Ministry for Primary Industries
Manatū Ahu Matua

Review of Microbial Pathogen Inactivation Relevant to Sous Vide Cooking at Temperatures below 55°C

MPI Technical Paper No: 2017/35

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ISBN No: 978-1-77665-561-8 (online)
ISSN No: 2253-3923 (online)

August 2016

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Scientific Interpretative Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers

Review of Microbial Pathogen Inactivation Relevant to Sous Vide Cooking at Temperatures below 55°C

ESR Report FW15038

Sous vide is a method of cooking food, vacuum sealed in plastic bags before heating, usually by immersion in a temperature-controlled water bath for a set period of time. Most regulators require that red meat and chicken is cooked to internal product temperatures above 55°C and above 60°C respectively, if it is to be served immediately. Cooking temperature/time combinations are designed to reduce the level of any pathogens present in the food to safe levels. In addition, the environment within the bag will become anoxic (little or no oxygen) which will also impact on microbial survival.

The focus of this report was to determine what scientific evidence was available to support sous vide cooking of meat, poultry, seafood and eggs at temperatures below 55°C; demonstrating inactivation of foodborne pathogenic bacteria and viruses.

Traditionally pathogen inactivation during heating of food has been described by D values, which are the times taken for a specific pathogen population at a specific temperature to reduce by 90% (undergo a 1 $\log_{10}$ reduction). D values are normally calculated from the linear portion of survival curves. It was noted in the report that the appearance of tails and shoulders on the curves, suggest that the calculated D value derived from the linear portion of a survival curve would potentially under-estimate the time required to achieve the required reduction in pathogen concentration, particularly at the lower cook temperatures. Furthermore as many of the sets of published data did not take into account these differences in inactivation rate, they could not be used in this report as valid temperature/time cook parameters.

Slow heating of the product to the cook temperature may induce a heat shock response by the bacteria, making them more heat tolerant to the cook temperature. This is the reason that good operating practice and the template FCP recommends pre-heating the water bath to the appropriate cook temperature. This is particularly important when the cook temperature is close to the upper growth temperature of a given bacterium as this may result in a decrease in the rate of microbial inactivation. Given that slow heating can result during individual sous vide runs (e.g. over-crowding of the water bath, placement of chilled product in the water bath, multiple-step sous vide cook processes or variable portion sizes), it is not possible to account for the impact of heat shock at the lower cook temperatures on survival.

Foodborne pathogens such as *Clostridium perfringens* and *Bacillus cereus* produce spores that allow them to survive when adverse conditions, e.g. sous vide cooking temperatures, threaten their survival. At temperatures below 55°C these spores germinate potentially leading to an increase in bacterial cell number during the cook process, hence increasing the risk of foodborne illness.

Very little data is available for inactivation or survival of viruses during cooking at temperatures below 60°C. This is due, in part, to difficulties in culturing and the reliance on molecular methodologies such as polymerase chain reaction (PCR) for detection which cannot distinguish viable from lethally damaged viral particles. Inactivation of viruses is via protein denaturation and disruption of the virus particle structure such

that it can no longer infect the host. High temperatures (≥80°C) with a short cook time have been shown to result in significant inactivation of viruses. Enteric viruses show limited inactivation at temperatures below 60°C, which further decreases at lower temperatures. Available information suggests that minimal inactivation would occur at temperatures of 50°C or below. This is particularly a concern when cooking shellfish or foods containing pork liver using lower temperature sous vide procedures as these foods may be contaminated with enteric viruses.

In conclusion, there is currently little to no scientific evidence to support any prediction model for inactivation for bacterial and viral foodborne pathogens in meat, poultry, seafood or eggs at cook temperatures at or below 55°C. Consequently, the safety of sous vide foods cooked at temperatures below 55°C cannot be assured.

MPI will maintain a watch for research studies that address this recognised gap in knowledge and for updated information from other international regulatory agencies.

In the meantime the recommended default cooking temperatures in the D value report ("Standardising D and Z values for cooking raw meat." http://www.foodsafety.govt.nz/elibrary/industry/standardisation-parameters-pathogen-control-food-processing.htm) should be applied to sous vide processes if the product is not to be further cooked at temperatures greater than 55°C.

Review of Microbial Pathogen Inactivation Relevant to Sous Vide Cooking at Temperatures Below 55°C

August 2016

PREPARED FOR:	**The Ministry for Primary Industries under project MFS/15/2 - Microbiological Food Safety**
CLIENT REPORT No:	**FW15038**
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ACKNOWLEDGEMENTS

The authors wish to acknowledge ESR scientists Wendy Williamson, Maurice Wilson, Louise Weaver and Elaine Moriarty for their assistance in sourcing information, evaluating bacterial experiment methodology and associated thermal inactivation data.

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SUMMARY

Background

Sous vide is a method of cooking where food is vacuum-sealed in plastic bags before heating. To be heated the plastic packs are submerged into a temperature controlled water bath for a set period of time. Eggs may be cooked in their shells without further packaging.

The Ministry for Primary Industries template Food Control Plan, Serve Safe (December 2015) requires that when cooking using the sous vide method, red meat is cooked at 55°C or above and that poultry is cooked at 60°C or above - if being served immediately. Some sous vide recipes suggest cooking at temperatures below 55°C. This report summarises the scientific evidence available to support the making of recommendations for time and temperature parameters for sous vide cooking at temperatures below 55°C.

The focus of the project was on the inactivation of potential foodborne bacterial and viral pathogens during sous vide cooking of beef including veal, pork, poultry, seafood and eggs. The project did not consider potential food safety risks following the cook step, such as bacterial growth during post-cook cooling or chilled storage.

Bacterial inactivation

The following conclusions have been made for bacterial pathogens:

- There are insufficient data in the literature at this time to allow prediction of the inactivation of bacterial pathogens at cook temperatures below 55°C. Therefore, this project has been unable to generate recommendations for safe cooking time/temperature combinations below 55°C.
- Most papers/experiments do not provide sufficient data to determine the shape of the survival curve (the relationship between $\log_{10}$ cfu/g concentration of bacterial pathogen and time during cooking), or have not been conducted for long enough to determine the time required to reduce pathogen concentration by 5 to 7 $\log_{10}$ cfu/g.
- Survival curves may be non-linear with increasing shoulder effects with decreasing cook temperature, increasing fat content and the addition of curing salts. Shoulders in the survival curve are an initial period of no or low rates of reduction in cell concentration which precede a higher rate of cell inactivation.
- Four papers have identified tails in the survival curve. Tails occur when there is either a reduction in the inactivation rate or no further inactivation, after a period of higher inactivation. A tail in a survival curve means a sufficient reduction in pathogen concentration may not be achievable irrespective of the time the product is held at that specific temperature. The underlying cause of tailing in two papers is unclear, but the other two papers related to experiments with very slow heating rates from 20°C to the cook temperature.
- Some *D* values given in the literature have been derived from the linear part of a non-linear survival curve and do not take into account shoulders or tails. *D* values alone should not be used to set sous vide cooking times unless the full survival curve have been shown to be linear.
- Heating of meat or eggs at sub-lethal temperatures may result in pathogens present undergoing a heat shock response. This makes the pathogen more heat tolerant during subsequent cooking at higher temperatures. Heat shocking of pathogens could occur

during sous vide cooking due to the time it takes for a portion of food to come up to the cook temperature, inadequate control of the water bath temperature, overloading the water bath or recipes that have a lower initial heating step before the main cook step.

- Spore forming bacteria such as *Bacillus cereus, Clostridium perfringens and Clostridium botulinum* can form spores which protect cells from unfavorable environmental conditions. If present in the food, a proportion of spores may germinate during sous vide cooking at temperatures below 55°C. Therefore such a cook step could increase, rather than decrease, the cell concentration of these species.

Virus inactivation

The following conclusions have been made for virus pathogens:

- There are insufficient data available on viruses to describe the survival or inactivation of viruses during cooking at temperatures below 60°C.
- Enteric viruses (those that inhabit the intestinal tract) show limited inactivation (<2 $\log_{10}$ cfu/g) at temperatures less than 60°C. At temperatures between 50°C and 56°C, only limited damage to the capsid of enteric viruses occurs, resulting in modest levels of virus inactivation. Temperatures above 56°C are likely to cause denaturation of the viral capsid proteins and will result in higher levels of inactivation.
- The lack of a cell culture system for norovirus and hepatitis E hampers the understanding of inactivation of these viruses. The current detection methods cannot determine if the detected virus components come from a viable virus or one that has been lethally damaged.

1. INTRODUCTION

1.1 SOUS VIDE COOKING AND FOOD SAFETY

Sous vide is a method of cooking where food is vacuum-sealed in plastic packs before heating. To be heated the plastic packs are submerged into a temperature controlled water bath for a set period of time. Eggs may be cooked within their shells without further packaging.

Sous vide cooking can be characterised by the following factors:

- The food is vacuum sealed, resulting in the food being cooked in a reduced oxygen or anaerobic environment.
- The maximum temperature in any part of the food will be no higher than the maximum temperature of the water bath.
- Cooking times range from minutes to multiple hours.

Sous vide is an increasingly popular cooking method in restaurants. A review by Stringer et al. (2012) of recipes used in restaurants in the UK, and recorded in books or on the internet suggested people may be cooking fish at temperatures as low as 38.5°C, red meat below 50°C and poultry at 50°C and above. At these low temperatures, the combination of cooking temperature and time may not reduce foodborne pathogenic micro-organisms sufficiently to avoid illness in consumers and may actually promote growth.

To ensure the food safety of sous vide cooked food it is important to understand how pathogenic micro-organisms will be affected during cooking. The maximum temperature at which many pathogenic micro-organisms can grow on food products is between 42°C and 49°C (Hudson, 2011a). *Bacillus cereus* and *Clostridium perfringens* have been observed to grow slowly at temperatures between 50 and 55°C[1]. Hence the temperatures being used for some sous vide cooking are close to, or overlap, the growth temperature ranges of foodborne pathogens.

Traditionally pathogen inactivation parameters, such as *D* values (section 2.4.3), have been used to determine the cook time-temperature combinations to achieve a required reduction in pathogen concentration. However, it is unclear if the *D* value methodology is appropriate for cooking at temperatures below 55°C.

1.2 PROJECT AIM

The aim of this project was to evaluate the scientific evidence available to support recommendations for time and temperature parameters for sous vide cooking at temperatures below 55°C.

[1] http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm

1.3 REPORT SCOPE

This project focuses on the inactivation of foodborne pathogens at temperatures below 55°C in the following raw food types:

- Beef including veal.
- Pork.
- Poultry including chickens, ducks and turkeys.
- Fish and Seafood including shell fish meat.
- Egg including whole egg as well as separated egg white or yolks.

Mutton and lamb was included in the initial project scope, but no relevant data was sourced.

Although the aim of the project was to make recommendations for cooking at less than 55°C, to capture all potentially relevant scientific information data were gathered from experiments up to 60°C.

The initial project scope included pathogens that are considered to be potentially foodborne in New Zealand (Horn *et al*, 2015). *Bacillus cereus* is not a pathogen normally associated with meat. However, this pathogen has been associated with seasonings (Jenson and Moir, 2003) which could be applied to meat prior to sous vide cooking.

The focus of the project is on the inactivation of foodborne pathogens during the main heating step of sous vide cooking. The project does not cover potential food safety risks immediately following the cook step, such as bacterial growth during post-cook cooling or chilled storage.

1.4 REFERENCE AND EXPERIMENTAL *D* VALUES

The rate of thermal inactivation of micro-organisms during cooking is dependent on a number of factors including; the species and strain of micro-organism, the intrinsic properties of the food (e.g. meat type, fat content, pH, flavouring or additives) and preliminary processing before cooking (Gilbert et al. 2011). Compilations of *D* values have shown there is a large variability in reported experimental *D* values for a given temperature and meat combination (Horn, 2015; van Asselt and Zwietering, 2006).

The reference *D* values provided by the Ministry for Primary Industries for cooking temperatures at or above 55°C are based on a linear regression of the 95th percentile values of the available data at each temperature. This approach provides reference *D* values which take into account the variability in heat resistance of pathogens due to characteristics of the food and incorporates data from the most heat resistant strains (Horn, 2015).

In contrast the *D* values and thermal inactivation data in this report are almost always derived from single sets of experiments. These limited data will capture only a part of the variability in *D* values for a given temperature-pathogen-food combination. So while *D* values are quoted to provide information, they should be used cautiously, recognising the limited data from which they are derived.

1.5 REPORT OVERVIEW

Section 2 of this report introduces; (i) how temperature can influence the survival properties (growth, survival or inactivation) of bacterial cells and (ii) the *D* value approach to defining the inactivation of cells. Section 3 summarises the effect of sous vide cooking at temperatures below 60°C on bacterial cells and Section 4 provides quantitative data on the reduction of bacterial cells observed in experiments. The final section of the report provides background information on foodborne viruses and a review of virus specific information in relation to cooking at temperatures below 60°C.

2. BACTERIAL BACKGROUND INFORMATION

2.1 INTRODUCTION

This section provides background information and introduces some of the terms and concepts that will be discussed in the following sections.

2.2 FOODBORNE BACTERIAL PATHOGENS

2.2.1 Mechanisms causing illness

Bacterial foodborne illness symptoms can be caused by two mechanisms; (i) ingestion of pathogen cells or (ii) the ingestion of toxin. The proportion of people becoming ill and their symptoms is dependent on the amount (or dose) of cells or toxin they ingest.

Ingestion of cells is the initial mechanism for illness caused by *Campylobacter, Clostridium perfringens, Listeria monocytogenes, Salmonella, Shigella*, Shiga Toxin-Producing *Escherichia coli* (STEC), *Yersinia enterocolitica and Vibrio parahaemolyticus* [2].

Given the right environmental conditions and cell populations, *Clostridium botulinum* and *Staphylococcus aureus* can produce toxin in food which can then cause symptoms when ingested. *Clostridium perfringens* can also produce a toxin in food, but this requires a very high cell concentration. Such high cell concentrations are usually associated with an advanced state of food spoilage (Bates and Bodnaruk, 2003).

Bacillus cereus can cause symptoms from ingesting pre-formed toxin in food or by the consumption of cells (Jenson and Moir, 2003).

2.2.2 Spore forming bacteria

Bacillus cereus, Clostridium botulinum and Clostridium perfringens can all produce spores. Spores are a resting and protective form of the micro-organism which allows the micro-organism to endure adverse conditions. When conditions improve, the spores germinate becoming actively growing bacteria (Setlow and Johnson, 2012).

If spores are present in the food at cooking, the cell concentration can be effected in the following ways:

- If the cook temperature supports germination of spores, an increase in the cell concentration which may cause illness will occur.
- When spores survive or form during the cook step, subsequent cooling of the food may result in germination of spores if cooling of the food is not sufficiently fast.

