

To simplify the graphical presentation of study results in the following sections, pressurised temperatures were grouped into the temperature ranges; 2 to 4, 8 to 10, 20 to 39, 40+ ºC. These groupings were chosen, as bacterial responses to high pressures were expected to be consistent within these temperature ranges.

Table 10: Adiabatic heating effect in milk

Reference	Milk Type	Pre-compression Temperature (°C)	Adiabatic heating effect (°C per 100 MPa)	Target Pressure (MPa)	Comments
Chen 2003	UHT milk	22	3.1 - 3.8	350 – 500	Pre-compression temperature was room temperature The highest temperature reached by samples was 37.5°C, but quickly dropped to room temperature.
Chen 2007	UHT milk	21.5	2.8	600	Temperature of pressure chamber and pressure medium were controlled by a water bath. Samples were pre-tempered in water bath.
Erkmen 2011	Raw milk	~13	3	400	Samples precooled to appropriate pre-compression temperatures to reach target temperature at target pressure
Gao 2006	Milk buffer	41	1	448	Samples pre-heated for 5 minutes prior to HPP
Garcia-Graells 2000	Ovine milk, pasteurised	20	2	350, 400	Temperature may temporarily reached up to 30°C during adiabatic compression
Guan 2005	UHT milk	21	3.1 (2.0)	350 – 600	2 different units were used for pressure treatment. Unclear which unit produced which results
Martinez-Rodriguez 2005	UHT milk	20	3.3	200 – 400	-
McClements 2001	UHT skim milk	8, 30	3	400	-
Patterson 1995	UHT milk	20	2	375, 600	Temperature was controlled by a heating device surrounding the pressure vessel.
Patterson 1998	UHT milk	10, 20, 50	2	400 – 700	Temperature was controlled by a heating device surrounding the pressure vessel.
Pina Pérez 2007	Rehydrated powdered infant formula	15	3.5	250 – 400	Temperature gains during pressure treatment were taken into account so that the temperature was controlled to remain below 30°C for all treatment conditions.
Yang 2012	Sterile milk	-	2	300, 400, 500	Samples were stored at 4°C for 24 hrs, unclear if bags were allowed to warm up to room temperature prior to HPP.

6.5 INTERPRETATION OF INACTIVATION DATA

The bacterial log reductions presented in the following sections are the observed reductions of the bacteria after a given time at a specified pressure. As stated in section 2.10.1 the time-inactivation relationship at a particular pressure may not be linear, so it is not known how quickly the inactivation occurred before the sampling point or how much depressurisation also provides some of the inactivation. For the majority of studies, the sampling points are at set holding times at the target pressure and are not designed to find the time taken to reach a given log reduction at a given pressure.

The inactivation results obtained in the studies are highly variable between studies and between strains of the same bacteria under the same experimental conditions. The point in time at which sampling is carried out will also have an impact on the variability seen within the results, as discussed above.

6.6 EFFECT OF HPP ON PATHOGENS IN MILK

6.6.1 *Bacillus cereus*

Studies:

Only one study investigating the effect of high pressure on *B. cereus* in UHT milk was identified in this literature search (McClements, Patterson, and Linton 2001). Two strains of psychrotrophic *B. cereus* were tested individually, both as vegetative cells or in a spore form.

For each strain, vegetative cells were grown at either 8 or 30°C to an exponential or stationary phase of growth before addition to UHT milk. The milk was then HPP treated at 400 MPa and with the pre-compression temperature set to the growth temperature (8 or 30°C) for holding times of 0, 3, 6, 9, 12, 15 or 18 minutes.

The zero holding times, represented a HPP treatment using compression at a rate of 200 MPa/minute, followed by immediate decompression (release time of 2 minutes). Therefore, there is no holding time. For the cells grown to an exponential or stationary phase at 8°C and with pre-compression temperature of 8°C, and cells grown to a stationary phase at 30°C, showed similar concentration reductions of ~1.5 to ~2.8 log cfu/ml (Table 11). However, the cells grown to exponential phase at 30°C before treatment where much more vulnerable to inactivation, resulting in a 5 to 6 log cfu/ml inactivation.

For HPP treatments with holding times of 3 minutes and longer, of milk inoculated with vegetative cells in stationary growth phase, the inoculum cells grown at 30°C were more pressure resistant than those grown at 8°C. For example, after HPP with a holding time of 9 minutes, inoculum cells grown at 30°C had been reduced by ~2.5 log cfu/ml, compared to a reduction of 5-6 log cfu/ml for inoculum cells grown at 8°C.

Table 11: Summary inactivation (log cfu/ml) from McClements et al (2001) for two *B. cereus* strains treated at 400MPa for zero holding time and pre-compression temperatures of 8 and 30°C.

		Cell growth phase	
		Exponential	**Stationary**
Inoculum cell growth and pre-compression temperature	**8°C**	1.5 to 2.3	1.5 to 2.8
	30°C	>5	1.8 to 2.2

For the spore experiments, UHT milk was inoculated with spores to a target concentration of 10^7 spores/ml.

B. cereus spores were more resistant to pressure than vegetative cells. Pressure treatment at 8°C did not inactivate spores. Spore numbers were reduced by 0.45 -1 log /ml following a 400MPa/25 minutes and pre-compression temperature of 30°C, though it is unclear if the reduction is due to inactivation or germination of spores.

The percentage germination of spores following a 30 minute pressure treatment at 400 MPa depended on the pre-compression temperature. The observed spore germination rate following pressurisation at 30°C was 75.8 and 18.7% germination for strain 1 and strain 2, respectively. Treatment at pre-compression temperature of 8°C induced 13.4 and 8.3% germination for the same strains.

A summary of *B. cereus* inactivation data can be found in Appendix A Table 15.

6.6.2 *Campylobacter* spp.

Two studies were identified on the effect of HPP treatment on *Campylobacter* spp. in milk. Both studies were conducted in UHT milk. A patent application was also identified.

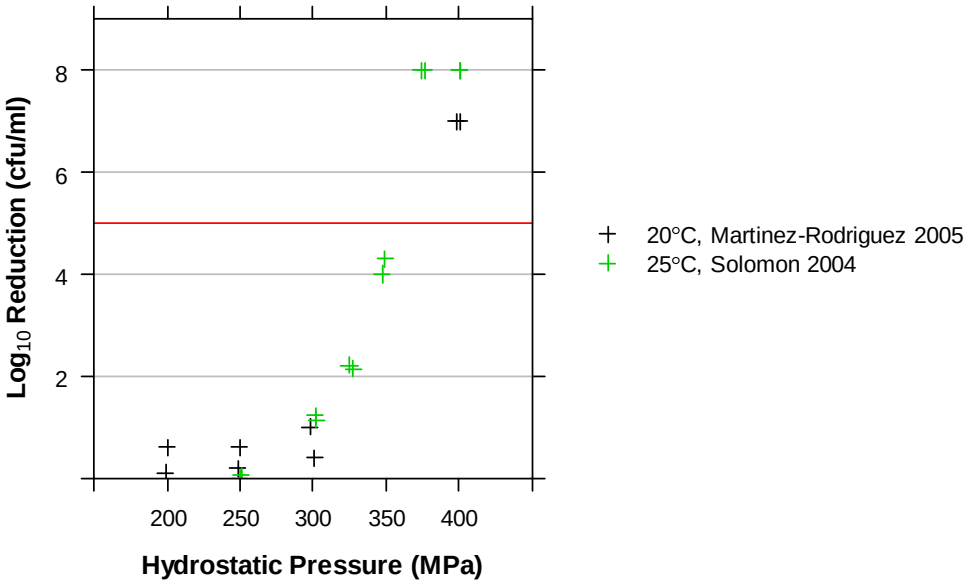
Studies:

According to a study by Solomon and Hoover (2004), HPP treatment at 350 MPa for 10 minutes was sufficient to cause at least a 5-$\log_{10}$ reduction in *C. jejuni* counts in UHT whole milk, while treatment at 325 MPa resulted in a 2–3 $\log_{10}$ decrease only. The pre-compression temperature was 25°C.

Another study by Martinez-Rodriguez and Mackey (2005) compared the pressure resistance of two strains of *C. jejuni* in UHT milk. Pressure treatment was carried out for 10 minutes at 200, 250, 300 and 400 MPa, with a pre-compression temperature of ~20°C.

In UHT milk, 300 MPa reduced *C. jejuni* counts by up to 1 $\log_{10}$ cfu/ml, but treatment at 400 MPa was sufficient to reduce bacterial numbers to below the detection limit (7 $\log_{10}$ reduction).

Figure 6: Inactivation of three different *C. jejuni* strains (ATCC 35921, 11351, 11322), after high pressure treatment with pre-compression temperature of 20-25°C, as a function of pressure applied for 10 minutes. The red line marks a 5 log_{10} reduction.



An Australian patent for a HPP method for the treatment of raw milk (application number AU2017101178) lists a challenge trial which tested *C. jejuni* for two cycles of 90 seconds duration at a pressure of 600 MPa (See section 6.7). Information on the number or name of the strains tested, the pressurisation temperature, the growth phase of the cells at the start of the pressurisation and the enumeration methods are not provided. From the general patent information, the temperature during pressurisation should have been between 45 and 60°C. An inactivation of 1.2 log_{10} cfu/ml reduction in cells was recorded.

A summary of *C. jejuni* inactivation data can be found in Appendix B Table 16.

Conclusion:
Although there are limited data available, combining data obtained from the two studies above, it appears that the strains of *C. jejuni* tested are inactivated by at least 5 log_{10} cfu/ml from 10 minute treatments at 400 MPa with pre-compression temperature of 20-25°C. However, the results from a patent application which tested *C. jejuni* at 600MPa for two cycles of 90s found only a 1.2 log_{10} cfu/ml reduction in cells.

6.6.3 *Clostridium* spp.

No studies on the inactivation of *Clostridium* spp. in milk by high pressure treatment were identified in this literature review.

6.6.4 *Cronobacter* spp.

In 2007, *Enterobacter sakazakii* was reclassified into eight distinct taxa of a new genus *Cronobacter* (Iversen et al. 2007). All four identified studies were conducted in rehydrated powdered milk/infant formula. The data from the studies are summarised in Figure 7 and Figure 8.

Studies:

Gonzales et al. (2006) reported >5 $\log_{10}$ inactivation of three out of four different strains of *C. sakazakii* after treatment at 600 MPa/1 minute/25°C (pressurised temperature). Inactivation of less than 2 $\log_{10}$ was observed with pressures of 200 and 400 MPa.

In the lower pressure range, Pina Pérez et al. (2007) conducted treatments with 200-400 MPa /1-20 minutes/23-29°C (pressurised temperature) and found low log reductions for short treatment times at all pressures used. However, if treatment lasted longer, >5 $\log_{10}$ reductions in *C. sakazakii* counts were achieved. These reductions were achieved at 350 MPa/27°C/ greater than 7 minutes and at 400 MPa/29°C/ greater than 2 minutes.

Koseki et al. (2009) conducted experiments to develop a model predicting the minimum processing conditions that would achieve a required $\log_{10}$ reduction of *C. sakazakii* cells. A single strain was chosen for the experiments, which they had found to be the most pressure resistant of four candidate strains. Rehydrated infant formula was inoculated with 3, 5 and 7 $\log_{10}$ cfu/ml. Infant formula was pressured treated at different pressures and holding times, following which a presence/absence test for viable *C. sakazakii* cells was conducted. Observed reductions of at least 5-$\log_{10}$ counts were recorded after treatment at 450, 500, 550, 600 MPa/40°C/20, 5, 3, 1 minutes and after treatment at 500, 550, 600 MPa/25°C/20, 10, 5 minutes.

The data was used to develop a logistic regression model which included the variables of pressure, holding time, temperature, and initial concentration of *C. sakazakii* cells. The model predicted the required pressure-holding times at 500 MPa for a 5-$\log_{10}$ reduction in *C. sakazakii* in infant formula with 0.9 achievement probability were 26.3 and 7.9 minutes at 25 and 40°C, respectively.

The most recent study (Arroyo et al. 2011), investigated the inactivation of *C. sakazakii* in reconstituted milk. For a 5-$\log_{10}$ reduction in viable counts of *C. sakazakii* treatment at 300 MPa had to be continued for 124 minutes (data point not plotted in Figure 8).

Figure 7: Inactivation of four different *C. sakazakii* strains after HPP at pressurised temperature of 20-29°C and 40°C as a function of pressure. The red line marks a 5 log_{10} reduction.

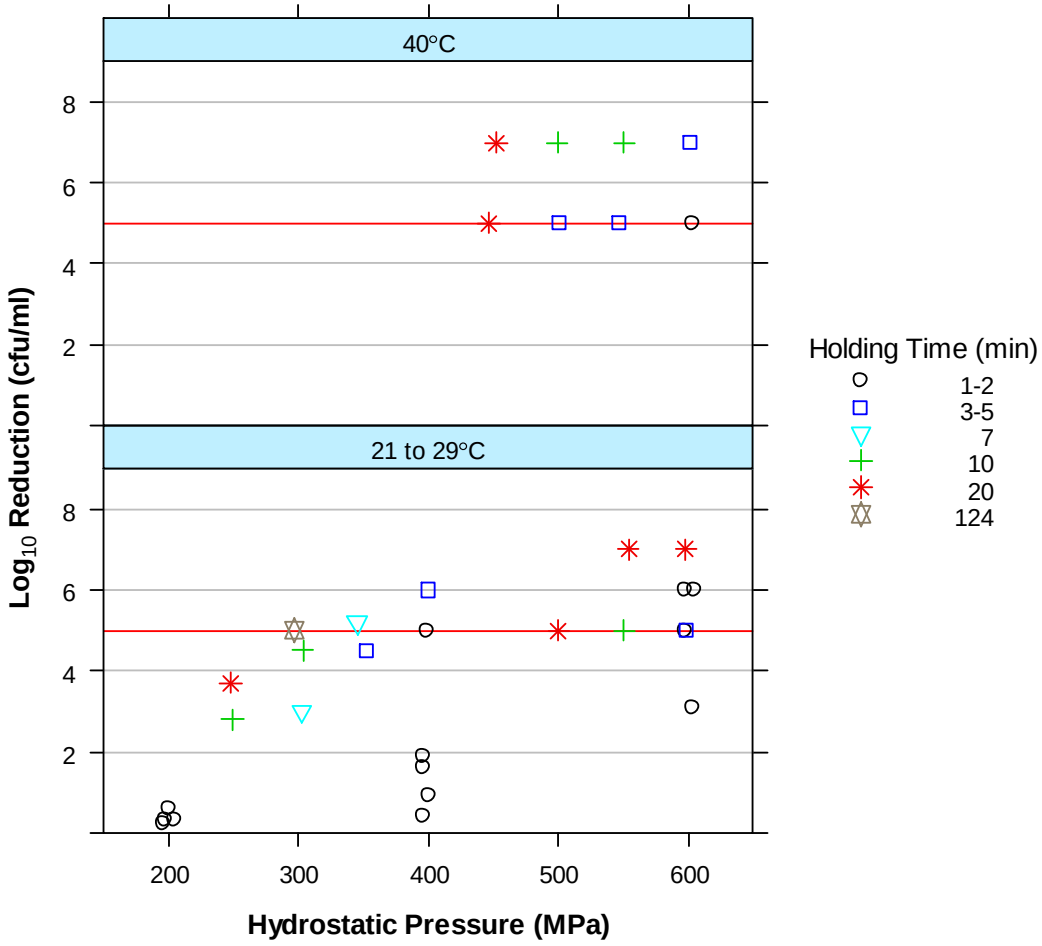
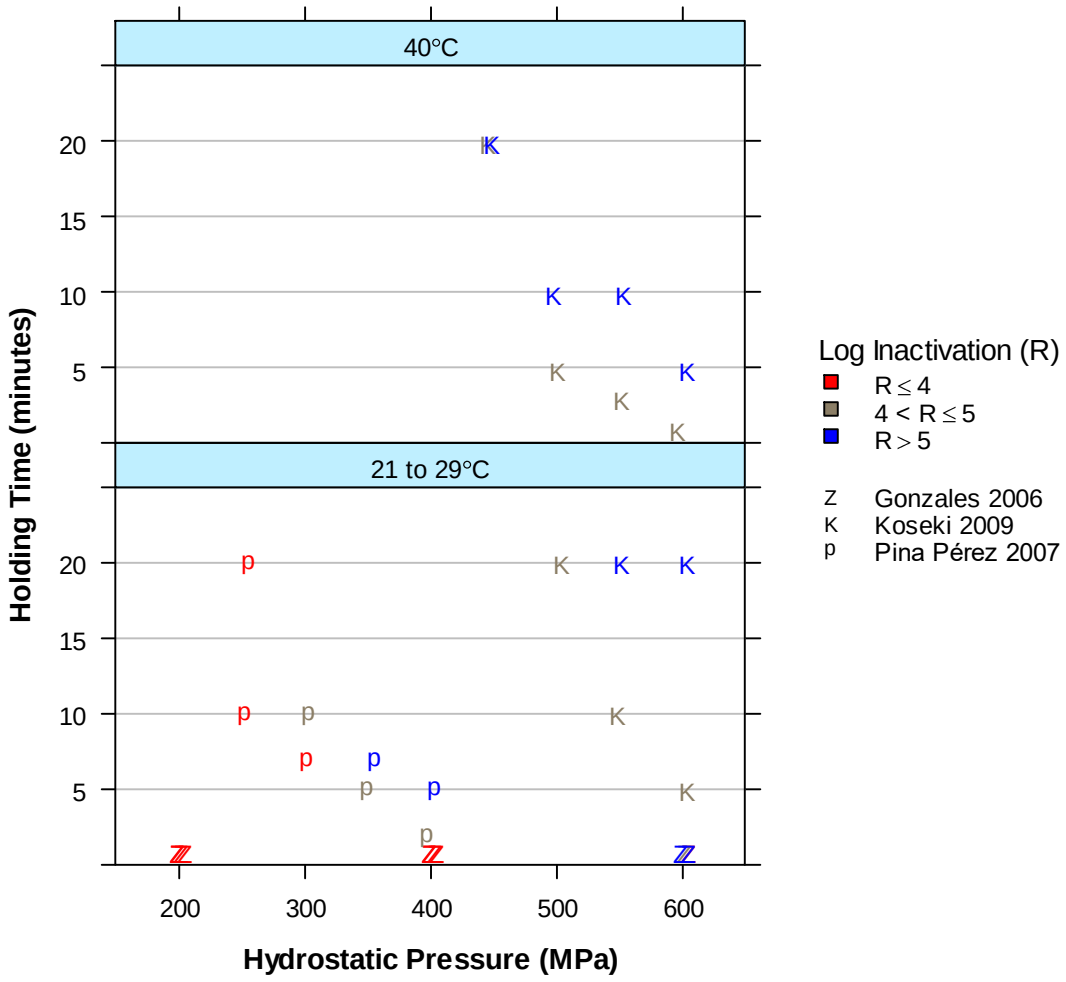


Figure 8: Effect of study, treatment pressure and holding time on the inactivation of four different *C. sakazakii* strains after HPP at pressurised temperature of 20-29°C or 40°C. The grey Koseki data points represent combinations for which a 5 log reduction was achieved for some but not all of the three replicate trials



A summary of *Cronobacter* spp. inactivation data can be found in Appendix A Table 17.