2.3 TEMPERATURE EFFECTS ON GROWTH AND TOXIN PRODUCTION

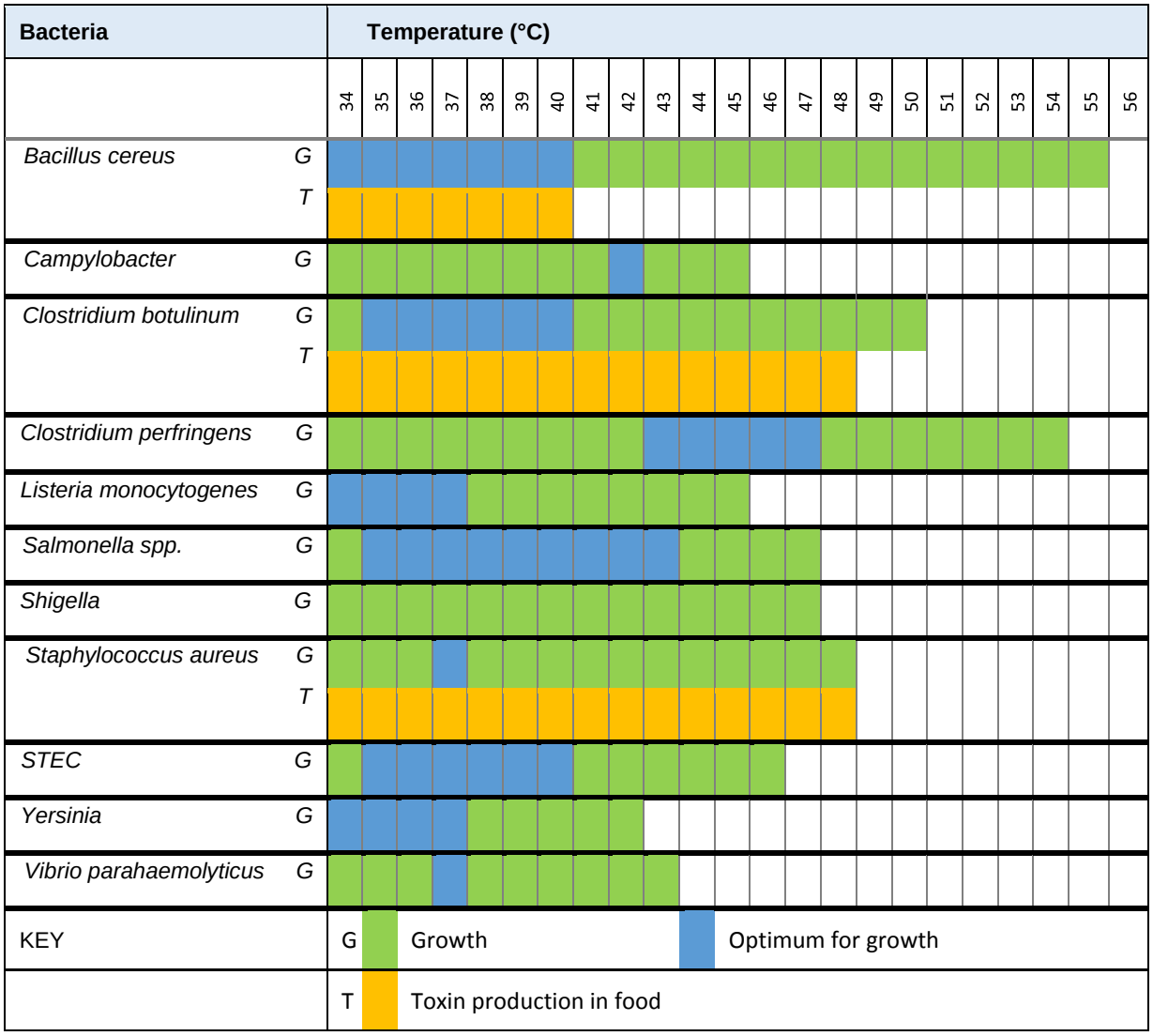
Temperature is one factor which determines if cells can grow and reproduce resulting in an increase in cell concentration. Figure 1 shows the temperatures at 34°C and above for which increases in cell concentrations or toxin production have been observed in experiments using broths or food (Setlow and Johnson 2001; Hocking 2003; ICMSF 1996; Ministry for Primary Industries pathogen datasheets[2]).

[2] http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm

Cells will grow at certain temperatures below 34°C, but the focus of Figure 1 is to consider the potential for growth during cooking below 55°C. At the optimum temperature for growth, the cell growth is greatest and most of the cells in a population will be actively growing.

The growth and toxin production temperature range for a specific pathogen-food combination is also dependent on other factors such as pH, water activity, atmosphere and food additives (Gilbert *et al*. 2011).

Figure 1: Temperature range (above 33°C)[a] of possible growth and toxin production for foodborne bacteria observed in experiments using broth or food



a: Growth will occur in temperature ranges below 34°C (Hudson, 2011b), the figure temperature range is shown to indicate the upper limits of temperature where growth has been observed to occur.

2.4 TEMPERATURE EFFECTS ON INACTIVATION

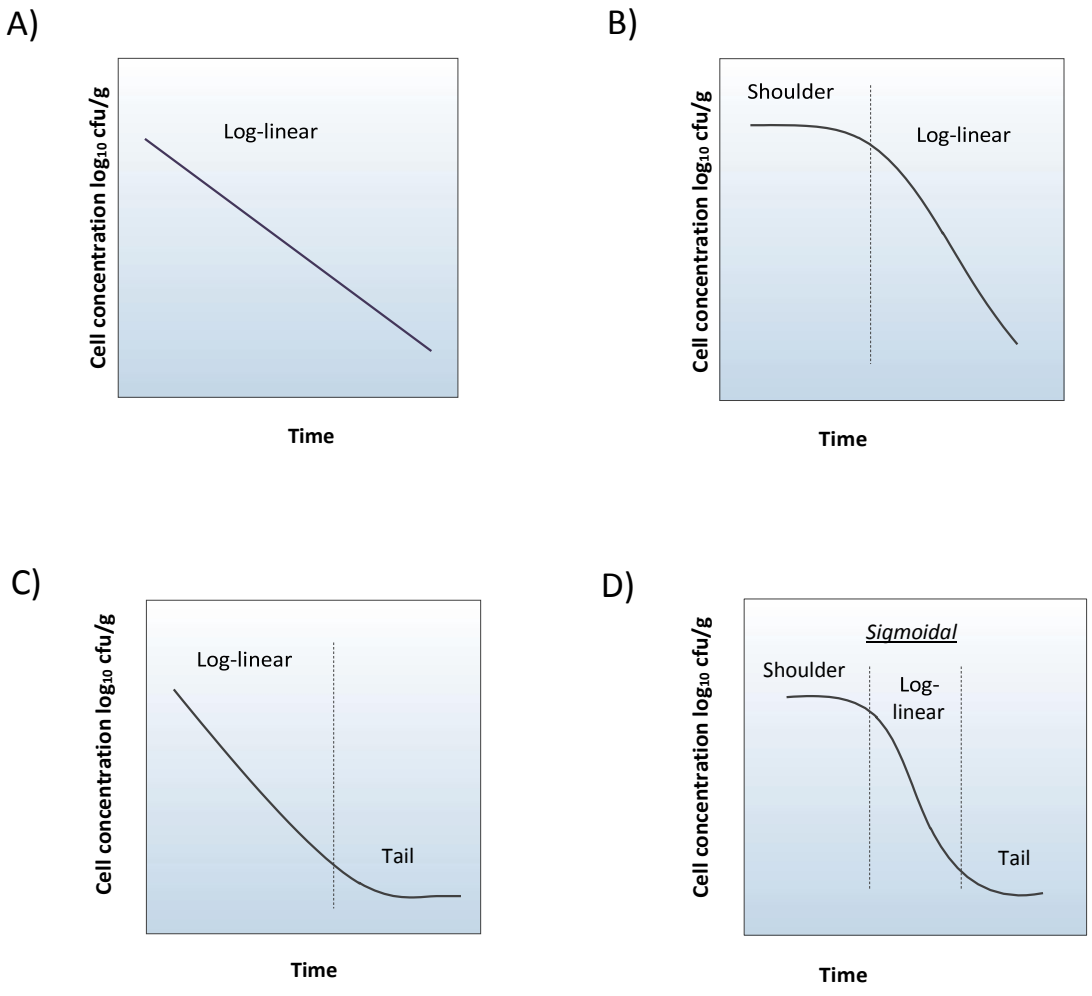
2.4.1 Rate of inactivation

As the temperature increases above the optimum for growth some cells in the population will continue to grow while in other cells growth will cease, reducing the rate of increase in the cell concentration. During this period, changes to the individual cell structure can occur.

As the temperature increases, changes in cell protein structure resulting in loss of cell function and the thermal break down of cell walls starts to occur. Above the maximum temperature for growth, cellular damage increases with increasing temperature until the level of cellular damage becomes sufficient to inactivate or kill the cells. The viable cell concentration decreases at a greater rate with increasing temperature (Adams and Moss 2000). The difference between growth and inactivation temperatures for a cell can be as little as a few degrees.

At a given temperature the changes in pathogen cell concentration in a food or broth can be measured over time to produce a survival curve. The shape of the survival curve can take a number of different forms (Devlieghere *et al*., 2009), the most common are shown in Figure 2.

Figure 2: Common types of survival curve for cell populations heated at a given constant temperature



The simplest survival curve relationship is for the cell concentration to decrease at a log-linear rate (Figure 2.A).

Sometimes the bacterial cells can survive the heat treatment of a food sample for a period of time before the cells are damaged sufficiently to be killed, producing a shoulder in the survival curve (Figure 2.B). Factors which could result in shoulders being observed in experiments (Juneja, 2000, Stringer, 2000) include:

- Temperature is low enough that cells can survive for a period of time before dying.
- Heat adaption of cells during the period the food is brought up to temperature or previous heating at sub-lethal temperatures.
- Protective effects of added ingredients or the food matrix.

Figure 2.C is an example of a survival curve where the cell concentration initially decreases log- linearly on heating, followed by a tail in the survival curve. Factors which could result in a tail being observed in the survival curve (Van Derlinden, 2010, Stringer, 2000) include:

- Different heat tolerance of strains within a cocktail of strains used in an experiment.
- Different heat tolerance of cells within a population of a single strain.
- Heat adaption of cells during the cooking process or from a prior heat shock (section 2.4.2).
- Clumping of cells, providing protection to cells in the centre of the clump, such as in biofilms.
- Localised heat protected areas within a food sample.

It is possible for a curve to exhibit both a shoulder and a tail as shown in Figure 2.D, this is known as a sigmoidal survival curve.

2.4.2 Heat shock

A heat shock process is one where bacteria are heated at temperatures between the optimum for growth and temperatures that would be lethal. Heating bacterial cells in this sub-lethal temperature range can cause changes to the cell structure and the synthesis of special proteins called heat shock proteins. This cellular response can make cells more resistant to damage from heating during subsequent cooking.

Heat shock proteins are believed to prevent miss-folding and aggregation of proteins during heating, i.e. the cook step, and to promote the cells to return to their normal physiological state once the temperature decreases (Richter *et al*. 2010, Juneja 2002).

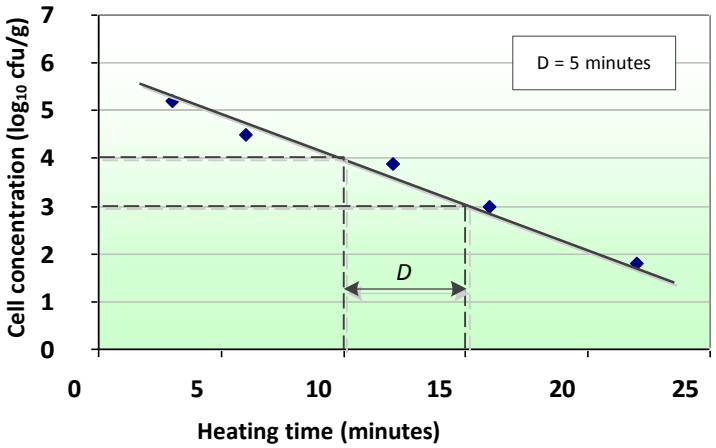
Heat shock of bacteria could arise during sous vide cooking due to a heat treatment prior to the main cook step, lack of sufficient control of temperature in the water bath, overloading the water bath or be due to the gradual increase in temperature of food during the cooking process.

2.4.3 *D* values

Traditionally pathogen inactivation during heating of food has been described by *D* values. The *D* value is defined as the time taken for a specific micro-organism population at a specified temperature and in a specified food to undergo a 90% or 1 $\log_{10}$ reduction in population.

D values are often calculated from experiments where small samples of food are inoculated with a micro-organism. The food is then heated at a constant temperature. At various time points after the food comes up to the target temperature, the pathogen concentration is determined. The pathogen concentration (log_{10} scale) is plotted against heating time as shown by the solid diamonds in Figure 3.

Figure 3: Example of a log linear cell concentration reduction during constant temperature cooking and associated *D* value



If the survival curve shows a linear relationship as seen in Figure 3, a *D* value can be calculated. In this report *D* values will be given in units of minutes unless otherwise stated.

For non-linear curves, such as shown in Figure 2; B,C and D, the *D* value alone does not fully describe the inactivation rate. A two or three component relationship may be required to describe inactivation; (i) the shoulder or initial slower rate of inactivation if present, (ii) the subsequent higher rate of inactivation and (iii) the tail if present.

The temperature at which the *D* value is calculated is sometimes indicated as a subscript to the '*D*'. For example a *D* value determined at 55°C will be written D_{55}.

3. GROWTH AND INACTIVATION OF BACTERIA DURING LOW TEMPERATURE SOUS VIDE COOKING

KEY FINDINGS

The *D* value approach to setting cook times may not be appropriate at temperatures below 55°C, due to the increased likelihood of shoulders in the survival curves.

The duration of shoulders can increase with decreasing temperature, heat shock prior to cooking, fat content and with the addition of curing salts.

Cooking products in the anaerobic environment of vacuum packaging will not increase the rate of inactivation of most foodborne pathogens during the cook step.

Germination of spores can occur at temperatures as high as 55°C and temperatures much higher than 55°C are required to inactivate the spores of *Bacillus cereus*, *Clostridium perfringens* and *Clostridium botulinum*.

3.1 INTRODUCTION

This section summarises the evidence in the literature for the inactivation of bacterial pathogens in beef, pork, poultry, egg or seafood during sous-vide cooking at temperatures below 60°C.

3.2 SURVIVAL AND GROWTH IN AN ANAEROBIC ENVIRONMENT

Vacuum packing of foods for sous vide cooking results in a low oxygen or anaerobic cooking environment. Most foodborne bacterial pathogens are facultative anaerobes meaning the cells are able to survive and grow in an environment with or without oxygen (Hocking 2003, ICMSF 1996).
Foodborne facultative anaerobes include; *Listeria monocytogenes*, *Salmonella*, *Shigella*, *Escherichia coli* including Shiga Toxin-producing *Escherichia coli* (STEC), *Yersinia* and *Vibrio*.

Staphylococcus aureus grows more slowly and produces less toxin in an anaerobic environment but may survive better. *Campylobacter* requires 3-5% oxygen for optimum growth.

The spore forming bacteria *Bacillus cereus*, *Clostridium perfringens* and *Clostridium botulinum* are also facultative anaerobes and the growth of *Clostridium perfringens* is optimum under anaerobic conditions. However, while *Bacillus* cereus can grow in an anaerobic environment, oxygen is needed for *Bacillus cereus* to produce toxin in the food (Jenson and Moir, 2003).

Cooking in an anaerobic environment will inhibit the growth of aerobic bacteria that are part of the natural flora of the food product, providing less competition for resources for the growth of facultative anaerobic bacteria (ICMSF 2000).

3.3 PATHOGEN GROWTH

3.3.1 Impact of temperature on growth

In the absence of non-temperature hurdles to the survival of cells, cell concentrations are unlikely to decrease during sous vide cooking at temperatures where growth is possible.

Most foodborne pathogens have maximum growth temperatures between 45 and 50°C (Figure1), however *Bacillus cereus* has a maximum growth temperature of 55°C and *Clostridium perfringens* has a maximum growth temperature of 54°C.

3.3.2 Spore germination

In experiments observing spores heated in an agar media, some spores germinated at temperatures as high as 55°C.

Lundgren (1966) found that *Bacillus cereus* spores heated in agar to 40°C for 5 minutes resulted in 90% germination and at 55°C over 50% germination. When heated for 60 minutes at 55°C, the germination rate increased to over 80%.