Conclusion:
Based on the above studies, in the pressurised temperature range 21-29°C, a treatment holding time would need to be greater than 20 minutes at 500 MPa, but a 5 log reduction may be possible after 20 minutes at 550 to 600 MPa.

Increasing the pressurised temperature increases the rate of inactivation in the pressure range 500-600 MPa.

6.6.5 *Listeria monocytogenes*

A number of studies on the effect of HPP treatment on *L. monocytogenes* and *L. innocua* were conducted in raw milk, skim and whole UHT milk and pasteurised ovine milk (summarised in Table 12). An examination of the data showed no difference between strains in reduction of *L. innocua* and *L. monocytogenes*, so the inactivation data is presented together in Figure 9 and Figure 10.

Table 12: Studies on the effect of HPP treatment on *Listeria* spp. with study HPP parameter ranges.

Reference	Milk Type	Pre-compression temperature (°C)	Target pressure (MPa)	Holding time (minutes)	Comments
Alpas and Bozoglu 2002	Past. milk, sterilised	50	345	5	-
Chen 2007	UHT milk	21.5	600	1 - 30	Model validation study.
Erkman and Dogan 2004	Raw milk	25	400, 600	5 - 65	-
Gao 2006	Milk buffer	30 - 50	300 - 500	5 - 15	-
Garcia-Graells 2000	Skim milk	20	350, 400	15	Combined treatment with HPP and the lactoperoxidase system.
Gervilla 1997b	Ewe's milk	2, 10, 25, 50	200 - 500	5, 10, 15	-
Gervilla 2000	Ewe's milk	4, 25, 50	100 - 500	15	Investigation of baroprotective effect of fat content.
Hayman 2007	UHT milk	20-25	400	0.01 - 30	Effect of growth temperature and growth phase on HPP effectiveness.
Huang 2015	Raw milk	25	250 - 450	5, 10	Cellular damage observed on microscopic images.
Koseki 2008	Sterile milk	25	400 - 600	5	Mild heat treatment (30-50°C) following HPP to prevent bacterial recovery. HPP inactivation and storage tests.
Liu 2017	Skim milk	25	300 - 500	5, 15	Effects of inhibitor-assisted HPP treatment.
McClements 2001	UHT skim milk	8, 30	400	8, 12	Effect of growth stage and growth temperature.
Misiou 2017	UHT milk	25	200 - 500	10	Challenge-lethality testing and storage testing.
Patterson 1995	UHT milk	20	300 - 700	5 - 30	-
Serment-Moreno 2017	UHT milk	20	300 - 600	≤10	Model validation study.
Sherer 2010	UHT milk	21	400	2	Effect of growth and recovery temperatures were investigated.
Vachon 2002	Raw milk	25	100 - 300	1	1 to 5 cycles of HPP.

Studies:

Pressure treatment at relatively low pressures (345 MPa), but high pre-compression temperatures (50°C) resulted in a reduction of 8 log_{10} cfu/ml for two *L. monocytogenes* strains inoculated into sterilised milk (Alpas and Bozoglu 2002a). In another modelling study (Gao, Ju, and Jiang 2006), in re-suspended milk buffer, the optimum process parameters for a 6-log_{10} reduction of *L. monocytogenes* cells were as follows: 448 MPa/11 minutes with a pre-compression temperature of 41°C.

A number of studies have been conducted with pre-compression temperatures in the range 20-25°C, with varying effectiveness of inactivation by HPP.

A recent study by Huang et al (2015) demonstrated that, at 25°C, a 450 MPa treatment for 5 minutes resulted in a reduction of 8 log_{10} cfu/ml viable *L. monocytogenes* cells. Application of 400 MPa for 5 and 10 minutes reduced the number of viable cells by 6.3 and 7.7 log_{10} cfu/ml, respectively.

In contrast, HPP was less effective in reducing cell counts in the pressure range 400 – 500 MPa in studies by Misiou et al. (2017), Shearer et al. (2010) and Erkmen and Dogan (2004).

Misiou et al (2017) reported that, after HPP treatment at 400 MPa and 500 MPa/10 minutes/25°C (pressurised temperature), *L. monocytogenes* cell counts decreased by about 4.8 and 6.2 log_{10} cfu/ml, respectively. Similar reductions of 4.5-4.9 log_{10} cfu/ml were reported by another group after HPP treatment at 400 MPa/2 min/21°C (Shearer, Neetoo, and Chen 2010).

Erkmen and Dogan (2004) found that *L. monocytogenes* was more sensitive to increased pressure than increased pressurisation time. In raw milk, 10 minute pressure treatment at 400 MPa resulted in reductions of about 2.7 log_{10} cfu/ml (pre-compression temperature 25°C). At a target pressure 600 MPa the inactivation of L. monocytogenes was more effective, increasing to about 6.47 log_{10} cfu/ml.

A study by Chen et al. (2007), to model the pressure response of different pathogens in UHT milk, found that a treatment of 600 MPa /0.5 minutes /21.5°C (pre-compression temperature) reduced the counts of *L. monocytogenes* by 6.7 log_{10} cfu/ml. Extending the treatment time to 6 minutes only increased reduction by an additional 0.9 log_{10}.

The impact of the pre-treatment cell condition of *L. monocytogenes* (growth temperature and growth phase) and the plating media on inactivation rates was investigated in UHT milk by Hayman et al. (2007). Cells grown at 15°C were most sensitive to HPP, followed by cells grown at 4, 25 or 35°C, with cells grown at 43 °C appearing to be the most resistant. Inactivation of cells grown at 4, 15 or 25°C followed first order kinetics, whereas cells grown at 35 or 43°C displayed non-linear inactivation kinetics. Growth phase and plating medium had significant effects on the inactivation of *L. monocytogenes* by HPP. Exponential cells

grown at 8°C were more resistant than those grown at 30°C, but for stationary-phase cells the reverse was true (McClements, Patterson, and Linton 2001).

Koseki et al (2008) studied the possibility of sub-lethal injury of cells after HPP. After a treatment of 550 MPa /5 minutes/25°C (pressurised temperature), no *L. monocytogenes* cells were detected in sterile whole milk, regardless of the inoculum levels (3, 5, and 7 $\log_{10}$ cfu/ml). Treatment for 5 minutes at 500 MPa led to a reduction of 5 $\log_{10}$ cfu/ml. Recovery after storage at 4 and 25°C, but not at 37°C was observed. Cells were recovered at a much higher rate when milk samples were stored post HPP at 25°C. The authors propose that storage temperature after HPP plays an important role in bacterial recovery and that mild heat treatment post HPP (37-50°C) would prevent bacterial re-growth. The authors postulate that the repair of HPP damaged membranes may be more difficult at higher temperatures due to weaker intermolecular forces among membrane molecules.

A 2017 study conducted in skim UHT milk, showed a 3.4 and >5 $\log_{10}$ reduction in *L. monocytogenes* counts after 5 minutes treatment with 400 and 500 MPa, respectively (Liu et al. 2017). Storage under refrigerated conditions allowed for re-growth of *L. monocytogenes* following treatment with 500 MPa (Liu et al. 2017).

Using *L. innocua* as a surrogate for *L. monocytogenes*, a 2 to 5 $\log_{10}$ cfu/ml inactivation, depending on the strain, was achieved with a treatment of 400 MPa / 15 minutes/ 20°C (pressurised temperature). The authors observed a strong synergistic interaction of treatments with the lactoperoxidase system[17] and HPP under the same conditions, with inactivation exceeding 7 $\log_{10}$ (Garcia-Graells, Valckx, and Michiels 2000). Another study of *L. innocua* in ewe's milk found low temperature (2°C) pressure treatments resulted in higher *L. innocua* inactivation than treatments at room temperatures (25°C) (Gervilla, Capellas, et al. 1997; Gervilla, Ferragut, and Guamis 2000). Pressures between 450 and 500 MPa for 10 to 15 minutes were needed to achieve reductions of 7 to 8 $\log_{10}$ (Gervilla, Capellas, et al. 1997). Serment-Moreno et al. (2017) validated a recently proposed model describing microbial inactivation by HPP. Complete inactivation of *L. innocua* was observed after 4 minutes at 500 to 600 MPa, when the initial cell concentration was approximately 6 $\log_{10}$ cfu/ml.

A summary of *Listeria* spp. inactivation data can be found in Appendix A, Table 18.

[17] "Lactoperoxidase is a native milk enzyme that catalyzes the oxidation of thiocyanate (SCN2) by peroxide into short-lived reactive oxidation products that in turn rapidly oxidize many biomolecules. Most relevant for microbial inactivation is probably the oxidation of enzymes and other proteins in the bacterial cell membrane that have exposed sulfhydryl groups (ASH)". Lactoperoxidase is being investigated as "an interesting additional hurdle to improve the safety of high pressure food preservation". (Garcia-Graells et al 2000)

Conclusion:

Overall, pressure/time/temperature combinations that have been shown to be effective in reducing *L. monocytogenes* and *L. innocua* viable cell numbers by at least 5 $\log_{10}$ cfu/ml varied between studies. Applying a 'safe harbour' strategy it appears that application of 450 MPa and higher for at least 10 minutes at ambient temperatures reduces *L. monocytogenes* levels by greater than 5 $\log_{10}$ cfu/ml. However, storage conditions and shelf lives still need to be considered. It has been suggested that mild heat treatment after HPP, such as 37°C, prevents bacterial re-growth during storage.

Figure 9: Inactivation of six *L. innocua* strains and 12 *L. monocytogenes* strains after high pressure treatment at different pressurised temperatures as a function of pressure.

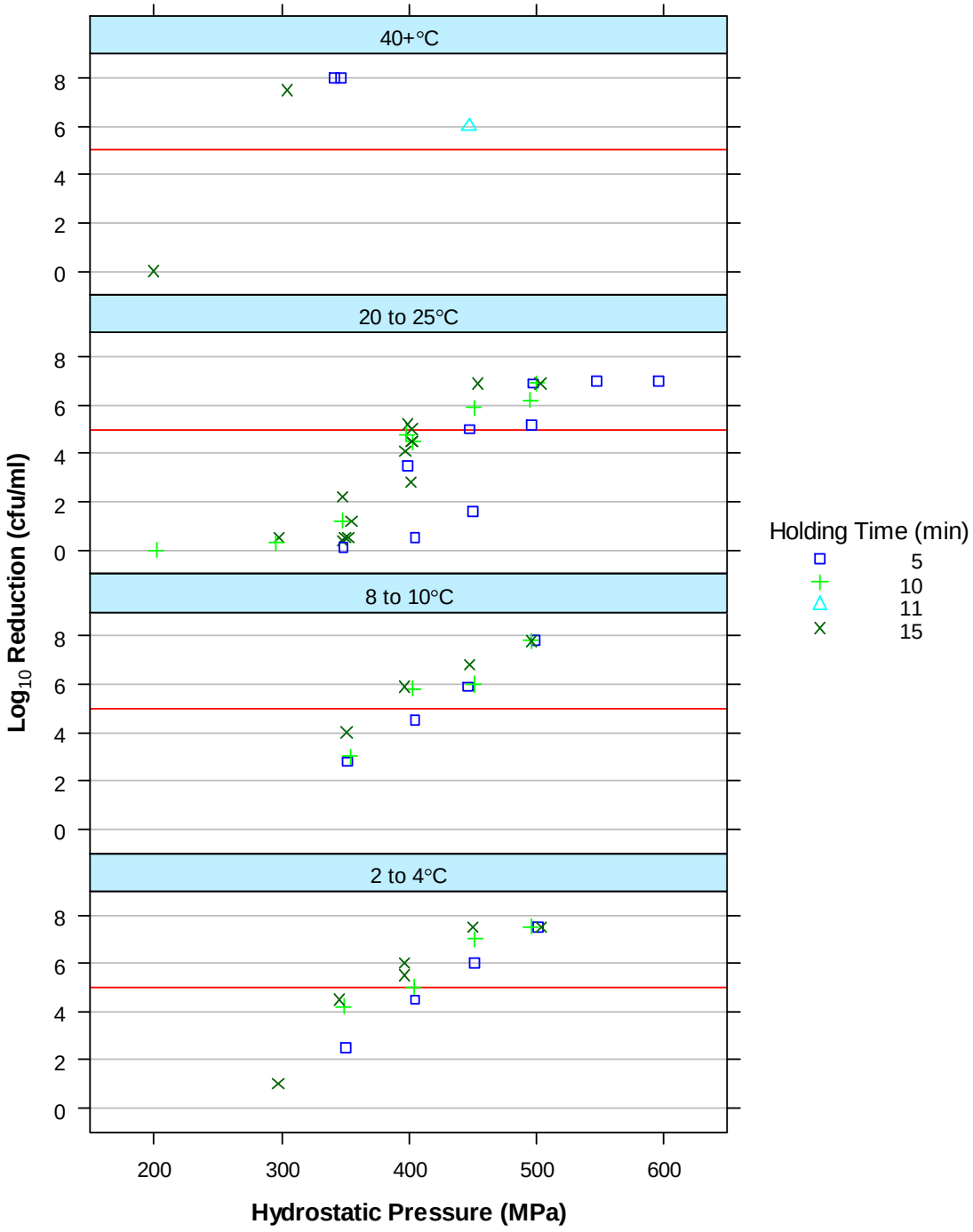
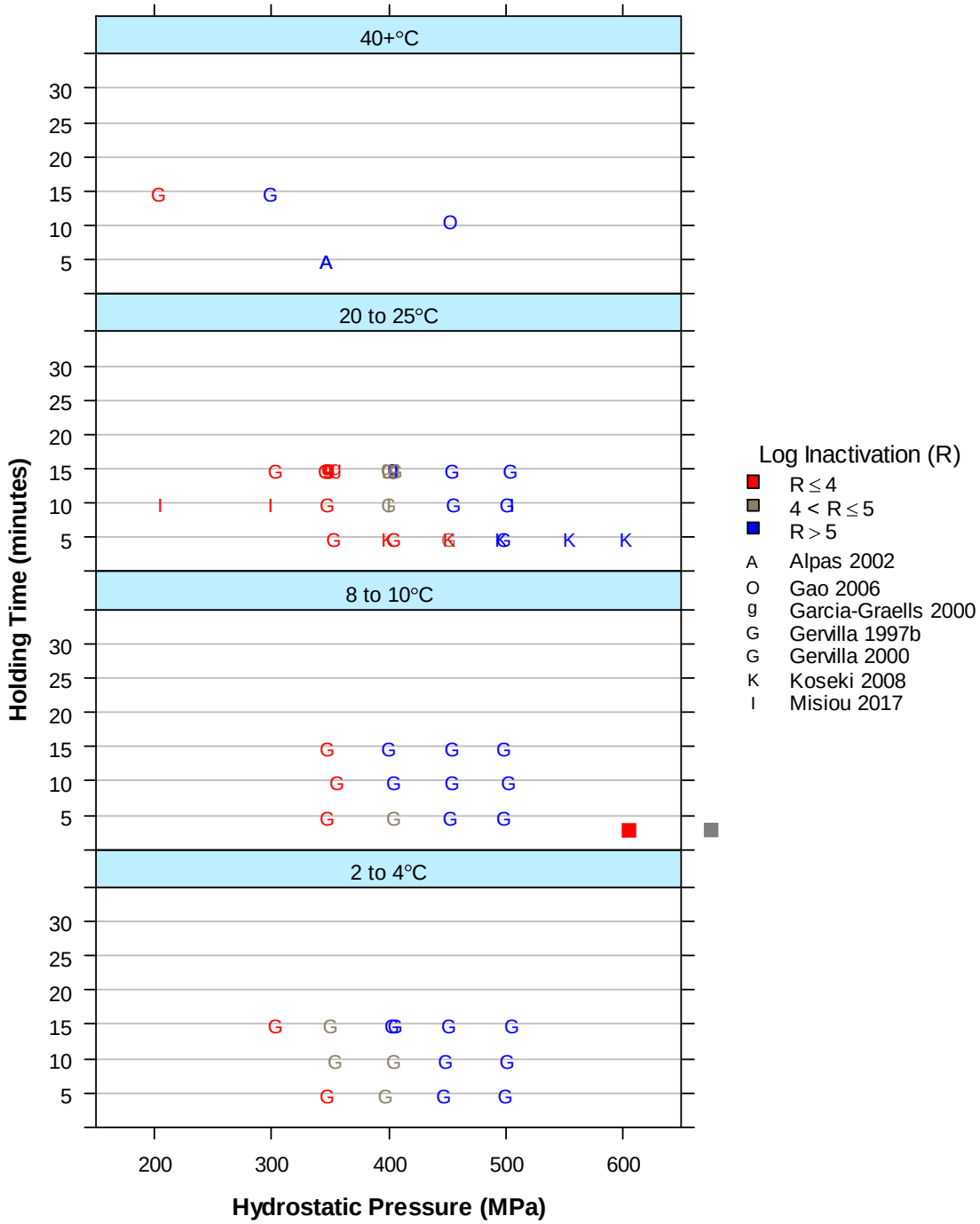


Figure 10: Effect of study and treatment holding time on the inactivation of six *L. innocua* strains and 12 *L. monocytogenes* strains after high pressure treatment at different pressurised temperatures as a function of pressure.



6.6.6 Mycobacterium avium subsp. paratuberculosis (MAP) (surrogate for M. bovis)

To the best of our knowledge no studies on the effect of HPP treatment on *Mycobacterium bovis* in milk have been published. However, there are a few studies available that examined the inactivation of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in UHT milk by HPP (Donaghy et al. 2007; Lopez-Pedemonte et al. 2006). MAP can cause illness in cattle and other ruminants and are closely related to *M. bovis*.

Studies:

In a 2006 study (Lopez-Pedemonte et al. 2006), UHT milk was inoculated with different MAP strains and subjected to HHP ranging from 300 to 500 MPa for 10 minutes with pre-compression temperatures of 20˚C and 5˚C. No difference in reductions between temperatures was noted, however, there were strong interactions between strain and enumeration medium, which indicates that assay conditions are very important for the assessment of inactivation of MAP.