Clostridium botulinum spores heated in agar at 50°C for two hours, resulted in 60% of spores fully germinated (Grecz 1982).

Spores from some *Clostridium perfringens* strains can germinate at temperatures which permit cell growth (below 54°C), while other strains germinate more readily after short heat treatments in the temperature range 60°C to 80°C (Labbe 1989).

These experiments suggest a proportion of spores, if present in the food, may germinate during sous vide cooking at temperatures below 55°C. Therefore such a cook step could increase, rather than decrease, the cell concentration of these species, which increases the risk of illness to the food consumer.

3.4 HEAT INACTIVATION

3.4.1 Inactivation shoulders

The literature shows examples of how survival curve shoulder duration (Figure 2.B) can be affected by the cook temperature, meat type, curing salts and fat content.

Ahmed et al. (1995) showed that low cooking temperatures resulted in shoulders on survival curves for *Escherichia coli* O157:H7 inoculated into 2 g portions of ground poultry, beef and pork sausage meat. Timing of the heat treatment started once the sample had come up to the cook temperature (< 1.0 minute). At a cook temperature of 50°C, shoulders of over 2 hours with less than 1 $\log_{10}$ cfu/g decrease were observed for *Escherichia coli* O157:H7 in beef, turkey and pork sausage. Furthermore the duration of the shoulder increased with increasing fat content of the

meat. Shoulders on the survival curves were not observed in beef, pork[3] and poultry samples heated at 55°C.

Line et al. (1991) investigated the effect of fat content of ground beef on the inactivation of *Escherichia coli* O157:H7. They observed a shoulder of 30 minutes in lean 1g ground beef portions (2% fat) cooked at 51.7°C. However ground beef with 30% fat did not exhibit a shoulder, but the population decreased at a slower rate than in the lean ground beef. The decrease in cell concentration for the 30% fat beef portion was 1 $\log_{10}$ cfu/g over the first 2 hours of heating. This is a greater decrease in cell concentration than observed by Ahmed et al. (1995) for 20% fat beef heated to 50°C.

Another study looked at the effect of fat content on *Salmonella* survival curves (Juneja and Eblen 2000). A *Salmonella* cocktail was inoculated into 5 g ground beef flattened portions, which was vacuum packed in plastic pouches and cooked at 58°C. The survival curves exhibited shoulders with duration dependent on the fat content. The shoulder was 4.4 minutes at 7% fat, increasing to 28 minutes at 24% fat.

Two grams flattened portions of fresh ground beef were inoculated with a single strain of *Listeria monocytogenes* and heated in sealed polythene bags placed in a water bath (MacKey 1990). A shoulder with less than 0.25 log inactivation lasting approximately 40 minutes was observed when the beef was cooked at 55°C, followed by a linear section with a *D* value of 21 minutes. At 60°C a shoulder with a 1 log inactivation lasted for less than 10 minutes, before a greater rate of inactivation occurred with a *D* value of 3 minutes. The addition of curing salts (3.5% w/v NaCl, 200ppm nitrite and 300ppm nitrate) resulted in a much longer shoulder at 55°C of 150 minutes compared to 40 minutes with no curing salts.

In all the above experiments, time zero was taken to be the time at which the middle of the meat sample reached or was close to the target temperature. It is therefore unlikely that the observed shoulders are due to some of the food not reaching the target temperature for a period of time.

The above examples from the literature show shoulders in pathogen survival curves may be present during sous vide cooking at low temperatures. The presence of a shoulder in a survival curve would require a shoulder (or lag time) parameter to be included with the *D* value to ensure a specific reduction in cell concentration is achieved during cooking (Juneja and Eblen 2000).

3.4.2 Inactivation tails

Examples of tails in survival curves (Figure 2.C) have been documented for ground beef samples cooked at 55°C.

Weigand *et al*. (2012) heated 25 g ground beef samples inoculated with a five-strain cocktail of *E.coli* O157:H7 at a temperature of 54.4°C. The samples were flattened to 0.3cm in sealed bags which were heated in a water bath. The *E.coli* concentration reduced from above 8 $\log_{10}$ cfu/g at the beginning of product heating to approximately 6 $\log_{10}$ cfu/g after about 60 minutes. After 120 minutes of heating the concentration of *E.coli* had reduced to approximately 5 $\log_{10}$ cfu/g. For the remaining 120 minutes of the cook step (total cook time of 240 minutes), the concentration of *E.coli* remained at approximately 5 $\log_{10}$ cfu/g. Hence a tail in the survival curve was observed

[3] Shoulders in cell concentration may have been present for pork sausage at 55°C, but the experiment duration was for 30 minutes which did not allow a full interpretation to be made.

after 2 hours of cooking at 54.4°C.

Huang and Juneja (2003) also found tailing in the inactivation of a four-strain *E.coli* O157:H7 cocktail in ground beef after 30 minutes of holding at 55°C. Five gram samples were flattened and vacuum sealed in bags and heated in a water bath. The cell concentration reduced from 7.7 $\log_{10}$ cfu/g to 3 $\log_{10}$ cfu/g over 30 minutes. Between 30 and 60 minutes after the start of heating the rate of inactivation reduced such that only a further 1 $\log_{10}$ cfu/g reduction in concentration was achieved by 60 minutes.

These examples suggest experiments used to define cooking times should be sufficiently long to establish if tailing off of the survival curve occurs before a sufficient pathogen reduction can be achieved.

3.4.3 Single temperature heat shock

A number of experiments have been published which consider the effect of holding bacterial cells at a sub-lethal temperature lower than the main cook temperature for a period of time before the main cook step. Appendix B provides examples of heat shock experiment results found in the literature for *Salmonella* spp., *Listeria monocytogenes, Yersinia* and *Clostridium perfringens.*

The extent of a heat shock effect on cook *D* values is dependent on the pathogen, food type, pH and the time-temperature combination of the pre-heating step. Up to a 4-fold increase in *D* values have been observed.

One example of the effect of the duration of heat shock process on the subsequent heat resistance of *Listeria monocytogenes* is described by Shen et al. (2014). The heat shock process used 10 ml inoculated samples of tryptic soy broth supplemented with 0.6% yeast extract in 15 ml test tubes. The tubes were immersed in a water bath at 48°C for periods of time ranging from 0 to 90 minutes. One milliliter samples were extracted and heated to 60°C. The concentration of *Listeria monocytogenes* at the beginning of the 60°C cook was approximately 7 $\log_{10}$ cfu/ml for all the experiments.

Table 1 summarises the survival of the 3 strains of *Listeria monocytogenes.* For all 3 strains, the greatest heat tolerance was observed for samples heat shocked at 48°C for 30 or 60 minutes. For heat shock durations of 5, 15 or 90 minutes, the heat tolerance was less. The experiments produced non-linear survival curves. The experiments showing greatest heat tolerance had slower initial rates of decrease in cell concentration than those with less heat tolerance.

Table 1: Effect of sub-lethal heating at 48°C for 0 to 90 minutes on the survival of 3 *Listeria monocytogenes* strains subsequently heated at 60°C (Values estimated from Figure 1 of Shen *et al*. 2014)

Heat shock duration at 48°C (minutes)	Approximate time to achieve a 6 $\log_{10}$ cfu/ml reduction at 60°C (minutes)		
	BUG 600 (serotype 1/2a)	NRRL B-33157 (serotype 4b)	F4260 (serotype 1/2b)
0	10	18	27
5	13	25	36
15	19	28	45
30	5.3 log reduction after 20 minutes	35	5 log reduction after 50 minutes
60	4 log reduction after 20 minutes	35	5 log reduction after 50 minutes
90	18	28	45

3.4.4 Rate of temperature increase to cook temperature

Meat is likely to be stored at refrigeration temperatures before cooking. The immersion of sous vide packaged cold meat into a water bath set to the target temperature is likely to initially decrease the temperature of the water. This cooling effect will be dependent on the size and number of the portions in comparison to the size and capability of the water bath. This means the meat and associated bacterial cells may have a period of gradual increase in temperature until the water bath and the slowest heating part of the food portion reaches the target cook temperature (Baldwin 2011).

Gradual heating of bacterial cells through the temperature range for growth and above can lead to both the growth and heat shock adaptation of the cells.

In experiments using autoclaved ground beef inoculated with *Clostridium perfringens,* growth of approximately 3 to 4 $\log_{10}$ cfu/g was observed in 15 g portions of meat heated from 25°C to 50°C at rates of 4 to 7.5 °C/h (Roy 1981). The meat portions were placed in screw capped test tubes and brought up to 25°C in a water bath before being inoculated with *Clostridium perfringens* at the bottom of the tube. Temperature was monitored at the centre of the meat portion and in the water bath throughout the experiment.

Heating of *Salmonella* in broth using a constant rate of rising temperature from 20°C to cook temperatures of 52°C, 55°C and 59°C also showed increased heat resistance at cooking temperatures with slower rates of temperature increase (MacKey and Derrick 1987). Capillary tubes containing 25µL of inoculated tryptone soya broth were immersed in a water bath and the sample temperature measured by placing a thermocouple inside a capillary tube.

The authors observed non-linear survival curves. The thermal resistance of *Salmonella typhimurium* at constant cook temperatures of 52, 55 and 59°C was measured after heating the broth at a rate of 10°C/min or 0.6°C/min. The time needed to reduce survival to 1% (2 $\log_{10}$ reduction) was approximately twice as long in cells heated at the slower 0.6°C/min rate compared to 10°C/min. In another experiment they reported increased heat resistance when cooking at 55°C for 20 minutes, following heating rates of 0.6°C/min to 2°C/min. No increase in thermal resistance was observed for more rapid heating of 5 to 38°C/min.

Other examples of heat adaptation over an extended cook time of multiple hours was shown in brain heart infusion broth by Cornet *et al*. (2011) and Van Derlinden *et al*. (2010). They conducted experiments looking at the survival of *Escherichia coli* K12 when heated from 42°C to 65°C where the temperature was increased slowly at a rate of 1, 2 or 4°C/h and then the temperature was held at 65°C. In these experiments there was an initial decrease in cell concentration as the temperature increased close to 50°C, followed by a slight increase or stationary cell concentration as the temperature increased to 65°C. Cell concentrations did not decrease, even after 15 hours at 65°C. Examination of the experimental procedure did not suggest the survival was an artefact of the experiment methodology.

3.4.5 Spore inactivation

Cooking at temperatures below 60°C will not inactivate spores present in the food. Heat resistant strains of *Clostridium perfringens* have a spore $D_{100°C}$ value of 30 to 124 minutes (Sarker *et a*l. 2000). $D_{85°C}$ values of 33 to 106 minutes have been observed for *Bacillus cereus* spores in a phosphate buffer (Jenson and Moir 2003) and a $D_{82.2°C}$ value of 483 minutes has been observed for *Clostridium botulinum* spores (Szabo and Gibson 2003).

3.4.6 Toxin inactivation

Cooking at temperatures below 60°C will not fully inactivate *Staphylococcus aureus* enterotoxin already present in the food (Stewart 2003). Toxin in beef bouillon heated to 104°C was still detected after 100 minutes (ICMSF 1996). Some *Bacillus cereus* toxins are also highly heat resistant, they are able to survive at 126°C for 90 minutes (ICMSF 1996, Jenson and Moir 2003).

Clostridium botulinum toxins are more readily inactivated by heat, a 99% reduction in toxin concentration has been observed in haddock heated at 60°C (Szabo and Gibson 2003).

3.5 HEAT INACTIVATION OF BACTERIA IN SOLID AND MINCED MEAT

Most meat experiments conducted to determine *D* values or thermal inactivation relationships use minced meat. Minced meat has the advantage of being able to incorporate the pathogenic bacteria throughout the meat sample either by the mincing process or by being mixed through meat that is already minced. However, minced meat is structurally different to solid muscle.

A series of experiments were conducted to compare the heat resistance of *Salmonella* in matched solid and minced pork, beef and turkey meat samples at temperatures from 55 to 63°C (Orta-Ramirez et al., 2005, Tuntivanich et al., 2008 and Velasquez et al., 2010).

A cocktail of 8 strains of *Salmonella* known to have moderate to high thermal resistance were suspended in a sterile marinade. The whole muscle (5-7g muscle core) samples were marinated for 20 minutes at 4°C and the amount of marinade uptake was determined by weighing the sample. The same amount of marinade was then hand-mixed into a matched ground meat sample.

Meat samples were placed in sealed 1.27cm diameter brass tubes which were heated in a water-bath. In these experiments, the time for the sample to reach the cook temperature in the middle of the sample was the same for both meat treatments and log-linear survival curves were observed.

Salmonella marinated solid beef, pork and turkey meat was more resistant to thermal inactivation than *Salmonella* mixed into minced meat as shown in Table 2.

However, the duration of the experiment at 55°C only resulted in a 2 $\log_{10}$ cfu/g drop in *Salmonella* concentration for beef and turkey whole muscle samples. Longer duration experiments (> 30 minutes) would be required to see if the log-linear inactivation continued on further heating. A 4 $\log_{10}$ cfu/g drop in *Salmonella* concentration was observed over 90 minutes for the pork samples.

Table 2: *D* values for *Salmonella* applied in a marinade to matched whole muscle and ground beef, pork and turkey samples.

Temperature (°C)	Experimental *D* value (minutes)					
	Beef shoulder clods[a] (Orta-Ramirez 2005)		Pork Loin[b] (Velasquez 2010)		Turkey Breast[b] (Tuntivanich 2008)	
	Solid meat	Minced meat	Solid meat	Minced meat	Solid meat	Minced meat
55	17.7	7.2	23.4	8.8	16.5	10.0
58			3.4	1.5		
60	1.5	0.7	1.7	0.6	1.8	0.9

[a]: marinade contained 80.7% water, 12.6% NaCl and 6.65% phosphate solution

[b]: marinade contained 96% water, 3.2% NaCl (wt/vol) and 0.8% phosphate solution (vol/vol).

This series of experiments showed that *D* values, for this temperature range, derived from minced meat may underestimate the *D* values required for solid muscle portions if the pathogens are internalised. The under-estimation of time to reduce bacterial concentration in whole muscle increases with decreasing temperature. It was not clear from the literature review how *D* values for folded or rolled meat are related to *D* values based on mince or solid muscle experiments.

Bolton *et al.* (2000) investigated how *D* values for *Listeria monocytogenes* and *Yersinia enterocolitica* were affected by inoculation into solid or minced beef. Samples were prepared by either: (1) dipping raw strips of beef into the bacterial suspension, which was then minced, and vacuum packed in 10 g portions, or (2) injecting a bacterial suspension into the centre of 50 g (2x2x5 cm) solid muscle portions, which were then vacuumed packed in bags. Both sets of samples were heated in a water bath at either 48°C, 52°C or 56°C.

The authors noted the solid meat samples took a longer time to reach the target temperature at the centre of the portion; the samples did not reach 48°C within 32 minutes, 52°C within 21 minutes or 56°C within 18 minutes. This is in contrast to the minced samples which all reached the internal target temperature within 2 minutes. This demonstrates the importance of timing the cook step from the point when the whole meat portion has reached the target temperature.

It was not clear from this paper if the meat had come up to temperature before the samples to establish *D* values were taken, if any shoulders were present in the survival curves or how long the samples were heated for. Given this lack of supporting information, *D* values from the paper are not reported here, however it is worth noting that the *D* values derived from solid muscle experiments were consistently longer than in minced samples.

4. BACTERIAL THERMAL INACTIVATION DATA

Key findings

The 12 survival curves for bacterial pathogens in meat heated at temperatures below 55°C do not provide sufficient data to define safe cooking times.

The 2 survival curves for bacterial pathogens in egg heated at temperatures below 55°C do not provide sufficient data to define safe cooking times.

For the limited data presented, the time for a 4 $\log_{10}$ cfu/g reduction in bacterial pathogen concentration was less during egg cooking compared to meat cooking.

4.1 INTRODUCTION

A review of the literature for quantitative data on the thermal inactivation of foodborne pathogens has been completed. The approach taken to the literature review and the extraction of data is given in Appendix A.

As discussed in section 3.4, inactivation survival curves may be non-linear. For this reason *D* values are not used to describe thermal inactivation in this section. Instead the time taken to achieve a specified $\log_{10}$ cfu/g reduction in bacteria concentration is reported.

The choice of $\log_{10}$ cfu/g reduction for a specific cooking process and food is dependent on a number of factors including; the initial pathogen concentration in the food, the intended purpose of the food (e.g. immediate consumption or ready to eat) and meeting any regulatory requirements. Reductions of 5 to 7 $\log_{10}$ cfu/g are commonly applied.

Restricting the dataset to experiments which achieved at least a 5 $\log_{10}$ cfu/g reduction in cell concentration would have provided only 9 data points at temperatures below 55°C. To increase the size of the dataset presented, while ensuring that any shoulders or tails in the survival curve were likely to have been captured, experiments resulting in at least a 4 $\log_{10}$ cfu/g reduction are reported below. The method used is provided in Appendix A.

The choice of 4 $\log_{10}$ cfu/g reduction is not based on any food safety criteria, and the cook times for a 4 $\log_{10}$ reduction cannot be extrapolated to estimate how long it may take to achieve pathogen reductions greater than 4 $\log_{10}$ cfu/g if the survival curve is non-linear.

4.2 MEAT AND FISH

A total of 249 experiments for the thermal inactivation of pathogens in different types of meat were initially collated for further investigation. Of these, 187 inactivation curves were removed from consideration, because the duration of 165 experiments resulted in pathogen reductions of less than 4 $\log_{10}$ cfu/g and 22 inactivation curves did not provide sufficient information to estimate the time taken to produce a 4 $\log_{10}$ cfu/g reduction.

The final set of 21 papers (Appendix C.3) resulted in 62 inactivation curves. Figure 4 shows the combination of meat type, cook temperature and pathogen from these selected studies. Four different pathogens were present in the data set; *Clostridium perfringens* (4 experiments), *Listeria monocytogenes* (10), *Salmonella* spp. (27) and STEC (21). From these data only 12 inactivation curves were for cook temperatures below 55°C. The 5 STEC data points at 50°C were for a single strain of STEC and come from the same set of experiments (Ahmed 1995). Four of the five pork data points also came from a single study (Velasquez 2010).

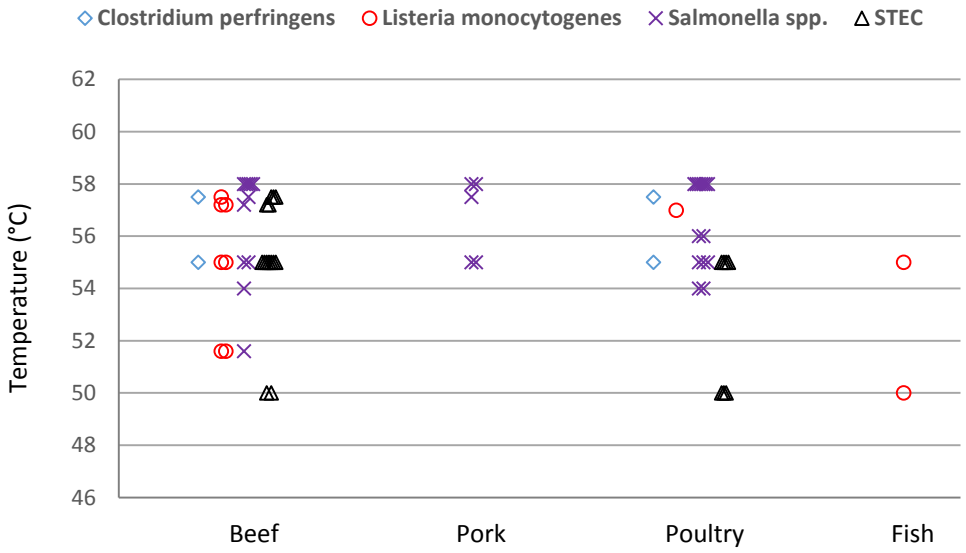


Figure 4: Combination of meat type, cook temperature and pathogen of the selected experiments which achieved a 4 $\log_{10}$ cfu/g reduction in pathogen concentration.

Given the known strain variability in cell inactivation from heating during cooking (Horn 2015, ICMSF 1996), these data are not sufficient to provide cook time guidelines for specific pathogen / meat type combinations. The cooking times to achieve a 4 $\log_{10}$ cfu/g reduction in cell concentration for most of the data in Figure 4 are plotted in Figure 5.

The fish experiment conducted at 50°C is not included in Figure 5, as the time to achieve a 4 log reduction was much longer than the other data (~1600 minutes). In the fish experiments, salmon was precooked in a water bath to eliminate native microflora, then flaked into bags. Portions of 60g of salmon inoculated with *Listeria monocytogenes* at a concentration of 6 $\log_{10}$ cfu/g were put into sealed containers and heated in an incubator. Experiments were conducted for 3 days at 50°C and 6 hours at 55°C (Hwang 2009). The authors also conducted experiments for 6 days at 40°C or 45°C,

In ideal laboratory conditions a single strain of *Listeria monocytogenes* has been shown to grow slowly in media at 45°C (ICMSF 1996). In the salmon experiments at 40°C no growth or inactivation was observed and at 45°C minimal inactivation occurred, with less than 1.5 $\log_{10}$ cfu/g decrease observed after 6 days at 45°C. The salmon heated to 40°C and 45°C was only sampled once every 24 hours. Therefore using this data, it is not possible to determine the shape of the growth/survival curve during the initial 24 hours and to know if a period of population growth followed by inactivation could have occurred within that period.

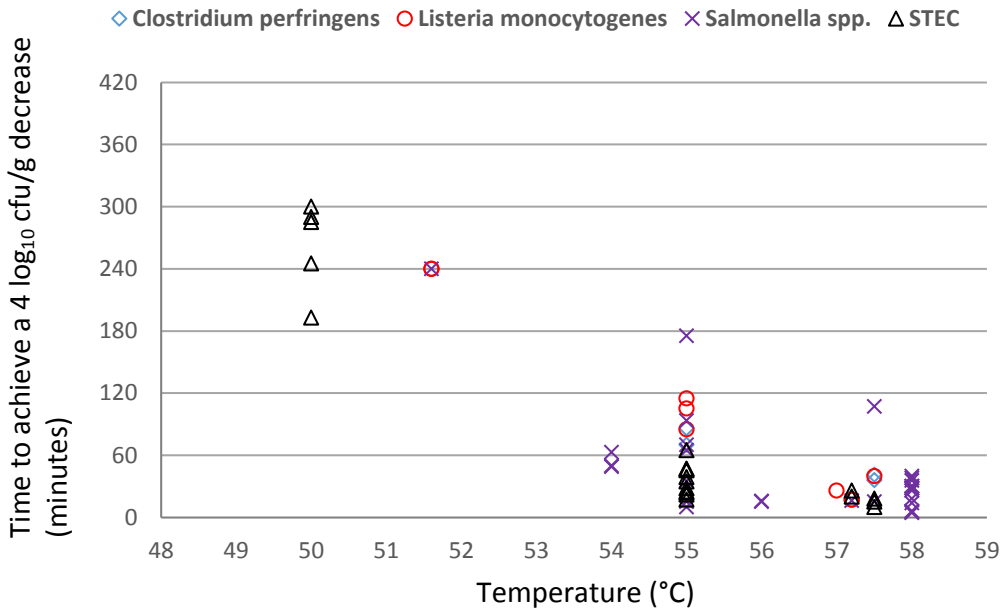


Figure 5: Published values of time to achieve a 4 log_{10} cfu/g reduction in bacterial concentration in beef, pork, and poultry.

The highest time point plotted for *Salmonella* at 55°C and 57.5°C in Figure 5 relate to experiments including a *Salmonella* Senftenberg strain which is known to be more heat resistant than most other *Salmonella* strains.

4.3 EGG

Data from a total of 92 experiments for the thermal inactivation of pathogens in eggs were collated for further investigation. Of these, 79 survival curves were removed from consideration. The duration of 53 experiments resulted in pathogen reductions of less than 4 $\log_{10}$ cfu/g and 26 survival curves did not provide sufficient information to estimate the time taken to produce a 4 $\log_{10}$ cfu/g reduction.

The final set of 5 papers (Appendix C.4) resulted in 13 survival curves. The cooking times to achieve a 4 $\log_{10}$ cfu/g reduction in cell concentration are plotted in Figure 6. There are insufficient data to give guidance on appropriate cooking time for eggs.

A shoulder of 2 to 3 minutes was observed in the inactivation of *Salmonella* in egg white at 52°C. It is not clear if the shoulder was due to the initial heat resistance of the bacteria or due to experimental factors such as the sudden temperature change from 0 to 52°C or the time for the 2 ml sample of egg white to uniformly come up to temperature.

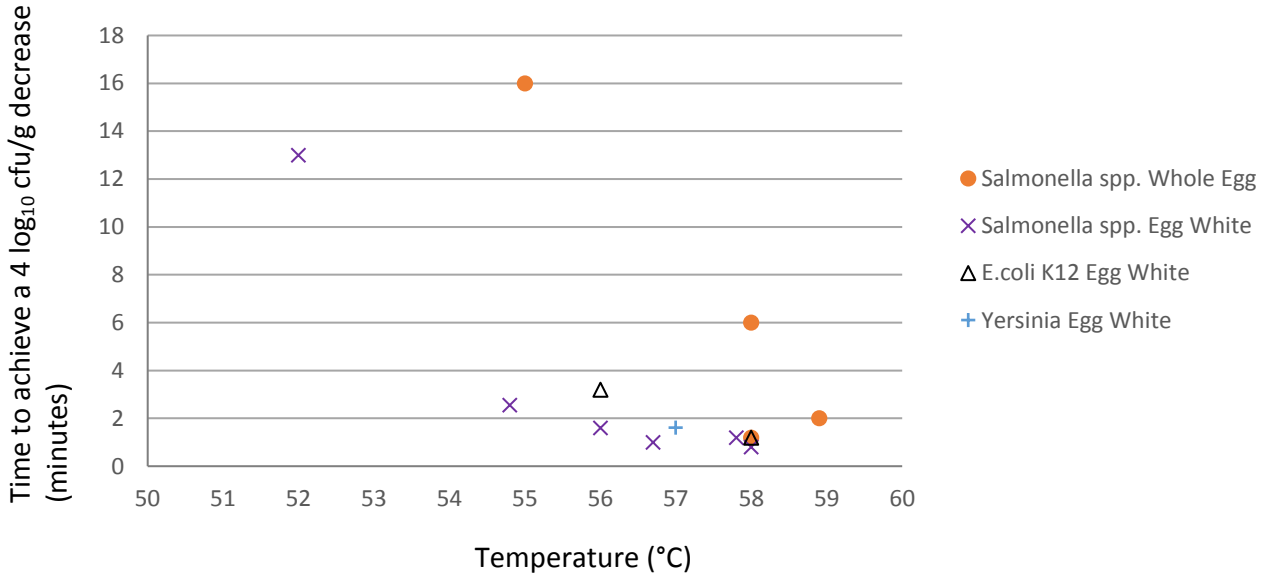


Figure 6: Published values of time to achieve a 4 $\log_{10}$ cfu/g reduction in bacterial population concentration in whole egg or egg white.

5. VIRUSES AND SOUS VIDE COOKING

Key findings

Viruses most relevant to foodborne human infections are noroviruses, hepatitis A virus and the emerging foodborne virus, hepatitis E.

Eggs, chicken, turkey and beef are foods not generally considered to be at risk from human enteric virus contamination.

Evaluation of the heat inactivation of norovirus and hepatitis E viruses is limited due to the lack of culture methods to determine viable virus particles in food products.

In enteric viruses, inactivation is significant at temperatures at 60°C or above (> 2 $\log_{10}$), however there is limited inactivation (< 2 $\log_{10}$) at temperatures less than 60°C.

Viruses do not grow on foods but with a low infectious dose (10-100 particles), low level contamination can readily result in infection following exposure.

5.1 INTRODUCTION

The aim of the project was to evaluate evidence available to support time and temperature parameters for sous vide cooking at temperatures below 55°C. As readers may be less familiar with foodborne viruses, this section first provides an introduction to foodborne viruses and then summarises the data on thermal inactivation of these viruses.

5.2 VIRUSES AND FOODBORNE DISEASE

Viruses most relevant to foodborne human infections are noroviruses and hepatitis A virus (HAV). Hepatitis E virus (HEV) is considered an emerging foodborne virus. Other relevant enteric viruses include rotaviruses (groups A to C), enteroviruses, astroviruses, sapoviruses and adenoviruses (types 40 and 41) but these are only occasionally linked to foodborne infections. Enteric viruses can be excreted in faeces in high numbers (10^{8-11} per g) from infected hosts and at least norovirus and HAV have a low (10-100) infectious dose.

Enteric viruses have a number of other properties that are significantly different to bacteria associated with foodborne infections. As enteric viruses require a host to replicate, they do not have the capability to grow on foods or in the environment but they do demonstrate environmental stability. For example, viruses are relatively stable at 37°C, can retain their infectivity for days or weeks at 4°C, and will remain infectious following freezing (Greening 2001). Storage at -20°C or lower will preserve viruses in the long-term, and therefore this is not an effective inactivation treatment (Butot et al., 2008). Foodborne outbreaks associated with the consumption of shellfish and soft berries that had been frozen are frequently reported (Chiapponi et al., 2014, Simmons et al., 2007, Webby et al., 2007).

In addition, enteric viruses show resistance to drying, pressure and UV inactivation and are highly acid resistant. They can withstand higher temperatures than even the most heat resistant bacteria (Lee *et al.* 2015). Guidelines designed to protect against bacterial foodborne

infections may therefore be unsuitable for enteric viruses. Key foodborne viruses grow poorly or not at all in cell culture, restricting the acquisition of precise inactivation data.

The following section describes some relevant background on the most significant foodborne viruses, noroviruses and HAV, with a description of HEV, an emerging foodborne virus.