The work of Donaghy et al. (2007) compared HPP of UHT milk for 5 and 10 minutes at 400, 500 and 600 MPa. All the 600 MPa treatments had a greater than 5 $\log_{10}$ reduction in MAP, while for 500 MPa one of the two strains tested did not reduce by 5 $\log_{10}$ after 5 minutes. At 400 MPa for 10 minutes the strains, showed $\log_{10}$ cell reductions in the range of 2.2 to 2.9.

Figure 11 shows the log reduction in cell concentrations achieved by the two studies. The data suggests that 10 minutes at pressures of 300 to 600 MPa is not necessarily sufficient to produce the required 6 log reduction.

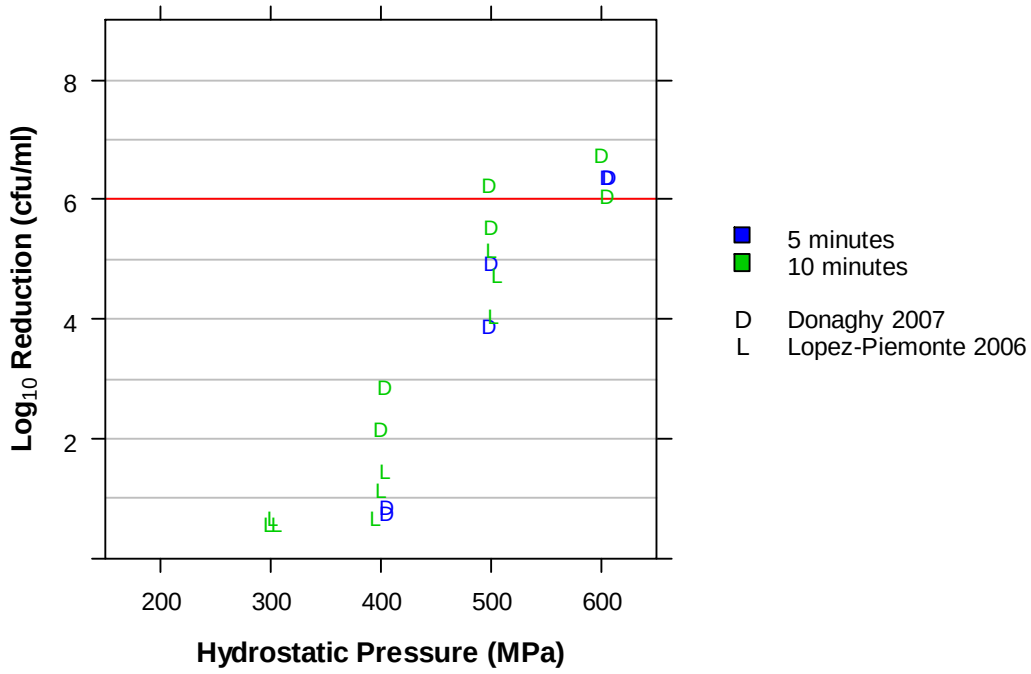
A summary of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) inactivation data can be found in Appendix A, Table 19.

Conclusions:

Both studies found significant differences in MAP recovery depending on the culture medium used, with 7H10 medium providing better recovery rates compared with HEYM.

The available information suggests that 10 minutes at 600 MPa might be sufficient to ensure a 6 $\log_{10}$ reduction in MAP concentration. Further research is necessary to investigate if application of pressures between 500 and 600 MPa to raw milk for longer time periods can provide sufficient inactivation of MAP.

Figure 11: Inactivation of four different MAP strains (NCEC 8578, 806R, 36644/02, ATCC 19698) after high pressure treatment as a function of pressure applied for 5 or 10 minutes. The red line marks a 6 log_{10} reduction.



6.6.7 *Salmonella enterica*

A total of six studies examined the effect of HPP treatment on *Salmonella enterica* in raw milk, skim and whole UHT milk, and sterile milk.

Studies:

Liu et al. (2017) found that 5-minute treatments at 400 and 500 MPa/25°C resulted in >5 log_{10} reduction of *S.* Typhimurium in UHT milk, while Chen et al. (2007) reported a 5.5 log_{10} reduction of *S.* Typhimurium and *S.* Enteritidis after treatment at 600 MPa /4 minutes/21°C (pressurised temperature). After HPP treatment at 500 MPa, no re-growth of *S.* Typhimurium was detected after 15 days under refrigerated conditions by Liu et al. (2017).

In contrast, Guan et al. (2005) found lower inactivation rates of *S.* Typhimurium in UHT milk, requiring at least 30 minutes at 450 MPa or greater than 20 minutes at 500 MPa or greater than 10 minutes at 550 MPa at pressurised temperatures of 21°C to achieve a 5 log_{10} reduction. For complete inactivation, pressurisation at 550 MPa for 50 minutes or 600 MPa for 30 minutes was necessary. Yang et al. (2015) and Erkmen (2011) reported similar reduction rates after treatments of 30 minutes at 300, 400 and 500 MPa and 20 minutes at 400 MPa, respectively (pre-compression temperature 25°C).

One group only investigated treatment at elevated temperatures, although at low pressures. Treatment at 345 MPa/5 minutes (pre-compression temperature 50°C) reduced *S.* Typhimurium and *S.* Enteritidis cell numbers from 8 $\log_{10}$ cfu/ml to non-detectable levels (Alpas and Bozoglu 2002a).

Figure 12: Inactivation of four *S.* Typhimurium strains and one *S.* Enteritidis strain after high pressure treatment at pressurised temperatures of 21 to 25°C and temperatures of 50+°C as a function of pressure. The red line marks a 5 $\log_{10}$ reduction.

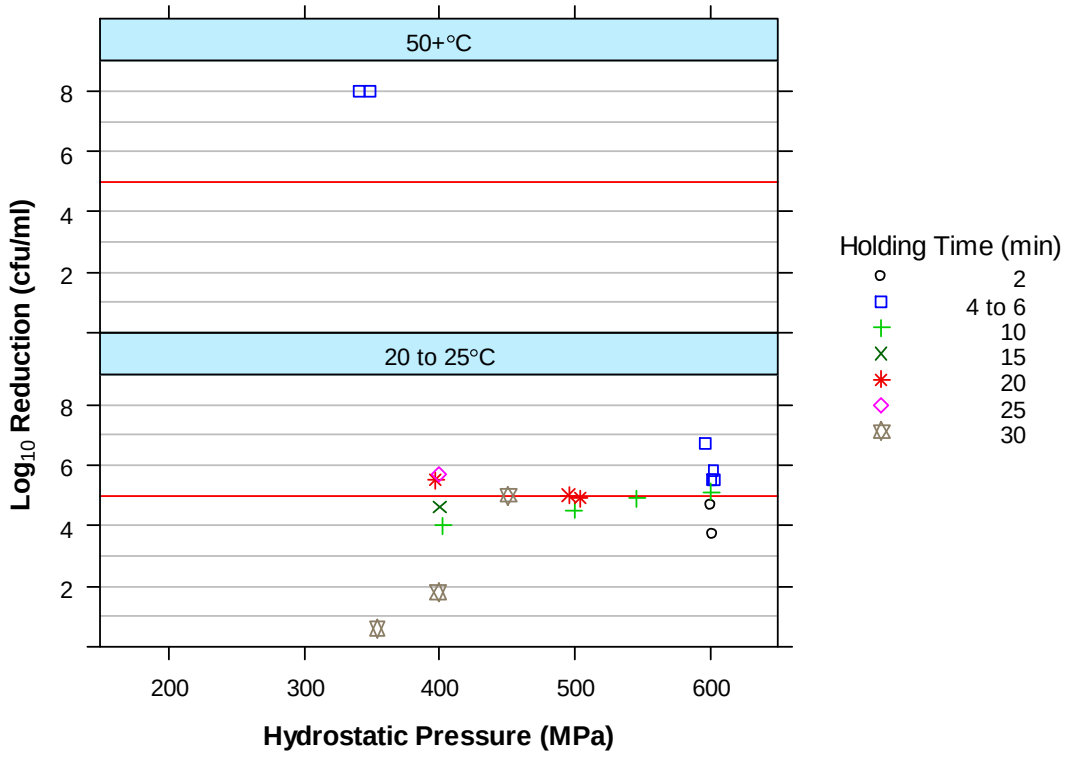
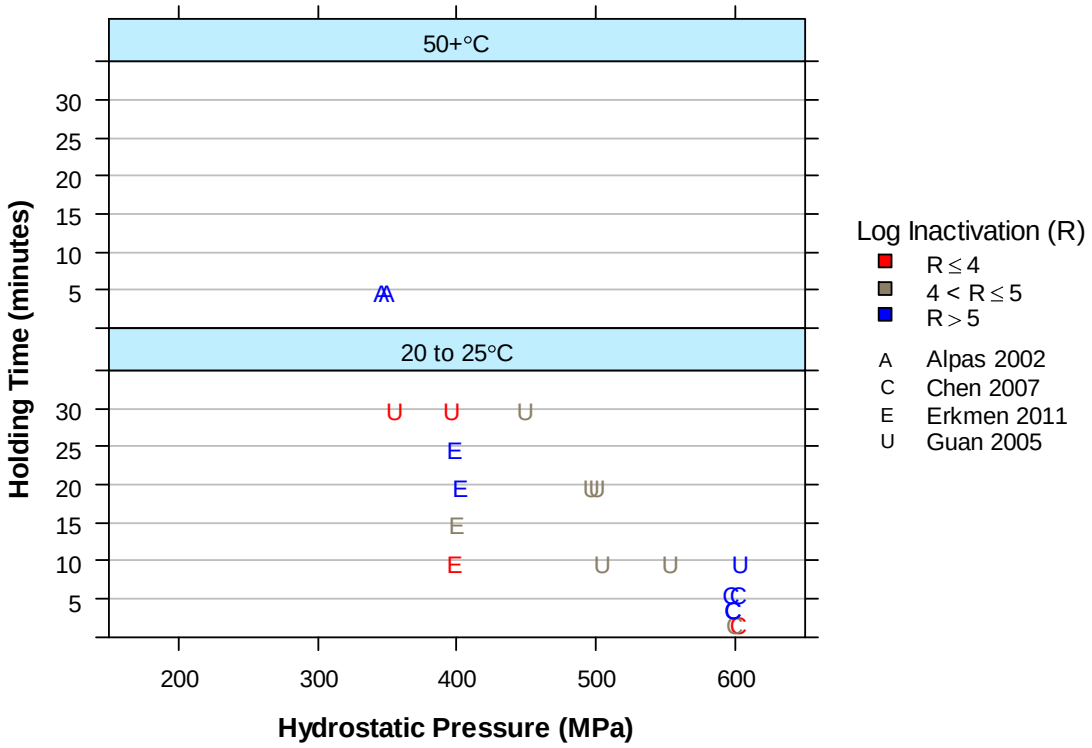


Figure 13: Effect of study, pressure and treatment holding time on the inactivation of four *S.* Typhimurium strains and one *S.* Enteritidis strain after high pressure treatment at pressurised temperatures of 21-25°C and temperatures of 50+°C.



A summary of *Salmonella* spp. inactivation data can be found in Appendix A, Table 20.

Conclusion:

A reduction in viable counts of *S.* Typhimurium and *S.* Enteritidis of at least 5 $\log_{10}$ has been reported after HPP treatments at 600 MPa for 10 minutes or 500 MPa for 30 minutes.

The results are highly variable at lower pressures. Some of the observed variability is most likely due to pressure resistance of individual strains, with *S.* Typhimurium LT2 appearing to be less pressure resistant, whereas DT104 appears to be more resistant at lower pressures (up to 400 MPa).

6.6.8 *S. aureus*

A recent review on the inactivation of *S. aureus* by HPP concluded that the efficiency of *S. aureus* HPP inactivation "depends on several factors resembling a cascade of effects rather than being dependent of only one specific factor" (Baptista et al. 2016). Monomeric proteins such as the staphylococcal enterotoxins (SE) are resistant to HPP, but strains of *S. aureus* which produce SE are more efficiently inactivated than those without SE.

Studies on the effect of HPP treatment on *S. aureus,* identified in this literature search, were conducted in raw milk, pasteurised milk (ovine and bovine), UHT milk and sterile milk.

Studies:
Guan et al. (2006) reported that treatment with 600 MPa at pressurised temperatures of 4, 21 and 45°C resulted in a reduction of greater than 5 $\log_{10}$ of *S. aureus* after holding times of 8, 6 and 6 minutes respectively. In this study, *S. aureus* showed less inactivation at 4°C than at 21 and 45°C. Similarly, treatment with 600 MPa/10 minute/21°C resulted in a 5.5 $\log_{10}$ reduction (Chen 2007). However, another study reported a 30 minute treatment at 600 MPa, with pre-compression temperature of 20°C to achieve a greater than 5 $\log_{10}$ reduction of *S. aureus* (Patterson et al. 1995). A later study by Patterson and Kilpatrick (1998) found that, overall, *S. aureus* was significantly more resistant than *E. coli* O157:H7 to the combined effects of pressure and temperature. HPP at 500 MPa /15 minutes/ 50°C resulted in a 6 $\log_{10}$ reduction of viable *S. aureus* cells.

At pre-compression temperature of 25°C, pressures between 400 and 500 MPa were found to result in greater than 5 $\log_{10}$ reductions after 30 minutes of treatment (Yang et al. 2012). Another study looking at shorter treatment times observed >5 $\log_{10}$ reduction at 500 MPa/25°C/5 minute (Liu et al. 2017).

In contrast, much lower inactivation was reported for treatments at 400-500 MPa/25°C/15 minutes by Gervilla in ovine milk (Gervilla, Ferragut, and Guamis 2000; Gervilla, Sendra, et al. 1999). In this pressure range, reduced temperatures at 2, 4 or 10°C appear to increase the pressure resistance of *S. aureus* (Tabla et al. 2012; Gervilla, Ferragut, and Guamis 2000; Gervilla, Sendra, et al. 1999). Higher temperatures, however, were found to increase the rate of inactivation, with >5 $\log_{10}$ reduction in *S. aureus* counts after treatments at 450-500 MPa /10-15 minutes/50°C (pressurised temperature) (Gervilla, Ferragut, and Guamis 2000; Gervilla, Sendra, et al. 1999).

Treatments at 300-350 MPa were found to reduce *S. aureus* ATCC 27690 counts by >5 $\log_{10}$ after 4-6 minute treatments at pressurised temperature of 20°C (Erkmen and Karatas 1997). HPP treatments at 345 MPa /5 minutes/50°C led to a >5 $\log_{10}$ decrease of *S. aureus* strains 485 and 765 (Alpas and Bozoglu 2002a). Morgan et al. (2000) reported on a 6 $\log_{10}$ reduction after treatment at 345 MPa /30 minutes/25°C (pressurised temperature), while 250 MPa achieved a reduction of only 2.8 $\log_{10}$.

Storage under refrigerated conditions following pressurisation allowed for re-growth of *S. aureus* following treatment at 500 MPa (Liu et al. 2017).

A summary of *S. aureus* inactivation data can be found in Appendix A, Table 21.

Conclusions:

At chilled and ambient temperatures results are variable between studies and even pressures of 600 MPa for holding times of 20 to 30 minutes may reduce the number of viable cells by less than 5 $\log_{10}$ cfu/ml. However, at pressurised temperatures of 45-50°C and above, inactivation of greater than 5 $\log_{10}$ cfu/ml of *S. aureus* viable cells was observed at pressures as low as 450 MPa after 15 minutes (see Figure 14).

Figure 14: Inactivation of *S. aureus* strains after high pressure treatment at low pressurised temperatures (2, 4, 10°C, three different strains), ambient temperatures (20-25°C, seven different strains) and elevated temperatures (45+°C), five different strains) as a function of pressure. The red line marks a 5 $\log_{10}$ reduction