- **Norovirus** infections are common in New Zealand and overseas with food being a significant transmission route. Globally approximately 15% of all reported norovirus outbreaks have been attributed as foodborne, most other outbreaks are attributed to person-to-person transmission (Verhoef *et al.* 2015). Despite extensive efforts, norovirus cannot be cultured in the laboratory and there is no validated model virus or system to assess their infectivity following heat inactivation studies (Duizer 2004; Papafragkou 2013). Instead, other viruses including murine norovirus have been used as surrogates to evaluate heat inactivation.
- **Hepatitis A virus** that is endemic in some countries is associated with shellfish and other food related, mainly soft fruits, outbreaks. In New Zealand, HAV is non-endemic and notified foodborne infections are uncommon. Approximately 20 cases are reported each year, most associated with travel to countries where the virus is endemic. Only two outbreaks associated with food have been reported in the last 15 years - one in 2002 with locally produced fresh soft berry fruit (Calder 2003) and one in late 2015 associated with imported frozen soft berries[4]. As wild-type strains of HAV are at best difficult to culture (they often show no evidence of cytopathic effect in cell assays), laboratory-adapted strains such as HM175 that grow relatively well in the laboratory have been used for heat inactivation studies. Hence, there is no heat inactivation data on wild-type HAV strains.
- There is increasing evidence of the role of **hepatitis E virus (HEV),** an important waterborne pathogen in developing countries, as a foodborne pathogen (Wilhelm 2015). While HEV is endemic mainly in Africa and Asia (genotypes 1 and 2), genotype 3 has a global distribution and is thought to be zoonotic. HEV has been detected in mammals including pigs, rabbits, deer and wild boar (Meng 2013; Tei *et al.* 2003). In New Zealand, the presence of HEV has been demonstrated in pigs (Garkavenko *et al.* 2001). As with other viruses that are shed enterically, HEV can be bioaccumulated by shellfish exposed to inadequately treated wastewater and shellfish can be a potential vehicle of infection (Crossan *et al.* 2012). As most HEV infections are asymptomatic and its transmission poorly understood, hepatitis E disease is probably underdiagnosed, and the amount of infection in the human population remains unclear. Symptomatic infections in New Zealand are rarely reported. Between one and seven cases were reported each year between 2011 and 2014. Most notified cases are currently related to travel to endemic countries[5]. Globally, seroprevalence is between approx. 5 to 25%. Although HEV culture assays are available, they are relatively difficult to use for heat inactivation studies due to low efficiency and problems achieving the high titre required to establish at least a 3-4 $\log_{10}$ reduction. Hence, animal inoculation experiments have been trialled to test for infectivity with some success (Barnaud et al., 2012; Feagins et al., 2008).

[4] Hepatitis A - New Zealand: Chinese frozen berries, alert, recall. Tue 1 Dec 2015. ProMED Digest, Vol 42, Issue 17
[5] https://surv.esr.cri.nz/surveillance/surveillance.php

With the possible exception of shellfish and soft berries, due to the lack of standardised methods and laboratory capabilities, food samples are not routinely tested for virus contamination following suspected foodborne outbreaks so their role may be under reported.

5.3 CASES ASSOCIATED WITH SOUS VIDE COOKING

Only one report was identified that describes enteric virus infections related to sous vide cooking[6]. In January 2014, 123 cases of norovirus gastroenteritis were associated with the consumption of norovirus-contaminated cockles consumed at a restaurant in London. The sous vide technique used involved the cooking of cockles in a water bath at 60°C or less for approximately 20 minutes.

5.4 FOODS

5.4.1 Shellfish

As enteric viruses are excreted in faeces of infected individuals, shellfish growing waters impacted by inadequately treated wastewater can be a source of virus contamination of shellfish. The detection of human noroviruses in shellfish have been widely reported and are associated with gastroenteritis outbreaks, including in New Zealand (Simmons *et al.* 2007). Outbreaks are generally associated with the consumption raw or lightly cooked shellfish (Smith *et al.* 2012).

HAV and HEV infection following consumption of shellfish has been reported overseas (Said 2009) but not in New Zealand. While the presence of HAV in the New Zealand water environment and commercially-available shellfish is unknown, the presence of HEV, albeit sporadically, in New Zealand fresh water has been demonstrated using reverse transcription real-time PCR (RT- qPCR) (Williamson 2011), demonstrating a potential transmission route to humans.

5.4.2 Pork products

In developed countries, HEV strains infecting mammals such as domestic pigs, wild boar, deer and rabbits are thought to be causative agents of zoonotic infection in humans and where studied, domestic pigs have a very high seroprevalence of HEV. Pigs are probably the important reservoir of HEV genotype 3 in terms of foodborne disease risk. One New Zealand study showed that the majority (20/22) of pig herds tested were positive for HEV IgG (Garkavenko 2001).

HEV infection in these animal hosts result in virus replication of hepatocytes (liver cells) and therefore virus may be present in food products containing infected liver. The presence of HEV RNA and/or infectious virus has been reported in retail pork liver and sausages overseas, with the prevalence of HEV RNA ranging from 0-11% in reported studies (Colson 2010, Song 2014, Szabo 2015, Wilhelm 2015). A French study reported that IgG seroprevalence (exposure to HEV) was significantly correlated with eating uncooked pork liver sausages ($p < 0.001$), offal ($p = 0.003$) or mussels ($p = 0.02$) (Mansuy *et al.* 2015).

[6] http://ecdc.europa.eu/en/ESCAIDE/programme/presentations/Documents/10.5%20Amy%20Mikhail%20Enteric%20virus%20outbreak%20-%20ESCAIDE%202014%20final.pdf

A case control study in Germany also indicated that autochthonous (locally acquired) HEV infections were significantly associated with consumption of raw or undercooked wild boar meat and offal (including liver) (Wichmann 2008). The presence of HEV in pig or wild boar products in New Zealand is unknown.

5.5 OTHERS

Eggs, chicken, turkey and beef are foods not generally considered to be at risk from human enteric virus contamination. Freshwater and ocean fish may become contaminated from contaminated waters, both externally on their scales and internally within their gills and guts but as these parts of the fish are not consumed, this significantly reduces the risk of infection.

Contamination of foods by infected food handlers before or after cooking is an important contamination route. This can apply to any foods handled and not heated, or not heated sufficiently to cause virus inactivation prior to consumption.

5.6 VIRUS INACTIVATION DATA

Koopmans and Duizer (2004) suggested that a reduction in viral infectivity of 1, 2, 3 and 4 $\log_{10}$ following a food manufacturing process equates to a high, medium, low and negligible risk of viral infection to the consumer. Therefore an inactivation of at least 4 $\log_{10}$ gives confidence on product safety. Due to experimental limitations associated with virus titres and limit of detection of efficiencies for a number of cell culture assays, it is often not possible to determine $\log_{10}$ reductions greater than 4 $\log_{10}$.

The survival and inactivation properties of the foodborne viruses, particularly noroviruses, are not fully understood. Most available data are based on studies in cell culture media. Data on foods including shellfish, dairy products (only milk studies are available) and meat are limited (Table 3). A review published in 2015 summarised the inactivation data available on foodborne viruses and potential viral surrogates (Bozkurt 2015b). Few studies are available for temperatures ≤50°C, and for those temperatures between 50 and 60 °C, time periods do not exceed 3 hours.

At or below 50°C available information indicates the amount of inactivation at those temperatures would be minimal. Generally, at temperatures between 50 and 56°C, only limited damage to the capsid of enteric viruses occurs, resulting in modest levels of virus inactivation. Higher temperatures (≥ 56°C) are likely to cause denaturation of the viral capsid proteins and will result in higher levels of inactivation. Achieving a temperature of 90-100°C for even a few minutes will result in a negligible risk of infection to the consumer.

As seen in Table 3, the number of matrices used in studies are limited. There is limited information on the thermal inactivation properties of viruses on meat. This is significant as data suggests that the amount of thermal inactivation may be affected by the composition of the matrix. Overall, thermal inactivation of viruses in solid matrices is lower than in liquids (such as cell culture media). As enteric viruses can be contained within the matrix (for example, HEV in pig liver, and noroviruses, HAV and HEV within the digestive gland of shellfish), data on inactivation in liquids should be used with caution. In addition, the amount of protein, fat and sugar in foods may have a protective role against heat inactivation (Croci 2012).

Table 3: Thermal inactivation studies on enteric viruses and viral surrogates (Bozkurt 2015b)

Matrix	Temperature range (°C)	No of studies	Viruses	Method
Meat	50-72	1[a]	HAV	Culture
	50-72	1[a]	Surrogates (FCV, MNV)	Culture
	56-71	2[b]	HEV	Swine bioassay and RT-PCR
Shellfish[c]	50-100	8	HAV	Culture
	60-100	2	NoV (genogroup II)	RT-PCR
	50-100	5	Surrogates (FCV, MNV)	RT-PCR and culture
Dairy (milk)	62.8-85	4	HAV	Culture
	63-72	2	Surrogates (FCV, MNV, poliovirus)	Culture
Fruit, vegetables & herbs	50-95	2[d]	HAV	Culture
	75-95	1[e]	NoV (genogroups I and II)	RT-PCR
	50-95	6[f]	Surrogates (FCV, MNV)	Culture
Cell culture media	50-100	6	HAV	Culture
	56-100	2	NoV (genogroup II)	RT-PCR
	37-100	15	Surrogates (FCV, MNV, Tulane, sapovirus)	Culture
Faecal suspension/PBS	45-70	1	HEV	Culture

HAV, Hepatitis A virus; FCV, Feline calicivirus; MNV, Murine norovirus; NoV, Norovirus. Tulane virus used as a surrogate.
PBS, phosphate buffered saline; reverse transcription PCR, RT-PCR

a: Turkey deli meat; b: Pork liver homogenates; c: Cockles, mussels, clams; d: Strawberry mashes (80-85°C), herbs (basil, chives, mint and parsley) (75-95°C) and spinach (50-72°C); e: Herbs (basil, chives, mint and parsley); f: Raspberry puree (65-75°C), herbs (basil, chives, mint and parsley) (75-95°C) and spinach (50-80°C)

5.6.1 Norovirus

As no cell culture assay (test) has been successfully established for norovirus, there are limited data on their inactivation following heat treatment. Some data are available from volunteer studies into infectivity. In the 1970s, it was shown that for the human norovirus strain, Norwalk virus (GI.1), there was incomplete inactivation at 60°C for 30 minutes (Dolin 1972). No other studies have been performed since.

As the only available method for the detection of norovirus, RT-PCR can be applied to determine the reduction of 'RT-PCR titres' following heat treatment. Using this approach, *D* values of 1.3 minutes at 100°C (Hewitt 2006), 25 minutes at 60°C and 5 minutes at 80°C, with a two point z-value of 28°C (Croci 2012) was determined. However, the usefulness of using RT-PCR titres for information on infectivity for viruses remains unclear.

5.6.2 Hepatitis A virus

As the most studied virus to date at temperatures between 60ºC and 90ºC, there is good evidence that HAV is the most heat resistant enteric virus. It is more resistant to heat than viral surrogates.
However, HAV can be readily inactivated at high temperatures (≥80^{0}C) for relatively short cook times. For instance, data often used as a guide for HAV inactivation was provided by Scheid et al., who reported that the HAV titre could be reduced by 4 logs when exposed to a temperature of 85ºC for 1 minute (Scheid 1981).

Similarly, Favero and Bond (1998) reported that a 5 $\log_{10}$ reduction could be achieved using those parameters. They also showed that although a 5 $\log_{10}$ reduction was achieved at 75ºC for 30 minutes, a 10 minute exposure was insufficient to reduce the titre by the same amount. Their data allowed for the development of recommendations for bivalve molluscan shellfish; for instance where an internal temperature of 90ºC for 90 seconds is recommended to give a 4$\log_{10}$ reduction of HAV.

There are limited data for HAV at ≤ 60ºC (Bertrand 2012) with little thermal inactivation data on meat and shellfish (Table 4). At 60ºC, a *D* value of up to 74.6 minutes was reported (Gibson 2011), which equated to a less than a 1 $\log_{10}$ reduction after 1 hour, and no more than a 3 $\log_{10}$ reduction after 2 hours. Similarly, a 1982 study reported that at 60ºC, a cook time of 2 hours was required for a 3 $\log_{10}$ reduction (reported by Millard 1987). Similarly, Favero and Bond (1993) reported incomplete virus inactivation (< 5 $\log_{10}$) in a liquid suspension at 60ºC for 6-12 hours.

Table 4: Range of *D* values for Hepatitis A virus in various matrices at 50 to 60ºC

Temperature (°C)	*Hepatitis A virus D* value range (minutes)			
	Cell culture media[a]	*Shellfish*[b]	*Meat*[c]	*Spinach*[d]
50	56-385	54.2	42.1	34.4
56	8.4	9.3	20.6	8.4
60	2.7- 74.6	3.3-6.5	5.9	4.6

a (Bozkurt 2014b; Croci 2012; Gibson 2011; Park 2015), b: (Bozkurt 2014c; Croci 2012), c: (Bozkurt 2015a), d (Bozkurt 2015c)

Most early studies on HAV and hence available data have assayed the inactivation properties of suspended viruses in liquid matrices. As previously discussed, the matrix can affect the inactivation and it is likely that protein rich matrixes such as in shellfish and meat/liver products would protect the viruses against heat inactivation (Millard 1987).

Figure 7 summarises *D* values from representative heat inactivation studies for cell culture media, shellfish, meat, dairy and soft fruit, spinach and herbs.

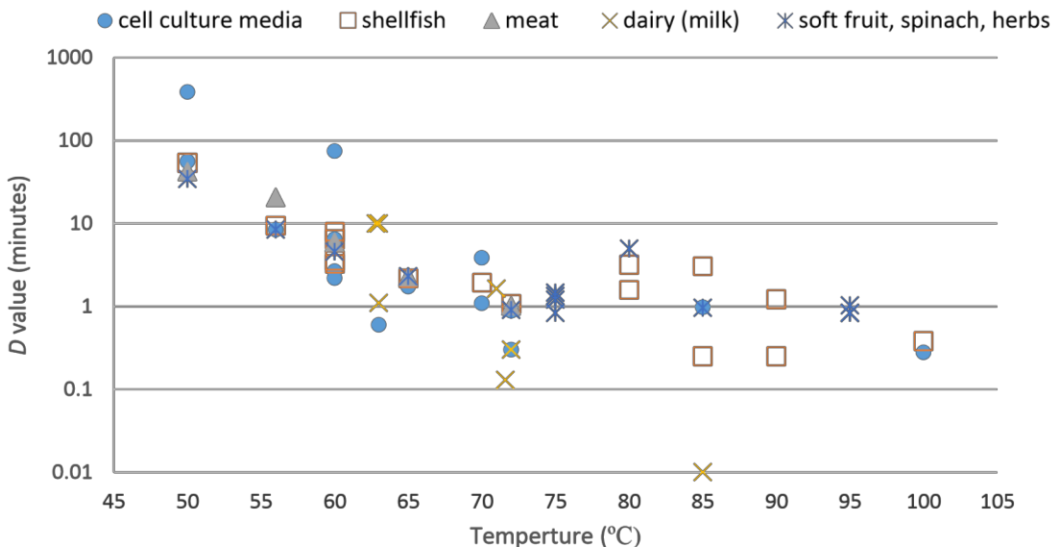


Figure 7: *D* values for hepatitis A virus in various matrices at 50 to 100ºC

5.6.3 Hepatitis E virus

Limited studies using faecal suspension of HEV, HEV-seeded buffers and pork liver homogenates have shown that HEV has good thermal resistance below 60ºC (Emerson 2005; Feagins 2008). In summary:

- HEV is not fully inactivated when exposed to temperatures between 45 and 56ºC for 1 hour, for example, only 50% of virus present is inactivated at 56°C for 1 hour
- a 2 $\log_{10}$ or less reduction occurs at 60ºC for 1 hour (i.e. 96-99% inactivation)
- HEV is almost completely inactivated at 66°C and temperatures greater than 71ºC for 20 minutes are sufficient to consider HEV inactivated (i.e. at least a 3 $\log_{10}$ reduction is achieved).

A study reported in 2012 looked more closely at the HEV inactivation profiles at 62ºC for 5, 20 and 120 minutes; 68ºC for 5, 10 and 20 minutes and 71ºC for 5, 10 and 20 minutes in pork liver. Due to the lack of a reliable cell culture assay, the heat inactivation in liver was investigated in two ways:

(1) The cooked liver was homogenized and a solution intravenously injected into pigs. Infectivity of the HEV was determined from host seroconversion and excretion. It was confirmed that an internal temperature of 71°C for 20 minutes was required to completely inactivate HEV in liver during cooking.

(2) The cooked liver was homogenized and tested by HEV RT-PCR. A reduction of 2.9 $\log_{10}$ genome equivalents/g was observed after the cooking at 71ºC for 20 minutes.