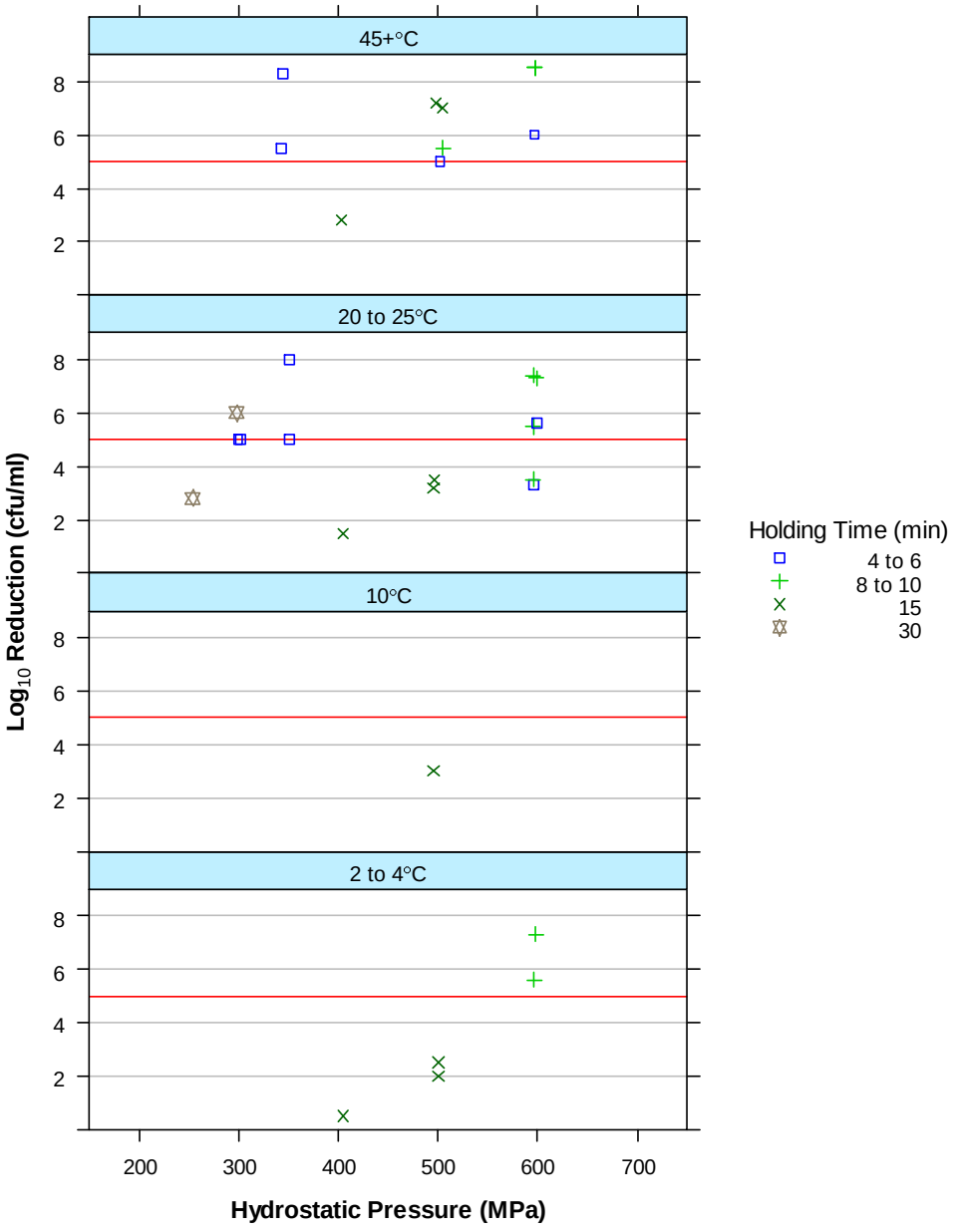
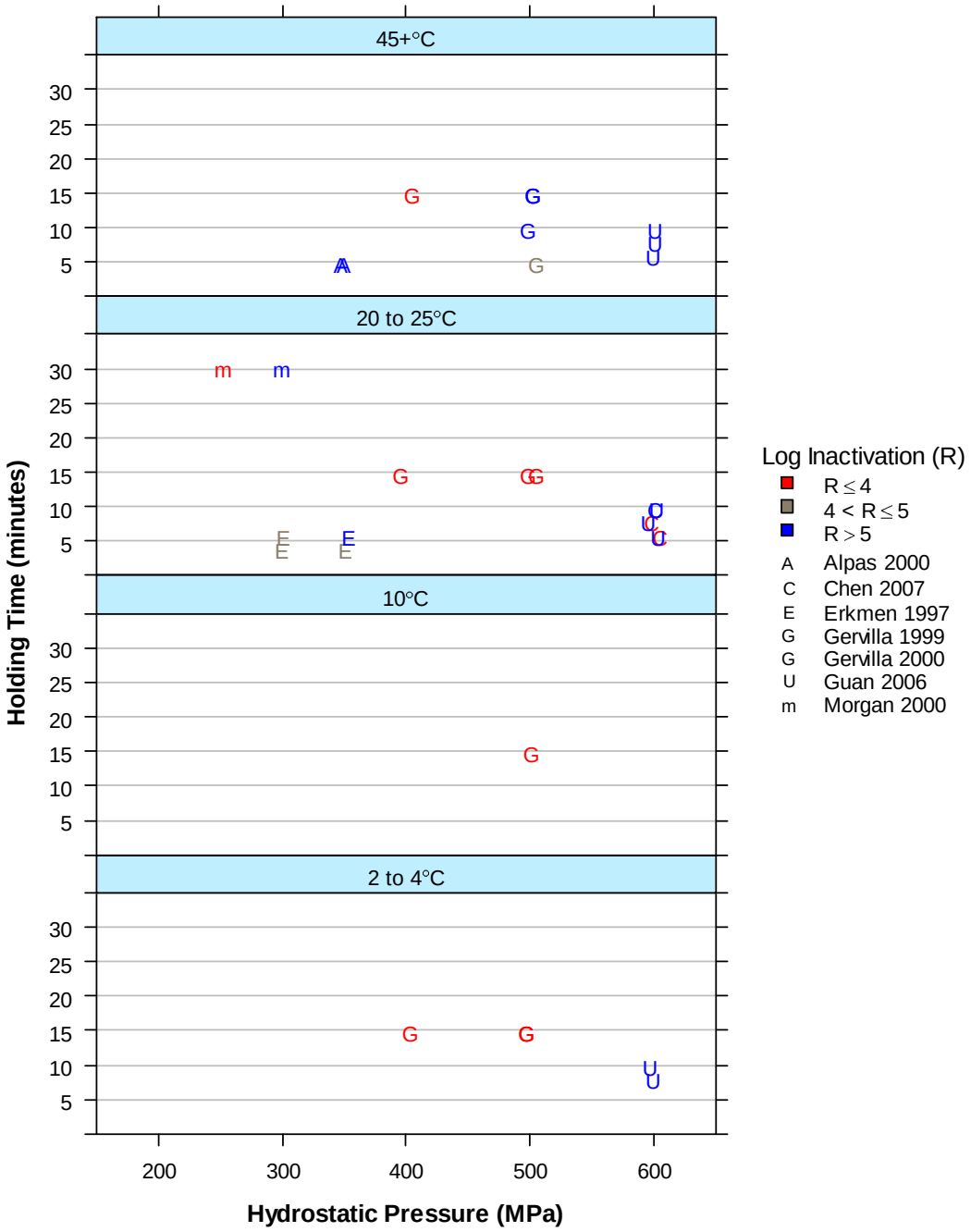


Figure 15: Effect of study, pressure and treatment holding time on the inactivation of *S. aureus* strains after high pressure treatment at different pressurised temperatures.



6.6.9 STEC and other *E. coli*

Studies on the effect of HPP treatment on *E. coli* identified in this systematic review were conducted in raw milk, ovine pasteurised milk, and skim, half-whole, and whole UHT milk. The inactivation of *E. coli* at different pressures and holding times are shown in Figure 16 and Figure 17.

All studies with shiga toxin-producing *E. coli* strains were conducted with O157 serotypes, no non-O157 serotypes were used. Examination of the data gave no indication of differences in the resistant to HPP treatment between O157 strains that produce shiga toxin and those that do not. Both are plotted together in the figures.

Studies:

Liu et al. (2017) reported a >5 $\log_{10}$ cfu/ml reduction of *E. coli* in UHT skim milk after high pressure treatment at 400 MPa or 500 MPa for 5 minutes at 25°C. After HPP treatment no re-growth of *E. coli* was detected after 15 days under refrigerated conditions. Raw milk inoculated with *E. coli* (*E. coli* KUEN 1504) and pressure treated at 400 MPa or 500 MPa at pressurised temperature of 25°C showed an inactivation of about 5 $\log_{10}$ cfu/ml after pressurisation for 25 minutes and 7 minutes respectively (Dogan and Erkmen 2003). In another study treatment at 400 or 500 MPa/30 minutes with pre-compression temperature of 25°C resulted in a 7 $\log_{10}$ reduction of *E. coli* counts (Yang et al. 2012).

Garcia-Graells et al. (1999) studied the inactivation of one *E. coli* strain in whole UHT milk with applied pressures ranging from 300 to 700 MPa[18]. Reductions of cell concentrations of the *E. coli* strain increased with increasing pressure from 0.5 $\log_{10}$ cfu/ml to 1.6 $\log_{10}$ cfu/ml for 300 and 600 MPa, respectively.

Another study by the same authors using skimmed milk comparing four strains of *E. coli* achieved a 0.5 to 3.9 $\log_{10}$ cfu/ml inactivation with 400 MPa/15 minutes/20°C (pressurised temperature) depending on the *E. coli* strain used. Increasing the pressure to 550 MPa resulted in inactivation ranging from 2 to 5 $\log_{10}$ cfu/ml reduction in viable cell counts (Garcia-Graells et al. 2000).

In pasteurised ovine milk inoculated with 6 $\log_{10}$ cfu/ml *E. coli,* HPP treatment at, 450 or 500 MPa/ 5 minutes/25°C (pressurised temperature) a 6 $\log_{10}$ cfu/ml reduction was observed. At refrigeration pressurised temperatures (2 and 10°C) treatment times of 15 minutes and pressures of 450 and 500 MPa achieved reductions of about 6 $\log_{10}$ (Gervilla, Felipe, et al. 1997). A later study by the same group compared *E. coli* inactivation at pressures up to 300 MPa with maximum inactivation of 3 $\log_{10}$ cfu/ml at room temperature (Gervilla, Mor-Mur, et al. 1999). At 400 MPa, inactivation of up to 6 $\log_{10}$ cfu/ml at pressurised temperature of 25°C were reported, (Gervilla, Ferragut, and Guamis 2000).

A study by Chen et al. (2007) of the pressure response of different pathogens in UHT milk, found that a treatment of 600 MPa /10 minutes /21.5°C (pressurised temperature) reduced the counts of *E. coli* O157:H7 by 5.9 $\log_{10}$. Guan et al.(2006) reported that treatment at 600 MPa and 4, 21 and 45°C (pressurised temperature) resulted in a drop of >5 $\log_{10}$ *E. coli* O157:H7 after 16, 10 and 10 min, respectively.

[18] Data is also given for pressure resistant mutants of the *E. coli* strain. This data is not presented as the data in the paper is not consistent suggesting potential typographical errors.

Three groups investigated treatment at elevated temperatures, although at low pressures. Treatment at 345 MPa/5 minutes with pre-compression temperature of 50°C reduced *E. coli* O157:H7 cell numbers from 8 $\log_{10}$ cfu/ml to non-detectable levels (Alpas and Bozoglu 2002b). Log-reductions between 5.4 and 6.5 $\log_{10}$ were reported by Gervilla et al. (1999) after HPP treatment at 300 MPa /5-15 minutes/50°C (pressurised temperature) of *E. coli* inoculated in ovine milk. In another study (Patterson and Kilpatrick 1998), pressures of 300 and 400 MPa for 15 minutes with a pre-compression temperature of 50°C resulted in 3.5 and 5 $\log_{10}$ reductions.

A summary of STEC and *E. coli* inactivation data can be found in Appendix A, Table 22.

Conclusions:

Figure 16 and Figure 17 show that inactivation rates are affected by study design with no clear indication of pressure/temperature/holding times to ensure a 5 $\log_{10}$ reduction. The studies summarised provide partly conflicting results. It appears that studies conducted by two groups reported surprisingly low inactivation rates (Garcia-Graells et al. 1999, 2000; Patterson et al. 1995), which might be due to the strains and/or equipment used. Excluding results from these studies, the following conclusions can be drawn:

At pressurised temperatures of 20-25°C, study results were too variable to draw conclusions. At low pressurised temperatures (2°C to 10°C), at least 450 MPa for 15 minutes was required to achieve >5 $\log_{10}$ cfu/ml reduction in *E. coli* counts. At elevated temperatures, 400 MPa achieved a 5 $\log_{10}$ reduction in about 15 minutes. Generally, HPP parameters found to reduce *E. coli* counts by at least 5 $\log_{10}$ cfu/ml varied widely between studies.

Figure 16: Inactivation of various *E. coli* strains after high pressure treatment at different pressurised temperatures as a function of pressure and holding time.

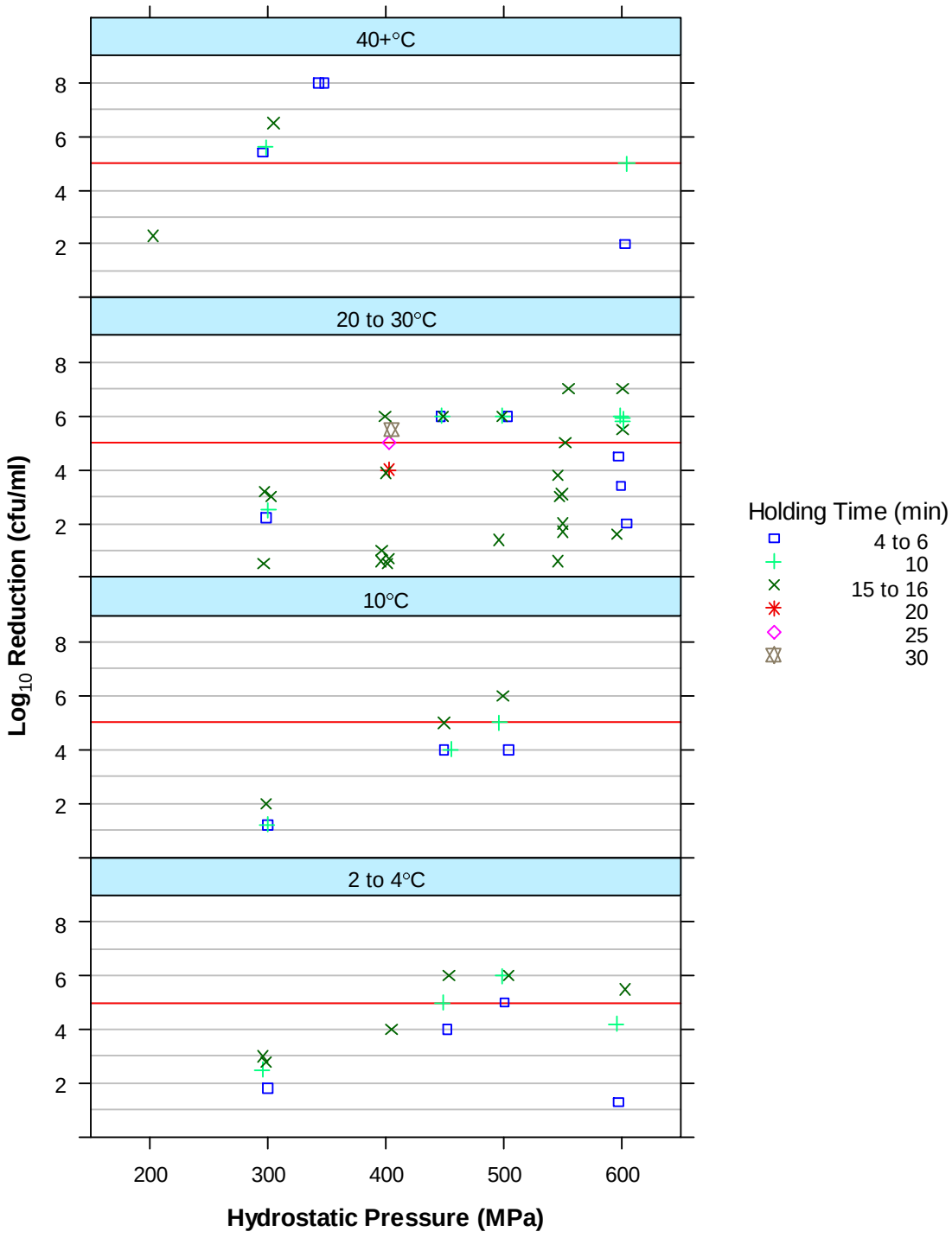
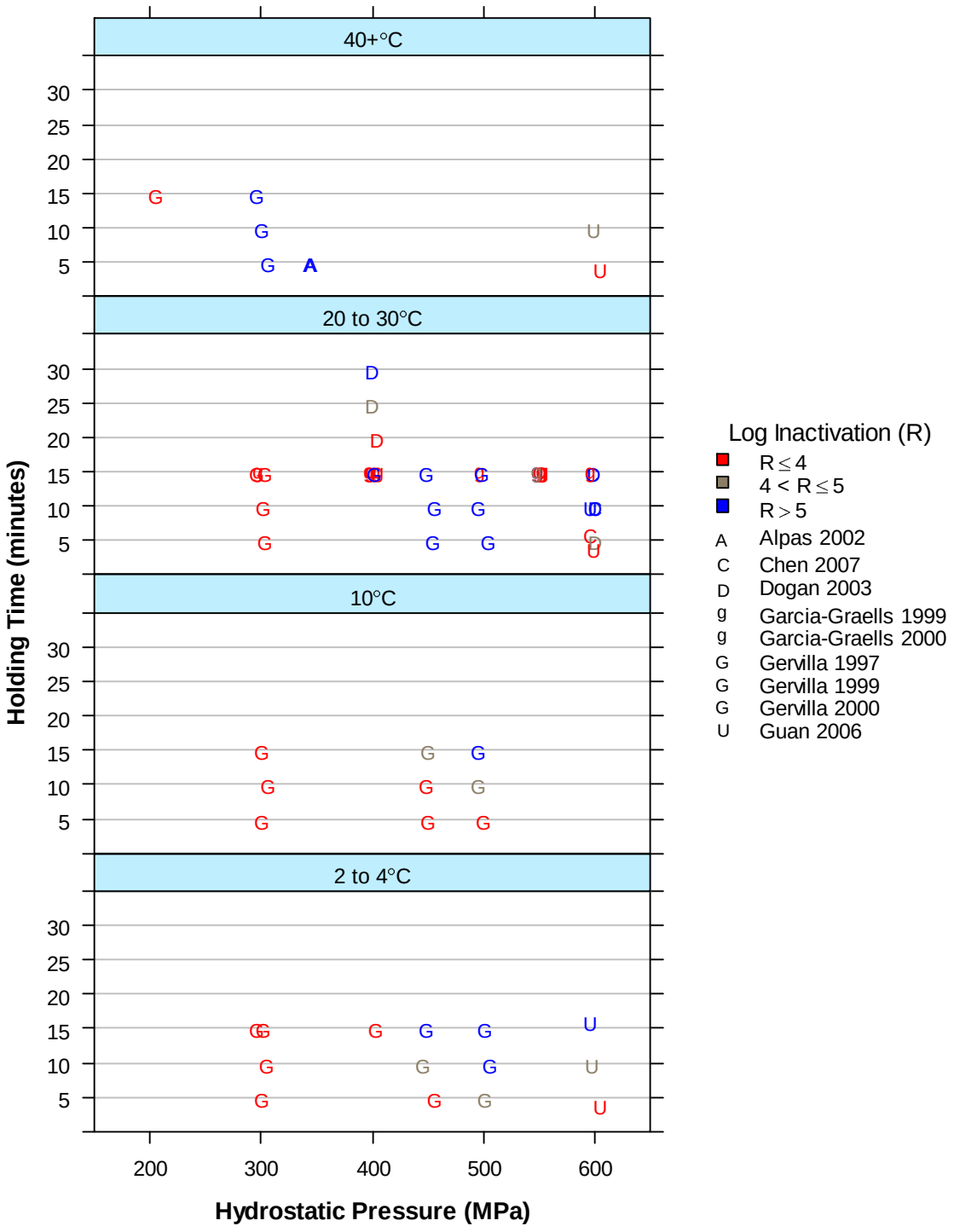


Figure 17: Inactivation of *E. coli* by study for high pressure treatments at different pressurised temperatures as a function of pressure and holding time



6.6.10 *Yersinia enterocolitica*

Two studies investigated the effect of HPP treatment on *Yersinia spp.*; one conducted in UHT skim milk and one in UHT whole milk.