A temperature of 62ºC for up to 2 hours had no measurable effect on HEV infectivity. The study also showed that inactivation of HEV may be affected by high (up to 30%) fat content in pork liver.

5.6.4 Viral surrogates

For surrogates, most data relates to liquids (buffers, faecal suspensions or cell culture media) (Table 4). Of the surrogates, murine norovirus, feline calicivirus and Tulane virus, *D* values are similar at approximately 1 minute at 72ºC when the virus is in suspension (Tian *et al.* 2013). Hirneisen and Kniel (2013) showed that Tulane virus is more heat sensitive than murine norovirus between 50-65ºC and so may not be a suitable surrogate for heat inactivation studies.

Murine norovirus and feline calicivirus have been reported to have similar heat resistance properties at lower (63ºC) temperatures (Cannon 2006). For feline calicivirus, while no reduction in virus titre was observed at 56ºC for 1 or 3 minutes in suspension, a reduction of 7.5 $\log_{10}$ occurred after 1 hour incubation. As may be expected, significant reductions were also determined of 3 $\log_{10}$ and 6.5 $\log_{10}$ for feline calicivirus when heated to 70ºC for 3 minutes (Doultree 1999).

Although potentially useful information, it is still unclear whether these viruses are suitable as surrogates in heat inactivation studies. For example, Slomka and Appleton (1998) examined their suitability by comparing to HAV and determined HAV was more heat stable than feline calicivirus for instance.

There are limited data on the thermal inactivation of enteric virus surrogates in shellfish, and even less for meat products. Table 5, Table 6 and Figure 8 shows available D values (minutes) as determined by culture assays for murine norovirus and feline calicivirus in shellfish and other matrices.

Table 5: Range of *D* values for murine norovirus in various matrices at 37 to 60ºC

Temperature (°C)	*Murine norovirus D* value range (minutes)			
	Cell culture media[a]	*Shellfish*[b]	*Meat*[c]	*Spinach*[d]
37	769	-	-	-
50	34.5- 106	20.2	21.0	14.5
56	3.5-12.4	6.12	7.3	3.3
60	1.1-13.7	2.64	2.7	1.0

a: (Bozkurt 2013; 2014b, Cannon 2006, Gibson 2011, Park 2015; Wang *e*2012), b: (Bozkurt 2014c), c:(Bozkurt 2015a), d:(Bozkurt 2014a)

Table 6: Range of *D* values for feline calicivirus in various matrices at 37 to 60ºC

Temperature (°C)	*Feline calicivirus D* value range (minutes)			
	Cell culture media[a]	*Shellfish*[b]	*Meat*[c]	*Spinach*[d]
37	480-599	-	-	-
50	20.0-50.6	5.20	9.9	17.4
56	2.7-8	3.33	3.0	5.9
60	0.56-14.1	0.77	0.8	0.8

a: (Bozkurt 2013; 2014b, Cannon 2006, Croci 2012, Doultree 1999, Duizer 2004, Gibson 2011), b: (Bozkurt 2014c), c: (Bozkurt 2015a), d: (Bozkurt 2014a)

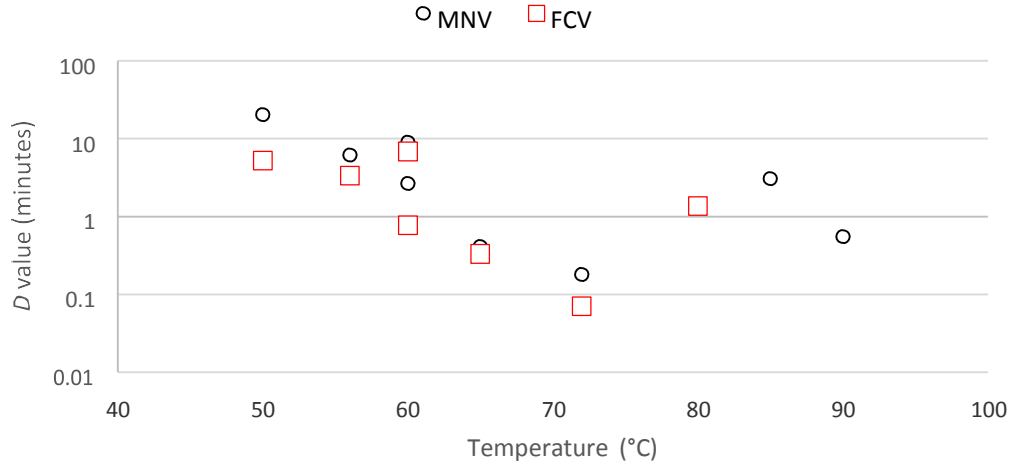


Figure 8: *D* values for murine norovirus (MNV) and feline calicivirus (FCV) in shellfish at 37 to 90ºC

An alternative surrogate virus, F-specific RNA (F-RNA) bacteriophage, is relatively easy to culture and is available at high concentrations. Flannery *et al.* (2014) determined the inactivation profile at approximately 70ºC and 90ºC using culture and RT-PCR in experimentally contaminated mussels. At 70ºC, concentrations of infectious F-RNA bacteriophage in mussels were not significantly reduced in simmering water. Temperatures higher than 90ºC for 3 minutes resulted in the non- detection of infectious F-RNA bacteriophage.

These results are similar to those using murine norovirus and feline calicivirus as described above, and shows its potential as a surrogate. The *D* value of infectious F-RNA bacteriophage at 90ºC was 42 seconds, with 126 seconds required at 90ºC to give a 3 $\log_{10}$ reduction in infectious virus.

6. CONCLUSIONS

A review of the literature regarding the survival of foodborne pathogens during the cooking of beef, pork, poultry, seafood and eggs at temperatures below 60°C has been conducted.

The following conclusions have been made for bacterial pathogens:

- There are insufficient data in the literature at this time to allow prediction of the inactivation of bacterial pathogens at cook temperatures below 55°C. Therefore, this project has been unable to generate recommendations for safe cooking time/temperature combinations below 55°C.
- Most papers/experiments do not provide sufficient data to determine the shape of the survival curve or have not been conducted for long enough to determine the time required to reduce pathogen concentration by 5 to 7 $\log_{10}$ cfu/g.
- Survival curves may be non-linear with increasing shoulder effects with decreasing cook temperature, increasing fat content and the addition of curing salts.
- Four papers have identified tails in the survival curve. A tail in a survival curve indicates that a sufficient reduction in pathogen concentration may not be achievable irrespective of the time the product is held at that specific temperature. The underlying cause of tailing in two papers is unclear, but the other two papers related to experiments with very slow heating rates from 20°C to the cook temperature.
- Some *D* values given in the literature have been derived from the linear part of a non-linear survival curve and do not take into account shoulders or tails. *D* values from this type of analysis should not be used alone to set sous vide cooking times below 55°C.
- Heating of meat or eggs at sub-lethal temperatures may result in pathogens present undergoing a heat shock response. This makes the pathogen more heat tolerant during subsequent cooking at higher temperatures. Heat shocking of pathogens could occur during sous vide cooking due to the time it takes for a portion of food to come up to the cooking temperature, inadequate control of the water bath temperature, overloading the water bath or recipes that have a lower initial heating step before the main cook step.
- Spore forming bacteria such as *Bacillus cereus, Clostridium perfringens and Clostridium botulinum* can form spores which protect cells from unfavorable environmental conditions. If present in the food, a proportion of spores may germinate during sous vide cooking at temperatures below 55°C. Therefore such a cook step could increase, rather than decrease, the cell concentration of these species.

The following conclusions have been made for virus pathogens:

- There are insufficient data available on viruses to describe inactivation or survival of viruses during cooking at temperatures below 60°C.

- Enteric viruses show limited inactivation (<2 $\log_{10}$) at temperatures less than 60°C. At temperatures between 50°C and 56°C, only limited damage to the capsid of enteric viruses occurs, resulting in modest levels of virus inactivation. Higher temperatures (above 56°C) are likely to cause denaturation of the viral capsid proteins and will result in higher levels of inactivation.
- The lack of a cell culture system for norovirus and hepatitis E hampers the understanding of inactivation of these viruses. The current detection methods cannot determine if the detected virus components come from a viable virus or one that has been lethally damaged.

GLOSSARY

Anaerobe	An organism that can grow without oxygen.
Autochthonous	Originating in the locality of the people becoming ill.
Capsid	A protein shell in a virus which encloses the genetic material of the virus.
Cyst	A microbial cyst is a resting or dormant stage of a microorganism that helps the organism to survive in harsh environmental conditions.
D value	The time taken for a specific organism at a specified temperature and in a specified substrate to undergo a 90% or 1 $\log_{10}$ reduction in population
Emetic Toxin	Toxin which causes nausea or vomiting
Enterotoxin	Toxin that targets the intestines. Thus it is associated with diarrhoeal symptoms.
F-specific RNA (F-RNA) bacteriophage	A group of male specific bacteriophage, the structure consists of proteins encapsulating a RNA genome.
Facultative anaerobe	An organism that can grow with or without oxygen
Generation time	The time taken for a bacterial cell population to double in size.
Heat Shock	A sub-lethal heat treatment.
Hepatocytes	Cells which make up 70-85% of liver tissue.
Homogenate	Tissues and cells that have been ground or mixed into a uniformly consistent substance.
Inactivation	Cell damage or death, such that the cell cannot grow or cause illness.
Log ($\log_{10}$) reduction	A log is a mathematical term that is short for logarithm. In this report a one log reduction of micro-organisms means to eliminate the micro-organisms by a factor of ten (10). If there were one thousand (1000) micro-organisms, they would be reduced to one hundred (100).
Microorganism	An organism which is too small to be seen by the unaided human eye. Microorganisms include bacteria and viruses.
Oocyst	A hardy, thick-walled spore, able to survive for lengthy periods outside a host. Organisms that create oocysts include *Cryptosporidium*.
Pathogen	An organism that causes disease or illness to its host.
Pathogenicity	The ability of an organism to cause disease.
Polymerase chain reaction (PCR)	A method for making enough copies of a segment of DNA for it to be detected or further analysed.
Reverse transcription PCR (RT-PCR)	A PCR test that is designed to detect and measure RNA. Many viruses use RNA as their genetic material instead of DNA.

Seroconversion	The time period during which antibodies develop and become detectable in the blood.
Seroprevalence	The number of persons in a population who test positive for a specific disease based on serology (blood serum) specimens, in this case given as a percentage of the population.
Shoulder (in survival curve)	A shoulder in a survival curve is an initial period of no or low rate of reduction in cell concentration which precedes a higher rate of cell inactivation.
Spore	A resting stage of bacteria which allows the organism to survive during harsh environmental conditions.
Sous vide	Translates from French to "under vacuum" and in this report refers to cooking where food is vacuum packed before cooking in a water bath.
Sub-lethal temperature	A temperature range above the optimum for cell growth and below the temperature at which cell death occurs.
Survival curve	A plot showing how cell or virus concentration changes over time.
Tail (in survival curve)	A tail in a survival curve is when there is either a reduction in inactivation rate or no further inactivation after a period of higher inactivation rate.
Thermocouple	An electrical device for measuring temperature
Titre	The concentration of virus particles in a given volume.
Tryptic soy broth (TSB)	A complex general purpose medium used to grow some types of pathogenic bacteria.
Virus particle	The name given to a virus that is not inside an infected cell or in the process of infecting a cell. These viruses exist in the form of independent particles.
z value	The increase in temperature required to decrease the *D* value by 90%.

APPENDIX A: METHOD

A.1 LITERATURE REVIEW

The literature review followed four different approaches:

- Extraction of journal papers with cook temperatures below 60°C from the existing ESR thermal inactivation database. Includes data up to 2010.
- Examination of Combase database to determine relevant papers.
- Literature review using the PubMed and Web of Science Databases for 2010 to September 2015. Initial search terms did not include specific pathogen names or temperatures, but included searches for;
    - sous vide
    - heat/thermal inactivation
    - thermal destruction
    - sub lethal heat
    - heat shock

    Papers which included cooking temperatures below 60°C in meat or egg were extracted for further examination.
- New references found through reading the extracted papers.

A.2 *D* TIME DATA

Thermal inactivation data of potential pathogens were collected from the scientific published literature. When review papers were located, the data were not considered unless the primary publications containing the relevant data for meat or eggs could be obtained. A list of the references are given in Appendix C.

Only data meeting the conditions below were included in the project:

- Cook temperatures below 60°C.
- Pathogen inoculated into/on raw beef, pork, poultry, fish, seafood or eggs.
- Pathogen cells where in a stationary growth phase at the time of inoculation. Stationary phase cells are more heat resistant than cell populations which are actively growing.
- Test product was of a form which allowed rapid heating throughout the sample to the target temperature, such as thin patties or glass tubes.
- Test product was held at a constant internal temperature once at the target temperature.
- Test product was rapidly cooled after the designated heating time to prevent further decline in viable cell concentrations.

Sixty four papers where located which listed *D*-values and met the above conditions. The resulting dataset included 341 *D* values including; 153 for beef, 55 for poultry, 20 for pork, 21 for fish and seafood and 92 for eggs.

Further examination of these papers, revealed that:

- Some inactivation curves exhibited shoulders before a log linear inactivation occurred. Or in other words there was an initial time period when no or a small rate of inactivation occurred, followed by more rapid inactivation with a constant decline rate in the logarithm of cell concentration.
- Some experiments where for short time periods relative to the inactivation rate. Therefore *D* values may have been calculated from experiments with limited cell concentration reductions. The experiment may have only captured an inactivation shoulder, and if the experiment had been conducted for longer, increased rates of inactivation may have been observed.
- Some papers did not provide sufficient information on the inactivation rate over the time period of the experiment to determine if shoulders, tails, or a linear inactivation rates in cell concentration where observed or included in the *D* value calculation.

To avoid presenting potentially misleading *D* values, which could result in over- or underestimating the *D* value, *D* values are not plotted in the section 4.

Instead the data was filtered to only include inactivation rates from experiments where at least a 4 $\log_{10}$ cfu/g reduction in pathogen numbers were observed. A 4 $\log_{10}$ cfu/g reduction was chosen to ensure the effects of any shoulder would likely to have been observed. It is usual to require a 5 to 7 log reduction in bacterial concentration when setting food safety targets, however requiring this level of reduction in the experiments would have reduced the dataset even further.

The filtered data is presented as the time taken to achieve a 4 $\log_{10}$ reduction in pathogen cell concentration from the start of cooking. This time does not assume a linear or non-linear (log scale) inactivation relationship and include any inactivation shoulders present in the experimental results.

A.3 FOODBORNE PARASITES

Potential foodborne pathogens include the parasites *Giardia* and *Cryptosporidium*. Both parasites will not grow outside an animal host and transmission between hosts is via (oo)cyst excreted with faeces. No thermal inactivation data in the temperature range between 40 °C to 60°C was located in the literature. Heating *Giardia cysts* to 60-70°C for 10 minutes[7] inactivates them and heating *Cryptosporidium* oocysts to 64.2°C for two minutes[8] has been reported to make (oo)cysts non- infective. *Giardia* and *Cryptosporidium* are not discussed further in this report.

[7] https://www.mpi.govt.nz/document-vault/11024 Accessed 2nd March 2016
[8] https://www.mpi.govt.nz/document-vault/11045 Accessed 2nd March 2016

APPENDIX B: HEAT SHOCK DATA

B.1 LISTERIA MONOCYTOGENES

Table 7: Examples of the effect on *D* values of prior heating before the main cook step: *Listeria monocytogenes*.