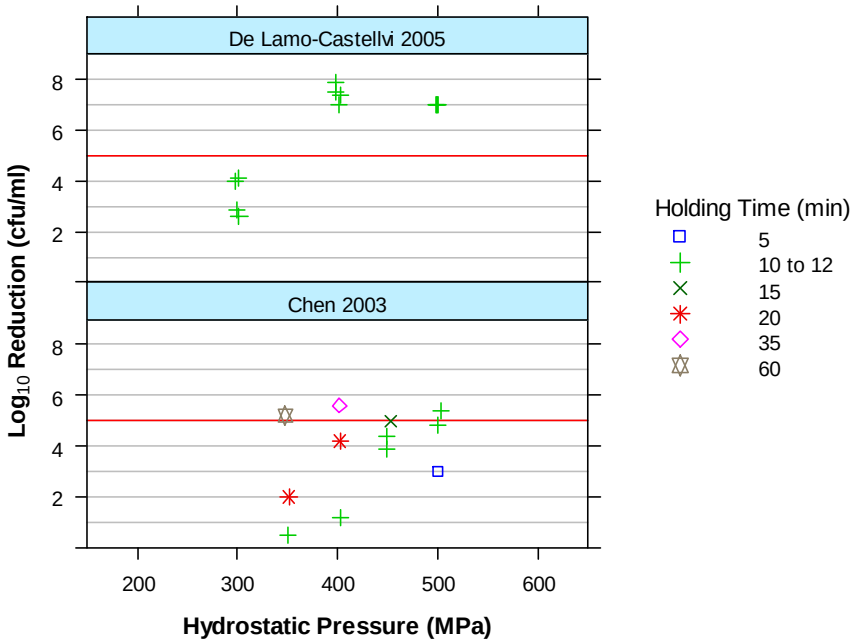
Studies:

HPP treatment of *Yersinia enterocolitica* ATCC 35669 inoculated in UHT whole milk at 350, 400, 450 or 500 MPa and pressurised temperature of approximately 22°C required treatment times of 60, 35, 15, and 12 minutes, respectively, to achieve >5 $\log_{10}$ reduction in bacterial counts (Chen and Hoover 2003).

Da Lamo-Castellvi et al. (2005) reported >7 $\log_{10}$ inactivation of four pathogenic strains of *Y. enterocolitica* in UHT skimmed milk after treatment at 400-500 MPa / 10 minutes/ 20°C (pressurised temperature). The increased inactivation compared to that observed in the Chen and Hoover study (Figure 18), could be due to strain variation and/or the reduced fat content of the milk.

The Da Lamo-Castellvi et al. (2005) study also investigated bacterial behaviour after HPP treatment, testing samples stored at 8°C for up to 15 days. Samples treated at 300 MPa showed an initial ~3-4 $\log_{10}$ reduction, followed by growth to over 8 $\log_{10}$ after 4 days of storage. After treatment at 500 MPa, no viable cells were detected, but cells started to grow between days 1 and 10, mostly at day 4, supporting the hypothesis that the treatment did not kill all the *Yersinia* cells and instead caused serious injury to some cells that needed time to repair.

Figure 18: Inactivation of five different *Y. enterocolitica* strains after high pressure treatment at pressurised temperatures 20-22°C as a function of pressure. The red line marks a 5 $\log_{10}$ reduction.



A summary of *Yersinia* spp. inactivation data can be found in Appendix A,Table **23**.

Conclusion:
The two studies do not provide sufficient data to make HPP inactivation parameter recommendations. However, the study data suggests it is possible to achieve a 5 $\log_{10}$ reduction in *Yersinia* concentration, if sufficient time at pressure is allowed. For the single strain considered in the Chen and Hoover study this was at least 60 minutes at 350 MPa/22°C (pressurised temperature), and at least 12 minutes at 500 MPa. It has been shown, however, that regrowth and repair can happen within a few days of storage at 8°C after the high pressure treatment.

6.7 DYNAMIC AND "CYCLIC" HIGH PRESSURE TREATMENT

The majority of studies located in the systematic review investigated the effect of high-pressure treatment using one cycle of treatment. Two publications and one patent (AU2017101178) looked into pressure treatments which used cycles of high pressure interrupted by brief decompressions (Vachon et al. 2002; Garcia-Graells, Masschalck, and Michiels 1999).

A study, in raw milk, looked into the effect of dynamic high pressure (DHP) and cyclic treatments on a single strain of *E. coli* and *L. monocytogenes* (Vachon et al. 2002). In contrast to the HPP batch system described in other studies in this report, DHP was conducted using a continuous flow homogeniser device operating at 25°C with a flow rate of 25 ml/minute. The dynamic pressure treatment enhanced the sensitivity of bacterial cells and facilitated inactivation rates of >5 $\log_{10}$ at pressures as low as 300 MPa for *L. monocytogenes* (25°C/5 passes) and 200 MPa for *E. coli* O157:H7 (25°C/3 passes), as shown in Figure 19. Viable counts of both pathogens were reduced with increased pressure and number of passes. The *E. coli* strain was more sensitive to cell inactivation than the *L. monocytogenes* strain. The authors also compared DHP to static HPP, although in a broth matrix. DHP was found to be more effective than static HPP when the same pressures were applied for the same total treatment time.

Another study (Figure 20), which used UHT milk, in a small 8-ml HPP vessel (500 MPa, 20°C), found an increase in inactivation of *E. coli* K12 (MG1655) from 4.5 $\log_{10}$ to 6 $\log_{10}$ for 2 and 3 10-minute treatment cycles, respectively (Garcia-Graells, Masschalck, and Michiels 1999). One pressure vessel was temperature controlled, while their second larger pressure vessel was not. The second vessel did not exceed 35°C for experiments at ambient pre-compression temperatures.

Repeated pressure treatments interrupted by brief decompressions always resulted in a higher inactivation than continuous treatment for the same total treatment time, i.e. cyclic treatment of 3 x 10 minutes versus continuous treatment of 1 x 30 minutes. Pressure resistant mutant strains were less susceptible to cyclic treatments than the parent strain.

Figure 19: Effect of number of pressure cycles during dynamic high pressure processing on the inactivation of *E. coli* O157:H7 and *L. monocytogenes* inoculated in raw milk (Vachon et al. 2002).

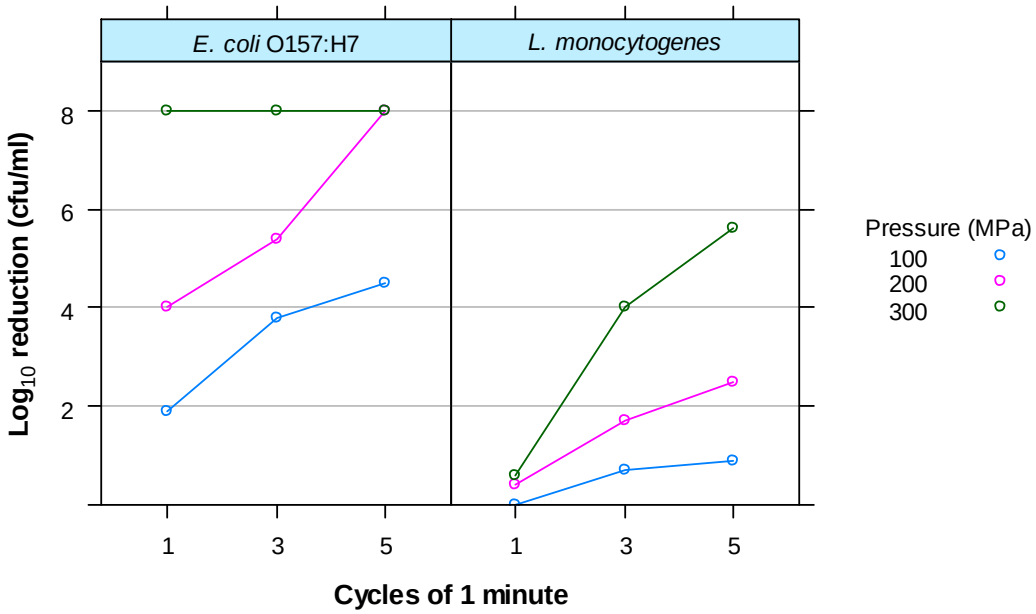
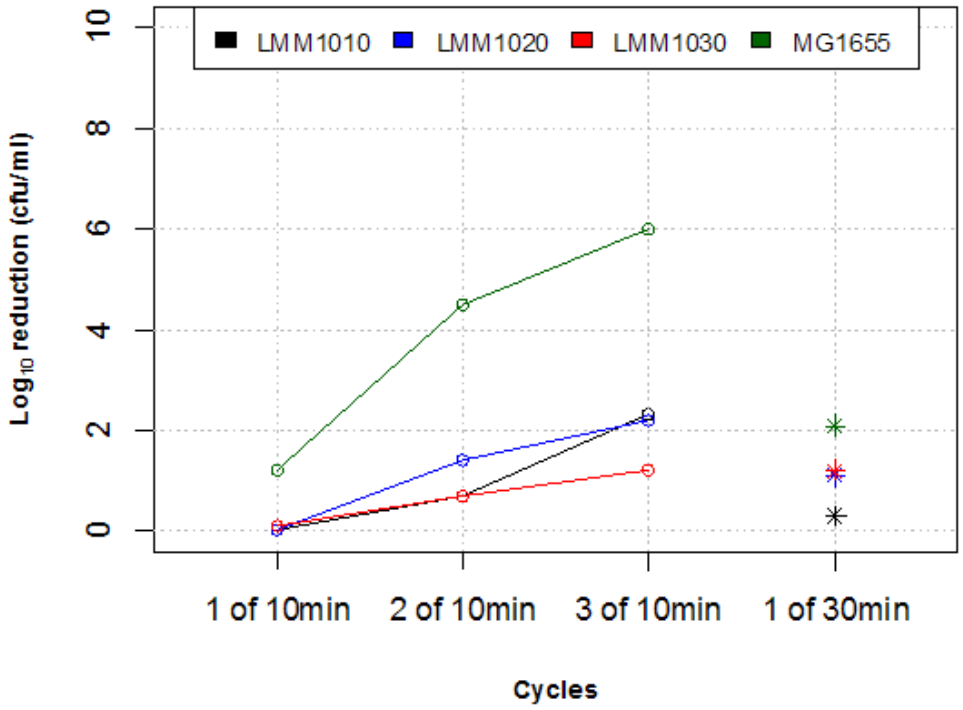


Figure 20: Effect of the number of cycles of high pressure on the inactivation of *E. coli* K12 parent strain MG1655 and pressure resistant mutants thereof (LMM1010, 1020, 1030) (Garcia-Graells, Masschalck, and Michiels 1999).



An Australian patent for a HPP method for the treatment of raw milk (application number AU2017101178) lists summary information on a raw milk challenge trial. Information on the number or name of the strains tested, the pressurisation temperature, the growth phase of the cells at the start of the pressurisation and the enumeration methods are not provided. From the general patent information, the temperature during pressurisation should have been between 45 and 60°C. Experiments with two cycles of 90 seconds duration, or one cycle of 180 or 240 second duration were conducted at a pressure of 600MPa (Table 13).

Table 13: Inactivation (Log_{10} reduction) of pathogens due to different processing durations at 600 MPa (Australian Patent: AU2017101178).

Pathogen	**Log_{10} reduction**		
Processing conditions	**600 MPa for 3 minutes**	**600 MPa for 4 minutes**	**600 MPa for 2x90 seconds**
E. coli	3.3	> 3	> 6
L. monocytogenes	4 to 5	5	>6
S. typhymurium	2.7	2 to 3	3 to 6
S. aureus	Not tested	2 to 3	1.3 to 3
C. jejuni	Not tested	Not tested	1.2

The above studies suggest that for some pathogen strains, multiple cycle pressure treatments may be more effective than single cycle treatments applied for the same total treatment time. However, the effects may be less pronounced for pressure resistant strains of pathogens, and in the patent challenge study, cyclic treatments of 2 x 90 seconds resulted in noticeably less inactivation of strain or strains tested of *S. aureus* and *Campylobacter* than observed for a strain or strains of *E. coli*, *L. monocytogenes* and *S. typhymurium*.

6.8 APPLICATION OF HPP IN MILK OVERSEAS

In the US, there are currently no companies using HPP for raw milk intended for retail sale. This is due to challenges in demonstrating equivalence to FDA pasteurised milk regulations (Alvin Lee, Institute for Food Safety and Health, IL, USA, personal communication).

HPP-treated raw milk has been approved for retail sale by the New South Wales Food Authority. The associated publicly available patent information (application number AU2017101178) points towards a “cycled” treatment process with the following parameters:

- Treatment pressures of between 350 and 750MPa.
- During pressurisation, the temperature of milk should be above 45°C, but below 60°C.
- Two or more cycles of pressure are applied, where each pass is between 60 and 150 seconds long and the pressure is released for between 1 to 10 seconds.

The patent provided test information on a challenge study using a pressure of 600MPa, with two cycles of 90 seconds. The 5 $\log_{10}$ reduction required by the NSW Food Authority was achieved for the *E. coli* and *L. monocytogenes* strains tested. However, the inactivation target was not achieved for *S.* Typhimurium, *S. aureus* or *Campylobacter* (see section 6.7). The patent argues that these three pathogens "can be controlled by applying hygienic raw milk production techniques and animal health strategies in combination with raw milk compliance testing prior to high pressure processing."

6.9 PATHOGEN RESISTANCE TO HPP IN MILK

6.9.1 Ranking pathogens for pressure sensitivity

The answer to the question which bacteria or strains of bacteria should be used for challenge studies for HPP of milk depends on both the bacteria and strains potentially present in New Zealand milk and also the pressure sensitivity of the bacteria.

Summarised in Section 6.6 and Appendix B of this report, the levels of inactivation of bacteria depend on a number of factors including differences in inactivation between strains of the same bacteria. To provide some guidance on which bacteria may be most important for trials based on the review data, the following approach has been taken. The inactivation of different bacteria which have been subjected to an increased pressure for a period of 10 minutes are compared in Figure 21. A time period of ten minutes was chosen, as this was a common test time in the experiments summarised in the systematic review and allowed the greatest amount of data to be compared.

At pressurised temperatures in the range 2-4°C, the available data are from *E. coli* and *L. innocua* experiments. The *E. coli* strains are slightly more pressure resistant for pressures in the range 300-600MPa.

In the pressurised temperature range 20 to 39°C there is no pathogen which is more pressure resistant than others across the range of pressures. *Y. enterocolitica* ATCC 35669 (skimmed UHT milk) is consistently one of the most pressure resistant pathogens for treatment pressures of 350 to 500 MPa, and *S. Typhimurium* strain DT104 for the pressures between 500 and 600MPa.

C. jejuni and *S. aureus* strains were identified as more pressure resistant than the strains of *E. coli, L. monocytogenes, S.* Typhimurium during the patent proving trials at 600 MPa/<45°C given in Table 13.

A study by Chen et al. (2007), to model the pressure response of different pathogens in UHT milk, found that a treatment of 600 MPa for 10 min, with pre-compression temperature of 21.5°C reduced the counts of *E. coli* O157:H7 by 5.9 $\log_{10}$. In this experimental setting, the order, least to most pressure-resistant pathogen, was: *L. monocytogenes* < *S.*Typhimurium = *S.* Enteritidis < *E. coli* O157:H7 = *S.aureus* < *Shigella flexneri.* However only one strain of each pathogen was tested in order to come to this conclusion.

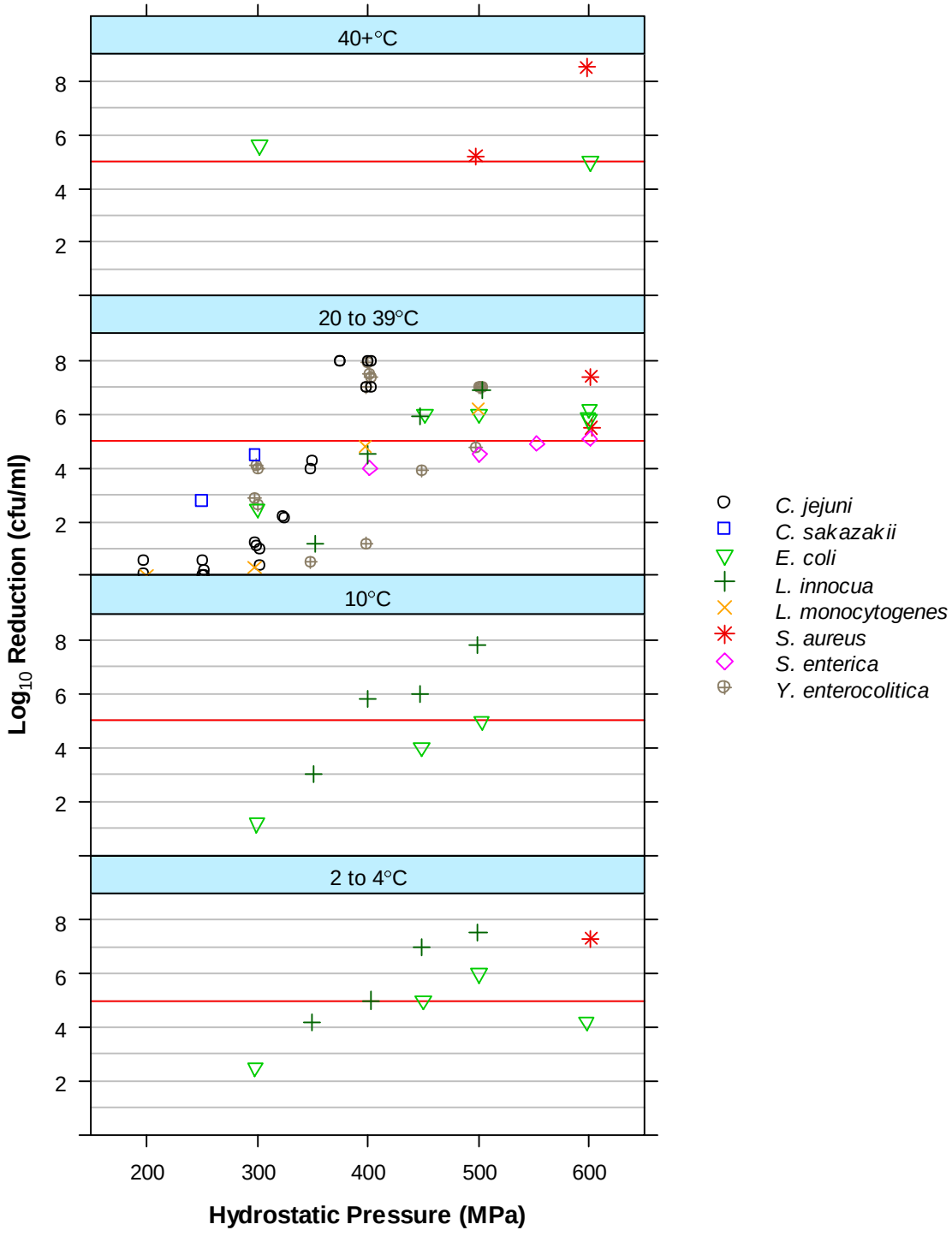
Yang et al. (2012) compared the inactivation of a single strain of *E. coli, S.* Typhimurium, *Shigella dysenteriae* and *S. aureus* in sterile milk[19]. Their study found the *S. aureus* strain to be the most pressure resistant for pressure treatments in the 100 to 300 MPa range, when treated at 25°C for 30 minutes. At 300 MPa and above, the four pathogens had similar concentration reductions.

The above results do not give a clear indication of which bacteria are more pressure resistant than others in milk. The range of inactivation for different pathogens overlap due to strain and HPP process variations. There is insufficient data to comment on how treatment temperature or holding time would affect the choice of pathogens to test.

A study of the inactivation of New Zealand relevant pathogen strains in milk due to HPP treatment may be required to establish suitable test strains for validation studies.

[19] This paper talks about inactivation in raw milk, but the methodology states sterile milk.

Figure 21: Inactivation of pathogens after high pressure treatment at different pressurised temperatures (2-4, 8-10, 20-39 and 40-50°C) as a function of pressure applied for 10 minute holding time. The red line marks a 5 log_{10} reduction.



6.9.2 Fat content

The effect of fat content on the inactivation of cells in milk by HPP has been considered in some studies, with mixed results.

In a study by Garcia-Graells (1999) increased fat content of milk provided a protective effect, with inactivation greater in skimmed milk than in whole milk. The authors compared the effect of HPP (600 MPa/20°C/15 minutes) on *E. coli* in whole, half-whole and skim milk, the results are given in Table 14. The LMM strains are pressure resistant mutants of strain MG1655, and an effect due to milk fat content was apparent, even for more pressure resistant strains.

Table 14: Logarithmic reduction of *E. coli* concentration after pressure treatment at 600 MPa for 15 minutes at 20°C in UHT milk of different fat contents

E.coli strain	Whole milk (3.6% fat)	Half-whole milk (1.55% fat)	Skim milk (0.05% fat)
MG1655	1.6	2.3	3.0
LMM1010	0.1	0.3	0.3
LMM1020	0.4	1.6	1.4
LMM1030	0.8	1.3	1.4

Gervilla et al. (2000) tested a single strain of *E. coli*, *L. innocua* and *S. aureus* in pasteurised ewes milk at temperatures of 4, 25 and 50°C, at 200-400 MPa for 15 minutes. Milk was tested with a 0, 6 and 50% fat content. No protective effect was identified for *E. coli* and *S. aureus*. A protective effect was seen for *L. innocua* treated at 4°C. The log reduction after treatment at 4°C was ~3, 2.5 and 1.5 for the 0, 6 and 50% fat content milk respectively. At higher temperatures it is not possible to say if there is an effect for *L. innocua* as the pressure/temperature combinations plotted in the paper either completely inactivated the cells or no significant inactivation occurs across the milk types.

Solomon and Hoover (2004) compared the inactivation of a *Campylobacter* strain in UHT whole and skimmed milk subjected to pressures in the range 0 to 375 MPa for 10 minutes at 25°C. They found no difference in the inactivation of cells for the two milk types.

The above data shows there is potential for fat content to have an effect on the outcome of pathogen reduction during HPP of milk, with increasing fat content decreasing inactivation of pathogen cells. However, the effect is not well pronounced for all pathogens and for all range of temperatures.

6.9.3 Milk storage temperature prior to pressure treatment

Several authors have suggested that it is important to establish defined growth conditions to be able to evaluate and compare the effectiveness of treatment conditions. Shearer et al.

(2010) observed an ~ 6 $\log_{10}$ difference in the number of *L. monocytogenes* cells that survived treatment at 400 MPa/21°C/2 minutes when the cells had been grown at 43°C for 16 hours prior to HPP, compared to cells grown in the range of 10 to 25°C for longer time periods prior to HPP. These results are in agreement with those reported by Hayman et al. (2007).

6.10 EFFECT OF HPP ON INDIGENOUS ENZYMES IN BOVINE MILK: POSSIBLE USE AS PROCESS INDICATORS?

Indigenous milk enzymes are inactivated by high pressure; the rate of inactivation depends on the pressure, holding time and the pre-treatment history of the raw milk (Koncza et al. 2007). Some research is being conducted to investigate the possible use of indigenous milk enzymes as process indicators for HPP.

Three indigenous milk enzymes are the main focus for HPP-treatment related effects on enzyme activity: alkaline phosphatase (ALP), γ-glutamyltransferase (GGT) and phosphohexoseisomerase (PHI). The pressure stability of these enzymes may be ranked in the following order: ALP>GGT>PHI (Rademacher and Hinrichs 2006). Their potential use as a process indicator requires a similar inactivation behaviour between microorganisms of concern and the enzyme.

The alkaline phosphatase (ALP), is always present in raw milk and its heat sensitivity is used as an indicator for the effectiveness of heat pasteurisation (Koncza et al. 2007). ALP is very pressure-stable compared with most pathogens, therefore it appears to be less suitable as a HPP process indicator. After treatment at 500, 600 or 700 MPa/5°C/10 minutes the relative activity of ALP was 80, 70 and 30%, respectively (Rademacher and Hinrichs 2006). Another study reported that treatment at 400, 500 and 600 MPa/20°C/10 minutes resulted in a relative enzyme activity of 80, 70 and 10%, respectively (Koncza et al. 2007).

Compared with ALP, GGT is less pressure-stable and complete inactivation of GGT was achieved by pressure treatment at 600 MPa/20°C/30 minutes (Rademacher and Hinrichs 2006). At 500 MPa the relative enzyme activity dropped to 70% after 10 minutes and to 50% after 30 minutes. The authors suggest that the kinetics of inactivation of GGT at 20°C and pressures above 500 MPa are sufficiently close to the inactivation of *L. monocytogenes* and *E. coli* to warrant consideration as a useful process marker for the destruction of these microorganisms. It should be noted that at low pressures (300 and 350 MPa) an initial increase in enzyme activity was observed. This increase in enzyme activity was found after the initial pressure pulse, followed by a clear inactivation of GGT activity with increasing holding time (Pandey and Ramaswamy 2004).

PHI is more pressure-sensitive than ALP and GGT. PHI was nearly completely inactivated after pressure treatment at 500 MPa/20°C/15 minutes. The relative activity was 10% after treatment at 500 MPa/20°C/2 minutes (Rademacher and Hinrichs 2006).

Detailed knowledge of pressure-temperature inactivation kinetics of pathogenic bacteria in milk is required to determine which enzyme would be a potential process indicator for HPP treatment. The following would be required:

- Determine HPP process parameters that lead to the desired inactivation of selected pathogens;
- Literature review targeted at pressure inactivation of indigenous milk enzymes, followed by challenge studies (if necessary) to compare enzyme inactivation kinetics with pathogen inactivation kinetics.

6.11 CONCLUSIONS FROM REVIEW OF HPP APPLIED TO MILK

While conducting this systematic review on the effects of HPP treatment on pathogens in milk it became apparent that published data show considerable variability, even within the same bacterial species. Different strains, as well as the culture conditions (media, temperature) prior to inoculating the milk, influence the resistance to HPP treatment.

The inactivation kinetics of bacteria during HPP do not always follow a linear relationship with time, with shoulders or tails in inactivation rates being observed for some studies. Most of the experiments reported in the literature are not designed to establish how the inactivation of cells changes over the holding time, with testing at a single or a set of sampling times. This will contribute to the variability of results observed.

These findings make it extremely difficult to effectively compare inactivation rates obtained in different experiments and to provide generic 'safe harbour' treatment conditions for HPP of raw milk.

The use of dynamic or cyclic pressurisation can increase inactivation rates for some pathogen strains when compared to the same total processing time as a single cycle of pressure treatment. Time-pressure treatment conditions may need to be different for single cycle and multiple cycle treatments.

From the systematic review the following considerations should be taken into account for milk challenge studies:

- The storage temperature of the milk prior to treatment by HPP may affect the inactivation rates of any pathogen originally present in the milk. *L. monocytogenes* grown at 35-43°C was found to be more pressure resistant than grown at 10-25°C. Any challenge studies should store inoculated milk at the same storage temperature and time prior to HPP as will be used during the commercial HPP.

- Inactivation of bacterial cells might depend on fat content of the milk. Challenge studies should use milk with similar fat characteristics to the proposed product.

- A study of the inactivation of New Zealand relevant pathogen strains in milk due to HPP treatment may be required to establish suitable test strains for validation studies. The literature does not provide a clear indication of which pathogens or pathogen strains should be used for validation studies.

- Some studies have shown that sub-lethally injured cells in milk that have not been detectable immediately following HPP, can repair and grow during storage. Challenge studies should include monitoring for the shelf life of the product.

APPENDIX A: SUPPLEMENTARY INACTIVATION DATA

Notes for following tables:

Where it is unclear if the temperature given in the papers is the pre-compression temperature or pressurised temperature, the temperature will be underlined.

Where multiple enumeration media/procedures have been compared the results of procedure resulting in the highest cfu counts are recorded.

Bacterial strains used in the publications included in this review were derived from a variety of culture collections. Please refer to the following abbreviations of culture collections for strains listed in the Appendix tables. Note that some studies used their own laboratory strains or derived strains elsewhere, in these cases refer to the original publication for details.

ATCC = American Type Culture Collection, Manassas, VA, USA

BCRC = Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan

CECT = Spanish Type Culture Collection, CECT, Universidad de Valencia, Valencia, Spain

CICC = China Center of Industrial Culture Collection, Xiao Yun Road Chao Yan District Beijing, China

CIP = Institut Pasteur Collection, Paris, France

KUEN = KUKENS – Center for Research and Application of Culture Collections of Microorganisms, Istanbul Faculty of Medicine, Turkey

LMG = Belgian Coordinated Collection of Microorganisms, Ghent, Belgium

NCFB = National Collection of Food Bacteria, NCIMB, UK

NCTC = National Collection of Type Cultures, Central Public Health England Laboratory, London, UK

Table 15: Effect of hydrostatic pressure on *B. cereus* vegetative cells and spores in UHT milk (McClements et al. 2001)

Strain / Type	Extra Info	Milk Type	Pre-compression temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (minutes)	Reduction in log count (log_{10} cfu/ml)
NCFB 578,	Inoculum cells in exponential growth phase	UHT skim milk	8	400	1	2	**5**
NCFB 1031	Inoculum cells in exponential growth phase		8	400	1	5	3
NCFB 578	Inoculum cells in stationary growth phase		8	400	1	6	**5**
NCFB 1031	Inoculum cells in stationary growth phase		8	400	1	15	**5**
NCFB 578	Inoculum cells in exponential growth phase		30	400	1	0.5	**6.04**
NCFB 1031	Inoculum cells in exponential growth phase		30	400	1	0.5	**5.25**
NCFB 578	Inoculum cells in stationary growth phase		30	400	1	15	3.2
NCFB 1031	Inoculum cells in stationary growth phase		30	400	1	15	2.5
NCFB 578	spores		8	400	1	30	0
NCFB 578	spores		8	400	1	30	13.4% induction of germination
NCFB 1031	spores		8	400	1	30	-0.2
NCFB 1031	spores		8	400	1	30	8.3% induction of germination
NCFB 578	spores		30	400	1	30	0.5
NCFB 578	spores		30	400	1	30	75.8% induction of germination
NCFB 1031	spores		30	400	1	30	0.5
NCFB 1031	spores		30	400	1	30	18.7% induction of germination

Log reductions of ≥5 cfu/ml are marked in **bold**

Table 16: Effect of hydrostatic pressure on *Campylobacter jejuni* in milk

Strain	Milk type	Pre-compression Temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (minutes)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
11351	UHT	20	200	1	10	0.1	Martinez-Rodriguez 2005
			200	1	10	0.6	
			250	1	10	0.2	
			250	1	10	0.6	
			300	1	10	0.4	
			300	1	10	1	
			400	1	10	**7**	
			400	1	10	**7**	
ATCC 35921	UHT skim milk	25	250	1	10	0.05	Solomon 2004
	UHT whole milk		250	1	10	0	
	UHT skim milk		300	1	10	1.2	
	UHT whole milk		300	1	10	1.2	
	UHT skim milk		325	1	10	2.2	
	UHT whole milk		325	1	10	2.2	
	UHT skim milk		350	1	10	4.3	
	UHT whole milk		350	1	10	4.0	
	UHT whole milk		375	1	10	**8**	
	UHT skim milk		375	1	10	**8**	
	UHT whole milk		400	1	10	**8**	
	UHT skim milk		400	1	10	**8**	

Log reductions of ≥5 cfu/ml are marked in **bold**

Table 17: Effect of hydrostatic pressure on *Cronobacter sakazakii* (formerly *Enterobacter sakazakii)*[#] in milk

Strain	Milk Type	Precompression Temperature (°C)	Pressurised Temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (minutes)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
ATCC 29544 [CECT 858]	Infant formula	20	20 (Peak <36)	300	1	124	5	Arroyo 2011
ATCC 29544		16.7	21.8	200	1	1	0.2	Gonzales 2006
ATCC 12868		16.7	21.8	200	1	1	0.3	
ATCC 29004		16.7	21.8	200	1	1	0.3	
ATCC 51329		16.7	21.8	200	1	1	0.6	
ATCC 29544		12.3	23.3	400	1	1	0.4	
ATCC 29544		12.3	23.3	400	1	1	0.9	
ATCC 12868		12.3	23.3	400	1	1	1.6	
ATCC 29004		12.3	23.3	400	1	1	1.9	
ATCC 29544		8.4	25.3	600	1	1	3.1	
ATCC 29004		8.4	25.3	600	1	1	**5**	
ATCC 51329		8.4	25.3	600	1	1	**6**	
ATCC 12868		8.4	25.3	600	1	1	**6**	
ATCC 29544	Infant formula	15	23	250	1	10	2.8	Pina Pérez 2007
			23	250	1	20	3.7	
			25	300	1	7	3	
			25	300	1	10	4.5	
			27	350	1	5	4.5	
			27	350	1	7	**5.2**	

			29	400	1	2	**5**	
			29	400	1	5	**6**	
ATCC 29544	Infant formula	25		500	1	20	**≥ 5**[§]	Koseki 2009
				550	1	10	**≥ 5**[§]	
				550	1	20	**≥ 7**[§]	
				600	1	5	**≥ 5**[§]	
				600	1	20	**≥ 7**[§]	
		40		450	1	20	**≥ 5**[§]	
				500	1	5	**≥ 5**[§]	
				500	1	10	**≥ 7**[§]	
				550	1	3	**≥ 5**[§]	
				550	1	10	**≥ 7**[§]	
				600	1	1	**≥ 5**[§]	
				600	1	5	**≥ 7**[§]	

Log reductions of ≥5 cfu/ml are marked in **bold**

[#] *Enterobacter sakazakii* was reclassified into eight distinct taxa of a new genus *Cronobacter* in 2007. The new classification has been adopted for this literature review and the name was changed accordingly (Iversen et al. 2007).
[§]This study determined death/survival only. Three different inoculum levels (3, 5 and 7 $\log_{10}$ cfu/ml) were used and results were obtained from survival/death interface figures in the original publication. The time to achieve 'death' at the specific inoculum level is presented in the table. The given times are likely to overestimate the required time for inactivation as only those results were included that showed no survival in any of the triplicates.

Table 18: Effect of hydrostatic pressure on *Listeria spp.* in milk (TCV: Temperature controlled Vessel, UNK: Unknown)

Species	Strain	Milk Type	Pre-compression temperature (°C)	Pressurised temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (minutes)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
L. monocytogenes	CA	Sterilised milk	50	UNK	345	1	5	**8**	Alpas 2002
	Ohio 2				345	1	5	**8**	
L. monocytogenes	ATCC 19115, Serotype 4b	UHT milk	21.5	UNK	600	1	1	**7.2**	Chen 2007
					600	1	6	**7.5**	
L. monocytogenes	KUEN 136	Raw milk	25	UNK	400	1	10	2.7	Erkman 2004
					400	1	15	4.3	
			25	UNK	600	1	5	**5.6**	
					600	1	10	**6.5**	
L. monocytogenes	LM 54004	Milk buffer	41	TCV	448	1	11	**6**	Gao 2006
L. innocua	LMG11387	Ovine milk, pasteurised	20	20 (Peak ≤30)	350	1	15	0.5	Garcia-Graells 2000
	CIP79.45				350	1	15	0.4	
	LMG13568				350	1	15	0.5	
	CIP78.44				350	1	15	2.2	
	LMG11387		20	20 (Peak ≤30)	400	1	15	2.8	
	CIP79.45				400	1	15	4.1	
	LMG13568				400	1	15	**5**	
	CIP78.44				400	1	15	**5.2**	

L. innocua	CECT 910	Ovine milk (6% fat), pasteurised	2	TCV	350	1	5	2.5	Gervilla 1997b
					350	1	10	4.2	
					350	1	15	4.5	
			10	TCV	350	1	5	2.8	
					350	1	10	3	
					350	1	15	4	
			25	TCV	350	1	5	0.1	
					350	1	10	1.2	
					350	1	15	1.2	
			2	TCV	400	1	5	4.5	
					400	1	10	**5**	
					400	1	15	**6**	
			10	TCV	400	1	5	4.5	
					400	1	10	**5.8**	
					400	1	15	**5.9**	
			25	TCV	400	1	5	3.5	
					400	1	10	4.5	
					400	1	15	4.5	

L. innocua	CECT 910	Ovine milk (6% fat), pasteurised	2	TCV	450	1	5	6	Gervilla 1997b
					450	1	10	**7**	
					450	1	15	**7.5**	
			10	TCV	450	1	5	**5.9**	
					450	1	10	**6**	
					450	1	15	**6.8**	
			25	TCV	450	1	5	**5**	
					450	1	10	**5.9**	
					450	1	15	**6.9**	
			2	TCV	500	1	5	**7.5**	
					500	1	10	**7.5**	
					500	1	15	**7.5**	
			10	TCV	500	1	5	**7.8**	
					500	1	10	**7.8**	
					500	1	15	**7.8**	
			25	TCV	500	1	5	**6.9**	
					500	1	10	**6.9**	
					500	1	15	**6.9**	

L. innocua	CECT 910	Ovine milk, 0, 6, 50% fat, pasteurised	50	TCV (Peak 53)	200	1	15	0	Gervilla 2000
			4	TCV	300	1	15	1	
			25	TCV	300	1	15	0.5	
			50	TCV (Peak 53)	300	1	15	**7.5**	
			4	TCV	400	1	15	**5.5**	
			25	TCV	400	1	15	4.5	
L. monocytogenes	ATCC 19115, Serotype 4b	UHT milk	20-25		400	1	4	2.8	Hayman 2007
					400	1	10	4.2	
L. monocytogenes	BCRC 15354	Raw milk	25		250	1	5	0	Huang 2015
					250	1	10	0.2	
					300	1	5	0.9	
					300	1	10	2.7	
					350	1	5	4.4	
					350	1	10	**5.7**	
					400	1	5	**6.3**	
					400	1	10	**7.7**	
					450	1	5	**8.7**	
					450	1	10	**8.7**	