Food/Broth	Reference	Heat Shock		Cook	D-value (minutes)		Cook step duration [reduction]
		Temperature (°C)	Duration/Rate	Temperature (°C)	With heat shock	Without heat shock	
Ham (brined and ground)	Carlier 1996	42	1 hour	55	19.2 ± 3.9	17.8 ± 6.01	120 minutes [4 log cfu/g]
		42	1 hour	60	3.5 ± 0.64	1.82 ± 0.06	20 minutes [3 log cfu/g]
Beef (surface)	Novak 2003	55	30 min	60	2.86	2.45	30 minutes [5 log cfu/g]
Beef (ground)	Hansen 1996	46	30min	60	D_{60C} = 18.7 shoulder for first 20 minutes then ~7 from linear plot	6.7	40 minutes [5 log cfu/g]
		48	2 hours	60		6.7	
Broth (TSYEB)	Bunning 1990	35	30 min	52	37.9	Not Stated	Not Stated
		48	30 min	52	49.8		
		42	30 min	57.8	11.1		
		48	30 min	57.8	10.3		
		52	30 min	57.8	7.0		
Broth (TYSEB)	Linton 1990	48	20 min	55	18.6	8.3	50 minutes [2 log cfu/ml]
Broth (BHI)	McMahon 2000	48	10 min	55	9.21	7.86	30-60 minutes [3-4 log cfu/ml *D* value does not take into account shoulders.
		48	60 min	55	7.92	7.86	
Broth (TSBYE)	Shen 2014	48	60 min	60	5.0 to 9.8	1.9 to 4.3	20-50 minutes [4-7 log cfu/ml]

Food/Broth	Reference	Heat Shock		Cook	D-value (minutes)		Cook step duration [reduction]
		Temperature (°C)	Duration/Rate	Temperature (°C)	With heat shock	Without heat shock	
Broth (TSB)	Agoston 2009	46	30 min	60	5.2	3.03	15 minutes [<1 to 6 log cfu/ml]
		48	30 min	60	6.7		
		50	30 min	60	13.9		
		46	60 min	60	16.2		
		48	60 min	60	14.8		
		50	60 min	60	11.2		

B.2 SALMONELLA

Table 8: Examples of the effect on *D* values or inactivation rates of prior heating before the main cook step: *Salmonella* spp.

Food/Broth	Reference	Heat Shock		Cook	D-value (minutes)		Cook step duration [reduction]
		Temperature (°C)	Duration/Rate	Temperature (°C)	With heat shock	Without heat shock	
Broth (TSB)	MacKey 1986	48	30 min	50	1 log decrease: ~20 hours	1 log decrease: ~1.5 hours	30 hours
				52	1 log decrease: 186 min	1 log decrease: 63 min	12 hours
				55	1 log decrease: 42 min 3 log decrease: 84 min	1 log decrease: 8 min 3 log decrease: 23 min	100 minutes
				59	1 log decrease: 3.9 min 3 log decrease: 6.1 min	1 log decrease: 0.9 min 3 log decrease: 1.8 min	7 minutes
Broth (TSYEB)	Bunning 1990	48	30 min	52	96.1	Not stated	Not stated
		35	30 min	52	21.3		
		42	30 min	57.8	0.9		
		48	30 min	57.8	2.1		
Broth (CASOYE)	Xavier 1997	42	60 min	52	16.9	5.3	Not stated
				54	4.5	2.7	
				56	2.5	1.3	
				58	1.3	0.9	
Whole Egg	Shah 1991	48	30 min	57.2	4.9	2.6	Not stated
Whole Egg	MacKey 1987	48	30 min	54	2 log decrease: 4 hours	>5 log decrease: 1 hour	4 hours
				60	4 log decrease: 4 minutes	4 log reduction: 2 minutes	4 minutes
Beef (minced)	MacKey 1987	48	30min	54	25	10.5	Not stated
				60	1.26	0.46	

B.3 YERSINIA

Two papers were located which showed the thermal resistance of *Yersinia enterocolitica* could be increased by prior heat shock.

McMahon (2000) conducted experiments in broth using stationary phase *Yersinia*. Cells subjected to heating at 55°C decreased by 5.5 $\log_{10}$ cfu/ml in 10 minutes. In contrast, cells pre-heated in food to 45°C for 30 minutes, decreased by 2 $\log_{10}$ cfu/ml in 18 minutes when heated at 55°C.

Similar results were observed in broth by Shenoy and Murano (1996) who conducted cell pre-heating at 45°C for 60 minutes, followed by heating at 55°C and 60°C. Increased thermal resistance was observed in experiments with *Yersinia* in broth and inoculated on ground pork (Table 9).

Table 9: Effect of preheating Yersinia cells on *D* values

	***D* values (minutes) [Shenoy and Muano, 1996]**			
	Broth (BHI)		**Ground Pork**	
Cook Temp	**Heat Shocked (45°C for 60 minutes)**	**Control**	**Heat Shocked (45°C for 60 minutes)**	**Control**
55°C	7.7	2.0	15.6	6.5
60°C	1.6	1.2	6.7	1.7

These papers only provide inactivation information at 55°C or 60°C for cooking times of 1820 minutes duration. It is not possible to say from these papers if the increased thermal resistance was a shoulder prior to greater thermal inactivation, or if the thermal resistance would be maintained for longer time periods.

B.4 CLOSTRIDIUM PERFRINGENS

*Clo*stridium *perfingens* inoculated into the beef surfaces was subjected to a heat shock at 55°C for 30 minutes. Subsequent cooking at 60°C, resulted in D_{60} of 18.21 minutes compared to D_{60} of 13.87 minutes without prior heat shock (Novak 2003). The 60°C cook step lasted a maximum of 30 minutes which provided information on less than a 2 $\log_{10}$ cfu/g reduction.

APPENDIX C: BIBLIOGRAPHY

C.1 BACTERIAL REVIEW

Adams M.R. and Moss M.O. (2000) Factors affecting the growth and survival of micro-organisms in foods, in Food Microbiology.(eds). Cambridge, UK: Royal Society of Chemistry.

Ágoston R. (2009) Understanding stress adaptive response in *Listeria Monocytogenes*. PhD thesis, Corvinus University of Budapest.

Ahmed N.M., Conner D.E. and Huffman D.L. (1995) Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. *Journal of Food Science* 60(3): 606-610.

Bates J.R. and Bodnaruk P.W. (2003) *Clostridium perfringens*. In: Hocking AD (Ed) Foodborne Microorganisms of public health significance, 6th Edition, Australian Institute of Food Science and Technology Inc, NSW Branch.

Bolton D.J., McMahon A.M. *et al*. (2000) Thermal inactivation of *Listeria monocytogenes* and *Yersinia enterocolitica* in minced beef under laboratory conditions and in sous-vide prepared mince and solid beef cooked in a commercial retort. *Journal of Applied Microbiology* 88:626-632.

Bunning V.K., Crawford R.G., *et al*. (1990) Thermotolerance of Listeria monocytogenes and Salmonella typhimurium after sublethal heat shock. *Applied and Environmental Microbiology* 56(10)3216-3219.

Carlier V., Augustin J.C. and Rozier J. (1996) Heat Resistance of *Listeria monocytogenes* (Phagovar 2389/2425/3274/2671/47/108/340):D- and z-values in Ham. *Journal of Food Protection* 59(6):588591.

Cornet I., Van derlinden, *et al*. (2011) The heterogeneous heat stress response of *Escherichia coli* K12. 11th International Congress on Engineering and Food in: *Procedia Food Science* 1:1060-1066

Devlieghere K., Francois K., Vermeulen A. and Debevere J. (2009) Predictive Microbiology. In: Costa R. and Kristbergsson K. (Eds). Predictive Modelling and Risk Assessment. ISEKI-Food Series, Vol 4:29-54.

Gilbert S., McIntyre L., Lake R., Wong T.L., Hudson A. (2011) Background document on factors influencing the heat inactivation of bacteria in foods. Client Report FW10045 for the Ministry for Primary Industries. Institute of Environmental Science and Research Ltd, Christchurch.

Grecz N. and Arvay L.H. (1982) Effect of temperature on spore germination and vegetative cell growth of *Clostridium botulinum*. *Applied and Environmental Microbiology* 43(2):331-337.

Hansan T.B. and Knøchel S. (1996) Thermal inactivation of *Listeria monocytogenes* during rapid and slow heating in sous vide cooked beef. *Letters in Applied Microbiology* 22:425-428.

Hocking A.D. (Ed) (2003) Foodborne Microorganisms of public health significance, 6th Edition, Australian Institute of Food Science and Technology Inc, NSW Branch

Horn B. (2015) D and z values for the heat inactivation of pathogens in raw meat. Client Report FW15001 for the Ministry for Primary Industries. Institute of Environmental Science and Research, Christchurch.

Horn B., Lopez, L., Cressey P. and Roos R. (2015) Annual report concerning foodborne disease in New Zealand 2014. Client Report FW15016 for Ministry for Primary Industries. Institute of Environmental Science and Research, Christchurch.

Huang L. (2004) Numercial analysis of growth of *Clostridium perfringens* in cooked beef under isothermal and dynamic conditions. *Journal of Food Safety* 24:53-70.

Hudson J.A. (2011a) Maximum growth temperatures of foodborne pathogens and appropriate temperatures for hot holding. Client Report FW10047 for the Ministry for Primary Industries. Institute of Environmental Science and Research, Christchurch.

Hudson J.A. (2011b) Minimum growth temperatures of foodborne pathogens and recommended chiller temperatures. Client Report FW11004 for the Ministry for Primary Industries. Institute of Environmental Science and Research, Christchurch.

ICMSF, International Commission of Microbiological Specifications for Food (1996) Micro-organisms in Food 5 Microbiological specifications of food pathogens, Blackie academic and Professional, London.

Jenson I., Moir C.J. (2003) Bacillus cereus and other Bacillus species. In: AD Hocking (Ed). Foodborne Micro-organisms of Public Health Significance. Australian Institute of Food Science and Technology.

Juneja V.K. (2003) A comparative heat inactivation study of indigenous microflora in beef with that of *Listeria monocytogenes, Salmonella* serotypes and *Escherichia coli* O157:H7. *Letters in Applied Microbiology* 37:292-298.

Juneja V.K. (2002) Thermal Inactivation of Microorganisms. In: Juneja V.K and Sofos J.N. (Eds). Control of Foodborne Microorganisms. Marcel Dekker Inc. New York.

Juneja V.K. and Eblen B.S. (2000) Heat Inactivation of *Salmonella typhimurium* DT104 in beef as affected by fat content. *Letters in Applied Microbiology* 30:461-467.

Labbe R. (1989) *Clostridium perfringens*. In: Doyle M.P (Ed) Foodborne bacterial pathogens, Marcel Dekker, New York.

Line J.E., Fain A.R. *et al*. (1991) Lethality of heat to *Escherichia coli* O157:H7: D-value and z-value determinations in ground beef. *Journal of Food Protection* 54(10): 762-766.

Linton R.H., Pierson M.D. and Bishop J.R. (1990) Increase in heat resistance of Listeria monocytogenes Scott A by sublethal heat shock. *Journal of Food Protection* 53(11):924-927.

Lundgren L. (1966) Effect of temperature on germination of spores from some *Bacillus* and *Clostridium species*. *Physiologia Plantarum* 19:403-410.

MacKey B.M., Pritchet C. *et al*. (1990) Heat resistance of *Listeria*: strain differences and effects of meat type and curing salts. *Letters in Applied Microbiology* 10:251-255.

MacKey B.M. and Derrick C.M. (1987) Changes in the heat resistance of *Salmonella typhimurium* during heating at rising temperatures. *Letters in Applied Microbiology* 4:13-16.

MacKey B.M and Derrick C.M. (1986) Elevation of the heat resistance of *Salmonella typhimurium* by sublethal heat shock. *Journal of Applied Microbiology* 61:389-393.

McClane B.A., Robertson S.L. and Li J. (2013) *Clostridium perfringens* In: Doyle M.P. and Buchannan R.L. (Eds) Food Microbiology: fundamentals and frontiers, 4th Edition, ASM Press, Washington D.C.

McMahon C.M.M., Byrne C.M. *et al*. (2000) The effect of culture growth phase on induction of the heat shock response in *Yersinia enterocolitica* and *Listeria monocytogenes*. *Journal of Applied Microbiology* 89:198-206.

Novak J.S. and Yuan J.T.C (2003) Viability of Clostridium perfringens, Escherichia coli and Listeria monocytogenes surviving mild heat or aqueous ozone treatment on beef followed by heat, alkali or salt stress. *Journal of Food Protection* 66(3):382-389.

Orta-Ramirez A., Marks B.P. *et al*. (2005) Enhanced thermal resistance of *Salmonella* in whole muscle compared to ground beef. *Journal of Food Science* 70(7): M359-M362.

Richter K., Haslbeck M. and Buchner J. (2010) The heat shock response: Life on the Verge of Death. *Molecular Cell* 40:253-266.

Roy R.J., Busta F.F. and Thompson D.R. (1981) Thermal inactivation of Clostridium perfringens after growth at several constant and linearly rising temperatures. Journal of Food Science 46:1586-1591.

Setlow P. and Johnson E.A. (2012) Spores and their significance. In: Doyle M.P. and Buchannan R.L. (Eds) Food Microbiology: fundamentals and frontiers, 4th Edition, ASM Press, Washington D.C.

Shah D.B., Bradshaw J.G. and Peeler J.T. (1991) Thermal resistance of egg-associated epidemic strains of *Salmonella enteritidis*. *Journal of Food Science* 56(2):391-393.

Shen Q., Jangam P.M., *et al*. (2014) Low, medium and high heat tolerant strains of Listeria monocytogenes and increased heat stress resistance after exposure to sublethal heat. *Journal of Food Protection* 77(8):1298-1307.

Shenoy K. and Murano E.A. (1996) Effect of heat shock on the thermotolerance and protein composition of *Yersinia enterocolitica* in brain heart infusion broth and ground pork. *Journal of Food Protection* 59(4):360-364.

Siegel L.S. and Metzger J.F. (1979) Toxin Production by Clostridium botulinum Type A Under Various Fermentation Conditions. Applied and Environmental Microbiology 38(4):606-611,

Stewart CM. (2003) *Staphylococcus aureus* and Staphylococcal Enterotoxins. In: AD Hocking (Ed). Foodborne Micro-organisms of Public Health Significance. Australian Institute of Food Science and Technology.

Stringer S.C., Fernandes M.A. and Metris A. (2012) Safety of sous-vide foods: feasibility of extending combase to describe the growth / survival / death response of bacterial foodborne pathogens between 40°C and 60°C. Institute of Food Research report for Food Standards Agency, UK. Report No FS102028.

Stringer S.C., George S.M. and Peck M.W. (2000) Thermal inactivation of *Escherichia coli* O157:H7. *Journal of Applied Microbiology Symposium Supplement* 88:79S-89S.

Szabo EA, Gibson AM. (2003) *Clostridium botulinum*. In: AD Hocking (Ed). Foodborne Micro- organisms of Public Health Significance. Australian Institute of Food Science and Technology.

Tuntivanich V., Orta-Ramirez A. et al. (2008) Thermal inactivation of *Salmonella* in whole muscle and ground turkey breast. *Journal of Food Protection* 71(12):2548-2551.

van Asselt ED, Zwietering MH. (2006) A systematic approach to determine global thermal inactivation parameters for various food pathogens. *International Journal of Food Microbiology*; 107(1): 73-82.

Van Derlinden E.V, Lule.I, et al. (2010) Quantifying the heterogeneous heat response of Escherichia coli under dynamic temperatures. *Journal of Applied Microbiology* 108:1123-1135.

Velasquez A., Breslin T.J. *et al*. (2010) Enhanced thermal resistance of *Salmonella* in marinated whole muscle compared with ground pork. *Journal of Food Protection* 73(2):372-375.