L. monocytogenes	**ATCC 19117**	**UHTmilk**	**25**	**25 (Peak ≤33)**	**400**	**1**	**5**	**0.5**	**Koseki 2008**
					450	1	5	1.6	
					500	1	5	**5.2**	
					550	1	5	**7**	
					600	1	5	**7**	
L. monocytogenes	CICC 21633	UHT, skim milk	UNK	UNK	300	1	5	1.5	Liu 2017
					400	1	5	3.4	
					500	1	5	**6**	
L. monocytogenes	NCTC 11994	UHT, skim milk	8		400	1	8	2.8	McClements 2001
					400	1	12	3.2	
			30		400	1	8	2	
					400	1	12	3.2	
	Scott A	UHT, skim milk	8		400	1	8	1.9	
					400	1	12	2.3	
			30		400	1	8	2.2	
					400	1	12	2.6	
L. monocytogenes	Cocktail of 5 strains	UHT milk	25	TCV	200	1	10	0	Misiou 2017
					300	1	10	0.3	
					400	1	10	4.8	
					500	1	10	**6.2**	

L. monocytogenes	NCTC 11994	UHT milk	20	UNK	375	1	25	1.2	Patterson 1995
					375	1	30	1.4	
L. innocua	ATCC 51742	UHT milk	20	UNK	500	1	5	**5**	Serment-Moreno 2017
					600	1	2.5	**5**	
L. monocytogenes	ATCC 19115	UHT milk	21		400	1	2	4.5	Sherer 2010
					400	1	2	4.9	
L. monocytogenes	LSD 105-1	Raw milk	25		100	1	1*	0	Vachon 2002
					100	3	1*	0.7	
					100	5	1*	0.9	
					200	1	1*	0.4	
					200	3	1*	1.7	
					200	5	1*	2.5	
					300	1	1*	0.6	
					300	3	1*	4	
					300	5	1*	**5.6**	

Log reductions of ≥5 cfu/ml are marked in **bold**

* length of 'one pass' not specified, however, another study of the same group stated the use of 1-minute cycles (Kheadr et al. 2002)

Table 19: Effect of hydrostatic pressure on *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in milk

Strain	Milk type	Pre-compression Temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (min)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
NCTC 8578	UHT	20	400	1	5	0.8	Donaghy 2007
806R (milk isolate)				1	5	0.9	
NCTC 8578				1	10	2.2	
806R (milk isolate)				1	10	2.9	
NCTC 8578			500	1	5	3.9	
806R (milk isolate)				1	5	**5**	
NCTC 8578				1	10	**5.6**	
806R (milk isolate)				1	10	**6.3**	
NCTC 8578			600	1	5	**> 7**	
806R (milk isolate)				1	5	**> 7**	
NCTC 8578				1	10	**6.1**	
806R (milk isolate)				1	10	**6.8**	
ATCC 19698	UHT	20	300	1	10	0.7	Lopez-Piemonte 2006
36644/02 (paratuberculosis in cattle isolate)			300	1	10	0.6	
36644/02 + ATCC 19698			300	1	10	0.6	
ATCC 19698			400	1	10	1.5	
36644/02			400	1	10	0.7	

36644/02 + ATCC 19698			400	1	10	1.2	
ATCC 19698			500	1	10	4.1	
36644/02			500	1	10	**5.2**	
36644/02 + ATCC 19698			500	1	10	4.8	

Log reductions of ≥5 log cfu/ml are marked in **bold**

Table 20: Effect of hydrostatic pressure on *Salmonella enterica* spp. in milk (TCV: Temperature controlled vessel, UNK: unknown temperature)

Serotype	Strain	Milk Type	Pre-compression temperature (°C)	Pressurised temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (min)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
Enteritidis	FDA	Past milk, sterilised	50	UNK	345	1	5	**8**	Alpas 2002b
Typhimurium	E21274				345	1	5	**8**	
Enteritidis	NR1	UHT milk	21.5	TCV	600	1	2	3.7	Chen 2007
Typhimurium	DT104				600	1	2	4.7	
Enteritidis	NR1				600	1	4	**5.5**	
Typhimurium	DT104				600	1	4	**5.5**	
Enteritidis	NR1				600	1	6	**6.7**	
Typhimurium	DT104				600	1	6	**5.8**	
Typhimurium	KUEN 1357	Raw milk	Chosen to reach 21	25	400	1	10	4	Erkmen 2011
			at target pressure		400	1	15	4.6	
					400	1	20	**5.5**	
					400	1	25	**5.7**	
Typhimurium	DT104	UHT milk	Chosen to reach 21	21	350	1	30	0.6	Guan 2005
			at target pressure		400	1	30	1.8	
					450	1	30	**5**	
					500	1	10	4.5	
					500	1	20	4.9	

					550	1	10	4.9	Guan 2005
					600	1	10	**5.1**	
Typhimurium	CICC 21484	UHT, skim milk	25		300	1	5	2.8	Liu 2017
					400	1	5	**6**	
					500	1	5	**6**	
Typhimurium	LT2	Sterile milk	25	UNK	300	1	30	**7**	Yang 2012
					400	1	30	**7**	
					500	1	30	**7**	

Log reductions of ≥5 cfu/ml are marked in **bold**

Table 21: Effect of hydrostatic pressure on *S. aureus* in milk (TCV: Temperature controlled vessel, UNK: Temperature unknown)

Strain	Milk Type	Pre-compression temperature (°C)	Pressurised Temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (minutes)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
485	Past. milk	50	UNK	345	1	5	**5.5**	Alpas 2002
765				345	1	5	**8.3**	
210	UHT milk	21.5	TCV	600	1	6	3.3	Chen 2007
				600	1	8	3.5	
				600	1	10	**5.5**	
ATCC 27690	Past. milk	20	20±2	300	1	4	**5**	Erkmen 1997
				350	1	4	**5**	
				300	1	6	**5**	
				350	1	6	**8**	
CECT 534	Ovine milk, 6% fat	2	TCV	500	1	15	2.5	Gervilla 1999b*
		10		500	1	15	3	
		25		500	1	15	3.2	
		50	TCV	500	1	5	**5.0**	
				500	1	10	**5.5**	
				500	1	15	**7.2**	
CECT 534	Ovine milk, 0, 6, 50% fat, pasteurised	4	TCV	400	1	15	0.5-1.5 #	Gervilla 2000

				500	1	15	2-3 #	
		25	TCV	400	1	15	1.5-2 #	
				500	1	15	3.5-4 #	
		50	TCV	400	1	15	2.8-3.2#	
				500	1	15	**7-7.5#**	
ATCC 12600	UHT	Chosen to reach	4	600	1	8	**5.6**	Guan 2006
		pressurised temperature	4	600	1	10	**7.3**	
		at target pressure	21	600	1	6	**5.6**	
			21	600	1	8	**7.3**	
			21	600	1	10	**7.4**	
			45	600	1	6	**6**	
			45	600	1	8	**8.5**	
			45	600	1	10	**8.5**	
ATCC 25923	UHT	25		300	1	5	0.5	Liu 2017
				400	1	5	4	
				500	1	5	**5.5**	
ATCC 6538	Reconstituted skimmed milk (10% RSM)	25	TCV	250	1	30	2.8	Morgan 2000
				300	1	30	**6**	
NCTC 10652	UHT milk	20	UNK	600	1	20	3.5	Patterson 1995

				600	1	25	4.5	
				600	1	30	**5**	
NCTC 10652	UHT milk	10	UNK	600	1	15	3	Patterson 1998
				700	1	15	**5.2**	
		20	UNK	500	1	15	2.5	
				600	1	15	**5.2**	
		50	UNK	400	1	15	4.5	
				500	1	15	**6**	
Sa9 (mastitic milk isolate)	Past. milk	10	UNK	400	1	5	2	Tabla 2012
				500	1	5	3	
ATCC 29213	Sterilised raw milk	25	UNK	**400**	**1**	**30**	**7**	Yang 2012
				500	**1**	**30**	**7**	

Log reductions of ≥5 cfu/ml are marked in **bold**

[#] Range includes results for different fat contents (0, 6 and 50% fat), which had almost no impact on the survival of *S. aureus*
***** Gervilla et al (1999b) paper contains results for HPP at 200, 300, 400, 450 and 500 MPa, and holding times of 5, 10 or 15 minutes.

Table 22: Effect of hydrostatic pressure on STEC and other *E. coli* in milk (TCV: Temperature controlled vessel, UNK: Unknown temperature)

Strain	Milk Type	Pre-compression temperature (°C)	Pressurised temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (minutes)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
O157:H7 933	Past milk, sterilised	50	UNK	345	1	5	**8**	Alpas 2002
O157:H7 931				345	1	5	**8**	
O157:H7	UHT	21.5	TCV	600	1	6	3.4	Chen 2007
				600	1	10	**5.9**	
				600	1	15	**5.5**	
KUEN 1504	Raw milk	25	TCV	400	1	20	4	Dogan 2003
				400	1	25	**5**	
				400	1	30	**5.5**	
				600	1	5	4.5	
				600	1	10	**5.8**	
				600	1	15	**7**	
K12, MG1655	UHT whole milk	20	TCV	300	1	15	0.5	Garcia-Graells 1999
				400	1	15	0.7	
				500	1	15	1.4	
				600	1	15	1.6	
		10	TCV	550	1	15	0.6	
		30	TCV	550	1	15	1.7	
		40	TCV	550	1	15	3.1	

		50	TCV	550	1	15	**7**	
K12, MG1655	UHT skim milk	20	TCV (Peak ≤ 30)	400	1	15	0.5	Garcia-Graells 2000
ATCC 43888; 0157:H7				400	1	15	0.6	
ATCC 11775				400	1	15	1	
ATCC 11303				400	1	15	3.9	
ATCC 11775				550	1	15	2	
ATCC 43888; 0157:H7				550	1	15	3	
K12, MG1655				550	1	15	3.8	
ATCC 11303				550	1	15	**5**	
CECT 405	Ovine milk (6% fat), pasteurised	Chosen to reach pressurised temperature at target pressure.	2	450	1	5	4	Gervilla 1997a
			2	450	1	10	**5**	
			2	450	1	15	**6**	
			10	450	1	5	4	
			10	450	1	10	4	
			10	450	1	15	**5**	
			25	450	1	5	**6**	
			25	450	1	10	**6**	
			25	450	1	15	**6**	
			2	500	1	5	**5**	
			2	500	1	10	**6**	

			2	500	1	15	**6**	
			10	500	1	5	4	
			10	500	1	10	**5**	
			10	500	1	15	**6**	
			25	500	1	5	**6**	
			25	500	1	10	**6**	
			25	500	1	15	**6**	
CECT 405	Ovine milk (6% fat), pasteurised	2	TCV	300	1	5	1.8	Gervilla 1999
				300	1	10	2.5	
				300	1	15	3	
		10	TCV	300	1	5	1.2	
				300	1	10	1.2	
				300	1	15	2	
		25	TCV	300	1	5	2.2	
				300	1	10	2.5	
				300	1	15	3.2	
		50	TCV	300	1	5	**5.4**	
				300	1	10	**5.6**	
				300	1	15	**6.5**	

CECT 405	Ovine milk, 0, 6, 50% fat, pasteurised	4	TCV	300	1	15	2.8 – 3.2	Gervilla 2000
				400	1	15	4.0 – 4.9	
		25	TCV	300	1	15	3.0 – 3.7	
				400	1	15	6.0 – 6.4	
		50	TCV	100	1	15	0.2	
				200	1	15	2.3 – 3.4	
ATCC 43895 0157:H7	UHT	Chosen to reach pressurised temperature at target pressure	4	600	1	4	1.3	Guan 2006
				600	1	10	4.2	
				600	1	16	**5.5**	
			21	600	1	4	2.0	
				600	1	10	**6.0**	
			45	600	1	4	2.0	
				600	1	10	**5.0**	
CICC 10305	UHT, skim milk	25		300	1	5	2.1	Liu 2017
				400	1	5	**6**	
				500	1	5	**6**	
NCTC 12079; O157:H7	UHT milk	20	UNK	600	1	25	2	Patterson 1995
				600	**1**	**30**	1.9	
NCTC 12079; O157:H7	UHT milk	10	UNK	600	1	15	0.5	Patterson 1998

		20	UNK	600	1	15	0.5	
		40	UNK	600	1	15	2.5	
		50	UNK	300	1	15	1.0	
		50	UNK	400	1	15	4.0	
		50	UNK	500	1	15	**8**	
O157:H7	Raw milk	25	UNK	100	1	1*	1.9	Vachon 2002
				100	3	1*	3.8	
				100	5	1*	4.5	
				200	1	1*	4	
				200	3	1*	**5.4**	
				200	5	1*	**8**	
				300	1	1*	**8**	
				300	3	1*	**8**	
				300	5	1*	**8**	
ATCC25922	Sterile milk	25	UNK	400	1	30	**7**	Yang 2012
				500	1	30	**7**	

Log reductions of ≥5 cfu/ml are marked in **bold**
* length of 'one pass' not specified, however, another study of the same group stated the use of 1-minute cycles (Kheadr et al. 2002)

Table 23: Effect of hydrostatic pressure on *Yersinia enterocolitica* in milk

Strain/serotype	Milk Type	Pre-compression temperature (°C)	Pressurised temperature (°C)	Target Pressure (MPa)	Number of Cycles	Holding Time (minutes)	Reduction in log count (log_{10} cfu/ml)	Reference (First Author, Year)
ATCC 35669	UHT whole milk	~22	22 (Peak ≤ 33)	350	1	10	0.5	Chen 2003
				350	1	20	2	
				350	1	60	**5.2**	
			22 (Peak ≤ 34)	400	1	10	1.2	
				400	1	20	4.2	
				400	1	35	**5.6**	
			22 (Peak ≤ 37)	450	1	10	3.9	
				450	1	12	4.4	
				450	1	15	**5**	
			22 (Peak ≤ 37)	500	1	5	3	
				500	1	10	4.8	
				500	1	12	**5.4**	
CECT 4055/O:3	UHT skimmed milk	20	Vessel controlled to 20	300	1	10	2.63	DeLamo 2005
CECT 4054/O:8				300	1	10	2.86	
CECT 559/O:1				300	1	10	4.01	
CECT 754/O:9				300	1	10	4.12	
CECT 559/O:1				400	1	10	**7**	
CECT 754/O:9				400	1	10	**7.38**	

CECT 4055/O:3				400	1	10	**7.51**	
CECT 4054/O:8				400	1	10	**7.9**	
CECT 559/O:1				500	1	10	**7**	
CECT 4055/O:3				500	1	10	**7**	
CECT 4054/O:8				500	1	10	**7**	
CECT 754/O:9				500	1	10	**7**	

Log reductions of ≥5 cfu/ml are marked in **bold**

APPENDIX B: METHODOLOGY

B.1 Systematic Literature Review

To capture as many relevant citations as possible, the scientific databases Pub Med and Web of Science were searched to identify primary studies of the effects of high pressure treatment on microorganisms potentially present in raw milk.

The two databases provided a good coverage of the topic and despite some overlap in results, each database provided enough unique material to warrant inclusion. The search strategy was the same for both databases with the same key words used for the search, results of which are summarised in **Error! Reference source not found.** below.

Table 24: Summary of search strategies and number of results

Database	Search field	Keywords	Number of search results	Comments
Pub Med	'All fields'	(hydrostatic pressure) AND milk	134	Reading titles/abstracts left 32 to be imported into EndNote
	'All fields'	pressure AND milk	4780	Many irrelevant hints, refined search
	'All fields'	(high pressure) AND milk	3456	Many irrelevant hints, refined search
	'All fields'	pressure*ed AND milk	117	All but one irrelevant
	'All fields'	(high hydrostatic pressure) AND milk	35	Six relevant, already included in 1st search
	'All fields'	(cold processing) AND milk	101	All but one irrelevant
	'All fields'	(high pressure processin)g AND milk	35	Already included in 1st search
Web of science	'topic'	(hydrostatic pressure) AND milk	666	Many irrelevant publications, changed search field to 'title'
	'title'	(hydrostatic pressure) AND milk	70	Reading titles/abstracts left 19 new titles to be imported into EndNote
	'title'	pressure AND milk	723	Many irrelevant hints
	'title'	(high pressure) AND milk	456	Many irrelevant hints

Searches were conducted on 9 and 10 November 2017, with no new publications identified 22 January 2018.

- Search results were imported into a bibliographic database (EndNote X8) and all duplicates removed. The literature search resulted in 74 citations from which potential relevant studies were selected for the review based on their title and abstract. Of these the full papers of 63 citations were assessed for relevance to the review on the effect of HPP on pathogens potentially present in New Zealand raw milk.

Inclusion and Exclusion Criteria for Data

Inclusion criteria:

- Treatment at temperatures ≤ 50°C.
- Plain milk – which is raw or previously heat treated to reduce local flora. Milk that is whole, or fully or partially skimmed. Reconstituted, powdered milk for *Cronobacter* spp.

Exclusion criteria:

- HPP effects on milk components (protein, fluidity etc.), biochemical and physiochemical properties of milk. These effects have been summarised in a number of reviews (Chawla, Patil, and Singh 2011; Pereda et al. 2007; Trujillo 2002).
- Combination of HPP with other treatments unless a 'HPP only' control treatment was included.
- Dairy products other than milk, such as cheese, yoghurt and other fermented milk products, ice cream, flavoured milks.
- Other foods.

B.2 Internet search

The following websites from government, commercial and research organisations were searched for applicable information:

Government webpages

NSW Food Authority, http://www.foodauthority.nsw.gov.au/

Canadian Food Inspection Agency, http://www.inspection.gc.ca/eng/1297964599443/1297965645317

US Food &Drug Administration (FDA), https://www.fda.gov/default.htm

Food Safety Authority of Ireland, https://www.fsai.ie/

European Food Safety Authority (EFSA); https://ec.europa.eu/commission/index_en

Commercial

Hiperbaric - manufacturers of HPP units; http://www.hiperbaric.com/en

https://www.dairynz.co.nz/

Cold press milk commercially available in Australia: http://www.madebycow.com.au/

Home grown juice company, HPP treated juice product commercially available in NZ: (http://www.homegrownjuice.co.nz/raw-cold-pasteurised-range.html)

Information on patents regarding HPP treatment of milk was obtained from https://worldwide.espacenet.com/ and https://www.ipaustralia.gov.au/patents

Research

Food Innovation Network, New Zealand; http://foodinnovationnetwork.co.nz/high-pressure-processing

CSIRO Australia; https://www.csiro.au/

Illinois Institute of Technology, United States; https://www.ifsh.iit.edu/services/product-development/high-pressure-processing

Cornell University, United States; https://foodscience.cals.cornell.edu/about-us/facilities/geneva-facilities/hpp-validation-lab

REFERENCES

Akhmazillah MFN, Farid MM and Silva FVM (2012) High pressure processing of Honey: Preliminary study of total microorganism inactivation and identification of bacteria. *Journal of Science and Technology* 4:1-12.

Alpas H, Kalchayanand N, Bozoglu F et al. (1999) Variation in pressure resistance of hydrostatic pressure among strains of food-borne pathogens. *Applied and Environmental Microbiology* 65(9): 4248-4251.

Alpas H, and Bozoglu F (2000) The combined effect of high hydrostatic pressure, heat and bacteriocins on inactivation of foodborne pathogens in milk and orange juice. *World Journal of Microbiology & Biotechnology* 16: 387-392.

Alpas H, and Bozoglu F (2002a) Inactivation of *Staphylococcus aureus* and *Listeria monocytogenes* in milk and cream of chicken soup by high hydrostatic pressure and bacteriocins.*High Pressure Research* 22: 681-684.

Alpas H, and Bozoglu F (2002b) Recovery of *Escherichia coli* O157: H7 and *Salmonella* in milk and cream of chicken soup from high hydrostatic pressure (HHP) and bacteriocin applications upon storage. *High Pressure Research* 22: 685-687.

Arroyo C, Cebrian G, Mackey BM, Condon S, and Pagan R (2011) Environmental factors influencing the inactivation of *Cronobacter sakazakii* by high hydrostatic pressure. *International Journal of Food Microbiology* 147: 134-143.

Balasubramaniam VM, Martinez-Monteagudo, SI and Gupta R (2015) Principles and application of high pressure-based technologies in the food industry. *Annual Reviews of Food Science and Technology* 6: 435-462.

Baptista I, Rocha SM, Cunha A, Saraiva JA, and Almeida A (2016) Inactivation of *Staphylococcus aureus* by high pressure processing: An overview. *Innovative Food Science & Emerging Technologies* 36: 128-149.

Barba FJ, Koubaa M, do Prado-Silva L, Orlien V and de Souza Sant'Ana M (2017) Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review. *Trends in Food Science & Technology* 66: 20-35.

Black EP, Setlow P, Hocking AD, Stewart CM and Hoover DG (2007) Response of spores to high-pressure processing. *Comprehensive Reviews in Food Science and Food Safety* 6: 103-119.

Buckow R and Heinz V (2008) High Pressure Processing – a database of kinetic information. *Chemie Ingenieur Technik* 80: 1081-1095.

Chawla, R, Patil GR, and Singh AK (2011) High hydrostatic pressure technology in dairy processing: a review. *Journal of Food Science and Technology* 48: 260-268.

Chen H (2007) Use of linear, Weibull, and log-logistic functions to model pressure inactivation of seven foodborne pathogens in milk. *Food Microbiology* 24: 197-204.

Chen H and Hoover DG (2003) Pressure inactivation kinetics of *Yersinia enterocolitica* ATCC 35669. *International Journal of Food Microbiology* 87: 161-171.

Cholewińska AE (2010) The legislation on high pressure processing and other factors that may have an impact on HPP applications in the EU food industry. Thesis, Wageningen University. Available from http://edepot.wur.nl/169062 (Accessed 2nd August 2018).

De Lamo-Castellvi S, Roig-Sagues AX, Capellas M, Hernandez-Herrero M, and Guamis B (2005) Survival and growth of *Yersinia enterocolitica* strains inoculated in skimmed milk treated with high hydrostatic pressure. *International Journal of Food Microbiology* 102: 337-342.

Dogan C and Erkmen O (2003) Ultra high hydrostatic pressure inactivation of *Escherichia coli* in milk, and orange and peach juices. *Food Science and Technology International*, 9: 403-407.

Donaghy JA, Linton M, Patterson MF and Rowe MT (2007) Effect of high pressure and pasteurization on *Mycobacterium avium* ssp. *paratuberculosis* in milk. Letters in Applied Microbiology 45: 154-159.

Erkmen O (2011) Effects of high hydrostatic pressure on *Salmonella* typhimurium and aerobic bacteria in milk and fruit juices. *Romanian Biotechnological Letters* 16: 6540-47.

Erkmen O, and Dogan C (2004) Effects of ultra high hydrostatic pressure on *Listeria monocytogenes* and natural flora in broth, milk and fruit juices. *International Journal of Food Science and Technology* 39: 91-97.

Erkmen O, and Karatas S (1997) Effect of high hydrostatic pressure on *Staphylococcus aureus* in milk. *Journal of Food Engineering* 33: 257-62.

European Commission (2004) Evaluation report on the novel food regulation 258/97.

Fauzi NA, Farid MM and Silva FV (2017) An insight on the relationship between food compressibility and microbial inactivation during high pressure processing. *Journal of Food Science and Technology* 54(3): 802-809.

Farkas DF and Hoover DG (2000) High Pressure Processing. *Journal of Food Science* 65(s8): 47-64.

Ferreira M, Almeida A, Delgadillo I, Saraiva J and Cunha A (2016) Susceptibility of *Listeria monocytogenes* to high pressure processing: a review. *Food Reviews International*, 32: 377-399.

Fletcher GC, Youssef JF, and Gupta S (2008) Research issues in inactivation of *Listeria monocytogenes* associated with New Zealand Greenshell mussel meat (Perna canaliculus) using high-pressure processing. *Journal of Aquatic Food Product Technology* 17: 173-194.

Food and Drug Association (2000) Overarching Principles: Kinetics and Pathogens of Concern for All Technologies *Journal of Food Science* 65(s8): 16-31.

Food Standards Australia New Zealand (2017) Regulation of novel foods, http://www.foodstandards.gov.au/industry/novel/Pages/default.aspx. (Accessed 2 March 2018).

Gao YL, Ju XR, and Jiang HH (2006) Statistical analysis of inactivation of *Listeria monocytogenes* subjected to high hydrostatic pressure and heat in milk buffer. *Applied Microbiology and Biotechnology* 70: 670-8.

Garcia-Graells C, Masschalck B, and Michiels CW (1999) Inactivation of *Escherichia coli* in milk by high-hydrostatic-pressure treatment in combination with antimicrobial peptides. *Journal of Food Protection* 62: 1248-54.

Garcia-Graells C, Valckx C and Michiels CW (2000) Inactivation of *Escherichia coli* and *Listeria innocua* in milk by combined treatment with high hydrostatic pressure and the lactoperoxidase system. *Applied Environmental Microbiology* 66: 4173-9.

Gervilla R, Felipe X, Ferragut V and Guamis B (1997a) Effect of high hydrostatic pressure on *Escherichia coli* and *Pseudomonas fluorescens* strains in ovine milk. *Journal of Dairy Science* 80: 2297-303.

Gervilla R, Capellas M, Ferragut V and Guamis B (1997b) Effect of high hydrostatic pressure on *Listeria innocua* 910 CECT inoculated into ewe's milk. *Journal of Food Protection* 60: 33-7.

Gervilla R, Ferragut V and Guamis B (2000) High pressure inactivation of microorganisms inoculated into ovine milk of different fat contents. *Journal of Dairy Science* 83: 674-82.

Gervilla R, Mor-Mur M, Ferragut V and Guamis B (1999a) Kinetics of destruction of *Escherichia coli* and *Pseudomonas fluorescens* inoculated in ewe's milk by high hydrostatic pressure. *Food Microbiology* 16: 173-84.

Gervilla R, Sendra E, Ferragut V and Guamis B (1999b) Sensitivity of *Staphylococcus aureus* and *Lactobacillus helveticus* in ovine milk subjected to high hydrostatic pressure. *Journal of Dairy Science* 82: 1099-107.

Gonzalez S, Flick GJ, Arritt FM, Holliman D and Meadows B (2006) Effect of high-pressure processing on strains of *Enterobacter sakazakii. Journal of Food Protection* 69: 935-7.

Guan D, Chen H, and Hoover DG (2005) Inactivation of *Salmonella* typhimurium DT 104 in UHT whole milk by high hydrostatic pressure. *International Journal of Food Microbiology* 104: 145-53.

Guan D, Chen H, Ting EY and Hoover DG (2006) Inactivation of *Staphylococcus aureus* and *Escherichia coli* O157:H7 under isothermal-endpoint pressure conditions. *Journal of Food Engineering* 77: 620-27.

Gupta S, Farid MM, Fletcher GC and Melton LD (2015) Color, Yield, and Texture of Heat and High Pressure Processed Mussels During Ice Storage. *Journal of Aquatic Food Product Technology* 24: 68-78.

Hauben KJ, Bartlett DH, et al. (1997) *Escherichia coli* mutants resistant to inactivation by high hydrostatic pressure. *Applied Environmental Microbiology* 63: 945-50.

Hayman MM, Anantheswaran RC and Knabel SJ (2007) The effects of growth temperature and growth phase on the inactivation of *Listeria monocytogenes* in whole milk subject to high pressure processing. *International Journal of Food Microbiology* 115: 220-6.

Health Canada (2016) Health Canada Position - High Pressure Processing (HPP) is no longer a novel process. https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/position-high-pressure-processing-no-longer-novel-process-treated-food-products-treated-food-products-2013.html Accessed 5 March 2018.

Health Canada (2017) Guidance on food products treated with high pressure processing (HPP). http://www.inspection.gc.ca/food/safe-food-production-systems/technical-references/high-pressure-processing/eng/1498504011314/1498504256677. Accessed 5 March 2018.

Huang HW, Wu SJ, Lu JK, Shyu YT and Wang CY (2017) Current status and future trends of high-pressure processing in food industry. *Food Control* 72: 1-8.

Huang HW, Lung HM, Chang YH, Yang BB, and Wang CY (2015) Inactivation of pathogenic *Listeria monocytogenes* in raw milk by high hydrostatic pressure. *Foodborne Pathogens and Disease* 12: 139-44.

ICMSF (1996) Micro-organisms in Foods 5: Microbiological specifications of food pathogens. Editors: Roberts TA, Baird-Parker AC and Tompkin RB. Blackie Academic & Professional Publishers. ISBN 0-412-47350-X.

IFT/FDA (2003) Evaluation and definition of potentially hazardous foods. *Comprehensive Reviews in Food Science and Food Safety* 2: 2-108.

Iversen C, Lehner A, et al. (2007) The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter* genomospecies 1. *BMC Evolutionary Biology* 7: 64.

Kheadr EE, Vachon JF, Paquin P and Fliss I (2002) Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. *International Dairy Journal* 12: 435-46.

Knoerzer K, and Chapman B (2011) Effect of material properties and processing conditions on the prediction accuracy of a CFD model for simulating high pressure thermal (HPT) processing. *Journal of Food Engineering* 104: 404-13.

Koncza A, Meszaros L et al. (2007) Pasteurisation of raw milk by high hydrostatic pressure. *Acta Alimentaria* 36: 471-81.

Koseki S, Matsubara M, and Yamamoto K (2009) Prediction of a required log reduction with probability for *Enterobacter sakazakii* during high-pressure processing, using a survival/death interface model. *Applied Environmental Microbiology* 75: 1885-91.

Koseki S, Mizuno Y, and Yamamoto K (2008) Use of mild-heat treatment following high-pressure processing to prevent recovery of pressure-injured *Listeria monocytogenes* in milk. *Food Microbiology* 25: 288-93.

Liu HB, Li P et al. (2017) Inhibitor-Assisted High-Pressure Inactivation of Bacteria in Skim Milk. *Journal of Food Science* 82: 1672-81.

Liu Y, Betti M and Ganzle MG (2012) High pressure inactivation of *Escherichia coli*, *Campylobacter jejuni*, and spoilage microbiota on poultry meat. *Journal of Food Protection* 75: 497-503.

Lopez-Pedemonte T, Sevilla I, et al. (2006) Inactivation of *Mycobacterium avium* subsp *paratuberculosis* in cow's milk by means of high hydrostatic pressure at mild temperatures. *Applied Environmental Microbiology* 72: 4446-49.

Margosch D, Ehrmann MA, Ganzle MG and Vogel RF (2004) Comparison of pressure and heat resistance of *Clostridium botulinum* and other endospores in mashed carrots. *Journal of Food Protection* 67: 2530-7.

Martinez-Rodriguez, A, and Mackey BM (2005) Factors affecting the pressure resistance of some *Campylobacter* species. *Letters in Applied Microbiology* 41: 321-6.

McClements JM, Patterson MF, and Linton M (2001) The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. *Journal of Food Protection* 64: 514-22.

Misiou O, van Nassau TJ, Lenz CA and Vogel RF (2017) The preservation of *Listeria*-critical foods by a combination of endolysin and high hydrostatic pressure. *International Journal of Food Microbiology* 266:355-362.

Morgan SM, Ross RP, Beresford T and Hill C (2000) Combination of hydrostatic pressure and lacticin 3147 causes increased killing of *Staphylococcus* and *Listeria*. *Journal of Applied Microbiology* 88: 414-20.

Muntean M-V, Marian O, et al. (2016) High Pressure Processing in Food Industry – Characteristics and Applications. *Agriculture and Agricultural Science Procedia*, 10: 377-83.

NACMCF (2010) Parameters for determining inoculated pack/challenge study protocols, *Journal of Food Protection* 73: 140-202.

New South Wales Food Authority. 2016. "High pressure processing - product considerations in HPP." Available from http://www.foodauthority.nsw.gov.au/_Documents/industry/high_pressure_processing.pdf (Accessed 28 August 2018)

Pandey PK and Ramaswamy HS (2004) Effect of high-pressure treatment of milk on lipase and γ-glutamyl transferase activity. *Journal of Food Biochemistry* 28: 449-62.

Patterson MF, Mackle A and Linton M (2011) Effect of high pressure, in combination with antilisterial agents, on the growth of *Listeria monocytogenes* during extended storage of cooked chicken. *Food Microbiology* 28:1505-1508.

Patterson MF and Kilpatrick DJ (1998) The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. *Journal of Food Protection* 61: 432-36.

Patterson MF, Quinn M, Simpson R and Gilmour A (1995) Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *Journal of Food Protection* 58: 524-29.

Pereda J, Ferragut V, Quevedo JM, Guamis B, and Trujillo AJ (2007) Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. *Journal of Dairy Science* 90: 1081-93.

Pina Perez, MC, Rodrigo Aliaga D, Saucedo Reyes D and Martinez Lopez A (2007) Pressure inactivation kinetics of *Enterobacter sakazakii* in infant formula milk. *Journal of Food Protection* 70: 2281-9.

Rademacher B and Hinrichs J (2006) Effects of high pressure treatment on indigenous enzymes in bovine milk: Reaction kinetics, inactivation and potential application. *International Dairy Journal* 16: 655-61.

Reineke K, Mathys A, Heinz D and Knorr D (2013) Mechanisms of endospore inactivation under high pressure. *Trends in Microbiology* 21:296-304.

Serment-Moreno V, Fuentes C, Torres JA and Welti-Chanes J (2017) A Gompertz Model Approach to Microbial Inactivation Kinetics by High-Pressure Processing (HPP): Model Selection and Experimental Validation. *Journal of Food Science* 82: 1885-91.

Shearer AE, Neetoo HS and Chen H (2010) Effect of growth and recovery temperatures on pressure resistance of *Listeria monocytogenes*. *International Journal of Food Microbiology* 136: 359-63.

Sheen S, Cassidy J, Scullen B and Sommers C (2015) Inactivation of a diverse set of shiga toxin-producing Escherichia coli in ground beef by high pressure processing. *Food Microbiology* 52:84-87.

Solomon EB and Hoover DG (2004) Inactivation of *Campylobacter jejuni* by high hydrostatic pressure. *Letters in Applied Microbiology* 38: 505-9.

Stollewerk K, Cruz CD, Fletcher G, Garriga M, and Jofre A (2017) The effect of mild preservation treatments on the invasiveness of different *Listeria monocytogenes* strains on Greenshell (TM) mussels. *Food Control* 71: 322-28.

Syed QA, Buffa M, Guamis B, and Saldo J (2016) Factors affecting bacterial inactivation during high hydrostatic pressure processing of foods: A review. *Critical Reviews in Food Science and Nutrition* 56: 474-83.

Tabla R, Martinez B, Rebollo JE et al. (2012) Bacteriophage performance against *Staphylococcus aureus* in milk is improved by high hydrostatic pressure treatments. International *Journal of Food Microbiology* 156: 209-13.

Tamber, S (2018) Population-Wide Survey of *Salmonella enterica* Response to High-Pressure Processing Reveals a Diversity of Responses and Tolerance Mechanisms. *Applied Environmental Microbiology* 84(2): e01673-17.

Tao, Y, Sun D-W, Hogan E, and Kelly AL (2014) Chapter 1 - High-Pressure Processing of Foods: An Overview. in, Emerging Technologies for Food Processing (Second Edition) (Academic Press: San Diego).

Institute of Food Technologists (2000) Kinetics of Microbial Inactivation for Alternative Food Processing Technologies. *Journal of Food Science* 65(S8): 6-31.

Trujillo AJ (2002) Applications of high-hydrostatic pressure on milk and dairy products *High Pressure Research* 22: 619-26.

United States Department of Agriculture (USDA) (2012) High pressure processing (HPP) and inspection program personnel (IPP) verification responsibilities. Edited by Food Safety and Inspection Service (FSIS) United States Department of Agriculture (USDA). Washington, DC. USA. Available from https://www.fsis.usda.gov/wps/wcm/connect/a64961fa-ed6f-44d1-b637-62232a18f998/6120.2.pdf?MOD=AJPERES (Accessed 28 August 2018).

Vachon JF, Kheadr EE, Giasson J, et al. (2002) Inactivation of foodborne pathogens in milk using dynamic high pressure. *Journal of Food Protection* 65: 345-52.

Wang Y, Ismail M and Farid M (2017) Processing of baby food using pressure-assisted thermal sterilization (PATS) and comparison with thermal treatment. *High Pressure Research* 37(4):579-593.

Yang BW, Shi Y, et al. Meng (2012) Inactivation of foodborne pathogens in raw milk using high hydrostatic pressure. *Food Control* 28: 273-78.

INSTITUTE OF ENVIRONMENTAL SCIENCE AND RESEARCH LIMITED

Kenepuru Science Centre
34 Kenepuru Drive, Kenepuru, Porirua 5022
PO Box 50348, Porirua 5240
New Zealand
T: +64 4 914 0700 **F:** +64 4 914 0770

Mt Albert Science Centre
120 Mt Albert Road, Sandringham, Auckland 1025
Private Bag 92021, Auckland 1142
New Zealand
T: +64 9 815 3670 **F:** +64 9 849 6046

NCBID – Wallaceville
66 Ward Street, Wallaceville, Upper Hutt 5018
PO Box 40158, Upper Hutt 5140
New Zealand
T: +64 4 529 0600 **F:** +64 4 529 0601

Christchurch Science Centre
27 Creyke Road, Ilam, Christchurch 8041
PO Box 29181, Christchurch 8540
New Zealand
T: +64 3 351 6019 **F:** +64 3 351 0010

www.esr.cri.nz