Warsaw C.R. (2003) Penetration of *Salmonella* spp. into whole muscle turkey breasts during vacuum tumbling margination. MSc thesis, Michigan State University, East Lansing, Michigan.

Weigand K.M., Ingham S.C. *et al*. (2012) Evaluating lethality of beef roast cooking treatments against *Escherichia coli* O157:H7. *Journal of Food Protection* 75(1):48-61.

Xavier I.J. and Ingham S.C. (1997) Increased D-values for *Salmonella enteritidis* following heat shock. *Journal of Food Protection* 60(2):181-184.

C.2 VIRUS REVIEW

Barnaud E., Rogee S., *et al.* (2012) Thermal inactivation of infectious hepatitis E virus in experimentally contaminated food. *Applied Environmental Microbiology* 78(15):5153-5159.

Bertrand I., Schijven J.F., *et al.* (2012) The impact of temperature on the inactivation of enteric viruses in food and water: a review. *Journal Applied Microbiology* 112(6):1059-1074.

Bozkurt H., D'Souza D.H. and Davidson P.M. (2013) Determination of the thermal inactivation kinetics of the human norovirus surrogates, murine norovirus and feline calicivirus. *Journal of Food Protection* 76(1):79-84.

Bozkurt H., D'Souza D.H. and Davidson P.M. (2014a) Thermal inactivation of human norovirus surrogates in spinach and measurement of its uncertainty. *Journal of Food Prot*ection 77(2):276-283.

Bozkurt H., D'Souza D.H. and Davidson P.M. (2014b) A comparison of the thermal inactivation kinetics

of human norovirus surrogates and hepatitis A virus in buffered cell culture medium. *Food Microbiol*ogy 42:212-217.

Bozkurt H., D'Souza D.H.and Davidson P.M. (2014c) Determination of thermal inactivation kinetics of hepatitis A virus in blue mussel (*Mytilus edulis*) homogenate. *Applied Environmental Microbiol*ogy 80(10):3191-3197.

Bozkurt H., D'Souza D.H. and Davidson P.M. (2015a) Thermal inactivation kinetics of human norovirus surrogates and hepatitis A virus in turkey deli meat. *Applied Environmental Microbiol*ogy 81(14):4850-4859.

Bozkurt H., D'Souza D.H., Davidson P.M. (2015b) Thermal inactivation of foodborne enteric viruses and their viral surrogates in foods. *Journal Food Protection* 78(8):1597-1617.

Bozkurt H, Ye X, Harte F, D'Souza DH, Davidson PM. (2015c) Thermal inactivation kinetics of hepatitis A virus in spinach. *International Journal of Food Microbiol*ogy 193:147-151.

Butot S., Putallaz T., Sanchez G. (2008) Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiol*ogy 126(1-2):30-35.

Calder L., Simmons G., *et al*. (2003) An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology and Infection* 131(1):745-751.

Cannon J.L., Papafragkou E., *et al*. (2006) Surrogates for the study of norovirus stability and inactivation in the environment: A comparison of murine norovirus and feline calicivirus. *Journal of Food Protection*. 69(11):2761-2765.

Chiapponi C., Pavoni E., *et al*. (2014) Isolation and genomic sequence of hepatitis A virus from mixed frozen berries in Italy. *Food and Environmental Virology* 6(3):202-206.

Colson P., Borentain P. *et al*. (2010) Pig liver sausage as a source of hepatitis E virus transmission to humans. *Journal of Infectious Diseases* 202(6):825-834.

Croci L., Suffredini E., *et al.* (2012) Detection of Norovirus and Feline Calicivirus in spiked molluscs subjected to heat treatments. *Food Control*. 25(1):17-22.

Crossan C., Baker P.J., *et al*. (2012) Hepatitis e virus genotype 3 in shellfish, United Kingdom. *Emerging Infectious Diseases* 18(12):2085-2087.

Dolin R., Blacklow N.R., *et al*. (1972) Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. *Proc. Soc. Exp. Biol. Med*. 140(2):578-583.

Doultree J.C., Druce J.D., *et al*. (1999) Inactivation of feline calicivirus, a Norwalk virus surrogate. *Journal of Hospital Infection* 41(1):51-57.

Duizer E., Schwab K.J., *et al*. (2004) Laboratory efforts to cultivate noroviruses. *Journal of General Virology*. 85(Pt 1):79-87.

Emerson S.U., Arankalle V.A. and Purcell R.H. (2005) Thermal stability of hepatitis E virus. *Journal of Infectious Disease*. 192(5):930-933.

Favero M. and Bond W. (1993) Disinfection and sterilization. In: Zuckerman A, Thomas H, editors. Viral hepatitis. London: Churchill Livingstone; p. 565–575.

Feagins A.R., Opriessnig T., *et al*. (2008) Inactivation of infectious hepatitis E virus present in commercial pig livers sold in local grocery stores in the United States. *International Journal of Food Microbiol*ogy 123(1-2):32-37.

Flannery J., Rajko-Nenow P., *et al*. (2014) Effectiveness of cooking to reduce norovirus and infectious F-specific RNA bacteriophage concentrations in Mytilus edulis. *Journal of Applied Microbiology* 117(2):564-571.

Garkavenko O., Obriadina A., *et al*. (2001) Detection and characterisation of swine hepatitis E virus in New Zealand. *Journal of Medical Virology* 65(3):525-529.

Gibson K. and Schwab K. (2011) Thermal inactivation of human norovirus surrogates. *Food and Environmental Virology* 3(2):74-77.

Greening G.E., Dawson J. and Lewis G. (2001) Survival of poliovirus in New Zealand green-lipped mussels, Perna canaliculus, on refrigerated and frozen storage. *Journal of Food Protection* 64(6):881-884.

Hewitt J. and Greening G.E. (2006) Effect of heat treatment on hepatitis A virus and norovirus in New Zealand greenshell mussels (Perna canaliculus) by quantitative real-time reverse transcription PCR and cell culture. *Journal of Food Protection* 69(9):2217-2223.

Hirneisen K.A. and Kniel K.E. (2013) Comparing human norovirus surrogates: murine norovirus and tulane virus. *Journal of Food Protection*. 76(1):139-143.

Koopmans M. and Duizer E. (2004) Foodborne viruses: an emerging problem. *International Journal of Food Microbiology* 90(1):23-41.

Lee S.J., Si J., *et al*. (2015) Effect of temperature and relative humidity on the survival of foodborne viruses during food storage. *Applied Environmental Microbiology* 81(6):2075-2081.

Mansuy J.M., Saune K. (2015) Seroprevalence in blood donors reveals widespread, multi-source exposure to hepatitis E virus, southern France, October 2011. *Eurosurveill*ance 20(19):27-34.

Meng X.J. (2013) Zoonotic and foodborne transmission of hepatitis E virus. *Seminars in Liver Dis*ease 33(1):41-49.

Millard J., Appleton H. and Parry J.V. (1987) Studies on heat inactivation of hepatitis A virus with special reference to shellfish. Part 1. Procedures for infection and recovery of virus from laboratorymaintained cockles. *Epidemiology and Infection*. 98(3):397-414.

Papafragkou E., Hewitt J., et al. (2013) Challenges of culturing human norovirus in three-dimensional organoid intestinal cell culture models. *PLoS One*. 8(6):e63485.

Park SY, Ha S-D. (2015) Thermal inactivation of hepatitis A virus in suspension and in dried mussels (*Mytilus edulis*). *International Journal of Food Science & Technology*. 50(3):717-722.

Said B., Ijaz S., *et al*. (2009) Hepatitis E outbreak on cruise ship. *Emerging Infectious Diseases* 15(11):1738-1744.

Scheid R., Deinhardt F., *et al*. (1981) Inactivation of hepatitis A and B and risk of iatrogenic spread. In: Szmuness W., Alter H., Maynard J., editors. Viral Hepatitis: International Symposium. Philadelphia: Franklin Institute Press; 1981. p. 627-628.

Simmons G., Garbutt C. *et al*. (2007) A New Zealand outbreak of norovirus gastroenteritis linked to the consumption of imported raw Korean oysters. *New Zealand Medical Journal* 120(1264):U2773.

Slomka M.J. and Appleton H. (1998) Feline calicivirus as a model system for heat inactivation studies of small round structured viruses in shellfish. *Epidemiology and Infection*. 121(2):401-407.

Smith A.J., McCarthy N., *et al*. (2012) A large foodborne outbreak of norovirus in diners at a restaurant in England between January and February 2009. *Epidemiology and Infect*ion 140(9):16951701.

Song Y.J., Park W.J., *et al*. (2014) Hepatitis E virus infections in humans and animals. *Clinical and Experimental Vaccine Reserch* 3(1):29-36.

Szabo K., Trojnar E., *et a*l. (2015) Detection of hepatitis E virus RNA in raw sausages and liver sausages from retail in Germany using an optimized method. *International Journal of Food Microbio*logy 215:149-156.

Tei S., Kitajima N., et al. (2003) Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* 362(9381):371-373.

Tian P., Yang D., *et al.* (2013) Inactivation of the Tulane virus, a novel surrogate for the human norovirus. *Journal of Food Prot*ection 76(4):712-718.

Verhoef L., Hewitt J., *et al*. (2015) Norovirus genotype profiles associated with foodborne transmission, 1999-2012. *Emerging Infectious Disease* 21(4):592-599.

Wang Q., Zhang Z. and Saif L.J. (2012) Stability of and attachment to lettuce by a culturable porcine sapovirus surrogate for human caliciviruses. *Applied and Environmental Microbiol*ogy 78(11):39323940.

Webby R.J., Carville KS., *et al*. (2007) Internationally distributed frozen oyster meat causing multiple outbreaks of norovirus infection in Australia. *Clinical Infectious Diseases* 44(8):1026-1031.

Wichmann O., Schimanski S., et al. (2008) Phylogenetic and case-control study on hepatitis E virus infection in Germany. *Journal of Infectious Diseases* 198(12):1732-1741.

Wilhelm B., Waddell L., *et al*. (2015) A scoping review of the evidence for public health risks of three emerging potentially zoonotic viruses: hepatitis E virus, norovirus, and rotavirus. *Preventative Vetinary Medicine* 119(1-2):61-79.

Williamson W.M., Ball A., *et al.* (2011) Enteric viruses in New Zealand drinking-water sources. *Water Science and Technology* 63(8):1744-1751.

C.3 MEAT DATA (SECTION 4.2)

Ahmed N.M., Conner D.E. and Huffman D.L. (1995) Heat-resistance of Escherichia coli O157:H7 in meat and poultry as affected by product composition. *Journal of Food Science* 60(3): 606-610.

Craven S.E. and Blankenship L.C. (1983) Increased heat resistance of *Salmonella* in beef with added soy proteins. *Journal of Food Protection* 46(5): 380-384.

Duffy G., Walsh C. *et al*. Survival of antibiotic resistant and antibiotic sensitive strains of *E. coli* O157 and *E. coli* O26 in food matrices. *International Journal of Food Microbiology* 109:179-186.

Fain A.R., Line J.E. *et al*. (1991) Lethality of heat to *Listeria monocytogenes* Scott A: D-value and zvalue determinations in ground beef and turkey. *Journal of Food Protection* 54(10):756-761.

Goodfellow S.J. and Brown W.L. (1978) Fate of *Salmonella* inoculated into beef for cooking. *Journal of Food Protection* 41(8):598-605.

Huang L. (2013) Determination of thermal inactivation kinetics of *Listeria monocytogenes* in chicken meats by isothermal and dynamic methods. *Food Control* 33:484-488.

Huang L. and Juneja V.K. (2003) Thermal inactivation of *Escherichia coli* O157:H7 in ground beef supplemented with sodium lactate. *Journal of Food Protection* 66(4):664-667.

Huang L. and Juneja V.K. (2001) A new kinetic model for thermal inactivation of microorganisms: Development and validation using *Escherichia coli* O157:H7 as a test organism. *Journal of Food Protection* 64(12):2078-2082.

Hwang C_A., Sheen S. and Juneja V.K. (2009) Effect of salt, smoke compound, and temperature on the survival of *Listeria monocytogenes* in Salmon during simulated smoking processes. *Journal of Food Science* 74(9):M522-M529.

Juneja V.K., Yadav A.S. *et al*. (2012) Kinetics of thermal destruction of *Salmonella* in ground chicken containing trans-Cinnamaldehyde and Carvacrol. *Journal of Food Protection* 75(2):289-296.

Juneja V.K. (2003) A comparative heat inactivation study of indigenous microflora in beef with that of *Listeria monocytogenes, Salmonella* serotypes and *Escherichia coli* O157:H7. *Letters in Applied Microbiology* 37:292-298.

Juneja V.K., Eblen B.S. and Marks H.M. (2001) Modeling non-linear survival curves to calculate thermal inactivation of *Salmonella* in poultry of different fat levels. *International Journal of Food Microbiology* 70:37-51.

Juneja V.K. and Eblen B.S. (2000) Heat Inactivation of *Salmonella typhimurium* DT104 in beef as affected

by fat content. *Letters in Applied Microbiology* 30:461-467.

Juneja V.K. and Marmer B.S. (1998) Thermal inactivation of *Clostridium perfringens* vegetative cells in ground beef and turkey as affected by sodium pyrophosphate. *Food Microbiology* 15:281-287.

Line J.E., Fain A.R. *et al*. (1991) Lethality of heat to *Escherichia coli* O157:H7: D-value and z-value determinations in ground beef. *Journal of Food Protection* 54(10): 762-766.

MacKey B.M., Pritchet C. *et al*. (1990) Heat resistance of *Listeria*: strain differences and effects of meat type and curing salts. *Letters in Applied Microbiology* 10:251-255.

McCann M.S., McGovern A.C. *et al*. (2006) Effects of storage and the presence of a beef microflora on the thermal resistance of *Salmonella typhimurium* DT104 in beef and broth systems. *Journal of Applied Microbiology* 102:1561-1569.

Murphy R.Y., Beard B.L. *et al*. (2004) Comparitive study of thermal inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ground pork. *Journal of Food Science* 69(4):97101.

Osaili T.M., Al-Nabulsi A.A. *et al*. (2013) Thermal inactivation of *Salmonella Typhimurium* in chicken shawira (gyro). *International Journal of Food Microbiology* 166:15-20.

Tuntivanich V., Orta-Ramirez A. et al. (2008) Thermal inactivation of *Salmonella* in whole muscle and ground turkey breast. *Journal of Food Protection* 71(12):2548-2551.

Velasquez A., Breslin T.J. *et al*. (2010) Enhanced thermal resistance of *Salmonella* in marinated whole muscle compared with ground pork. *Journal of Food Protection* 73(2):372-375.

C.4 EGG DATA (SECTION 4.3)

Corry J.E.L. and Barnes E.M. (1968) The heat resistance of *Salmonellae* in egg albumen. *British Poultry Science* 89:253-260.

Favier G.I., Escudero M.E. and De Guzman A.M.S. (2008) Thermal inactivation of *Yersinia enterocolitica* in liquid egg products. *Journal of Food Safety* 28:157-169.

Garibaldi J.A., Straka R.P. and Ijichi K. (1969) Heat resistance of Salmonella in various egg products. *Applied Microbiology* 17(4):491-496.

Jin T., Zhang H. *et al.* (2008) Thermal resistance of *Salmonella enteritidis* and *Escherichia coli* K12 in liquid egg determined by thermal-death-time disks. Journal of Food Engineering 84:608-614.

Michalski C.B., Brackett R.E. *et al*. (1999) Use of capillary tubes and plate heat exchanger to validate U.S. Department of Agriculture Pasteurisation Protocols for elimination of *Salmonella Enteritidis* from liquid egg products. *Journal of Food Protection* 62(2):112-117.

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