# EFFECT OF LOW TEMPERATURE ON

# *CAMPYLOBACTER* ON POULTRY MEAT

**August 2005**

Prepared as part of a New Zealand Food Safety Authority
contract for scientific services

by

R J Whyte

J A Hudson

and

N J Turner

**Institute of Environmental Science & Research Limited**
**Christchurch Science Centre**
**Location address**: 27 Creyke Road, Ilam, Christchurch
**Postal address**: P O Box 29 181, Christchurch, New Zealand
**Website:** www.esr.cri.nz

A CROWN RESEARCH INSTITUTE

Client Report
FW0593

# EFFECT OF LOW TEMPERATURE ON CAMPYLOBACTER ON POULTRY MEAT

August 2005

Kevin Taylor
Food Safety Programme Leader

Rosemary Whyte
Project Leader

TeckLok Wong
Peer Reviewer

**DISCLAIMER**

This report or document ("the Report") is given by the Institute of Environmental Science and Research Limited ("ESR") solely for the benefit of the New Zealand Food Safety Authority, District Health Boards and other Third Party Beneficiaries as defined in the Contract between ESR and the New Zealand Food Safety Authority, and is strictly subject to the conditions laid out in that Contract.

Neither ESR nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for use of the Report or its contents by any other person or organisation.

**ACKNOWLEDGMENT**

The authors would like to thank the Poultry Industry for their valuable contribution to this project.

**CONTENTS**

## LIST OF TABLES



## LIST OF FIGURES

# 1 INTRODUCTION

Campylobacteriosis is the most frequently reported gastrointestinal illness in New Zealand. Because of the common perception that it is "foodborne" in origin, particularly through poultry meat, it ranks highly for NZFSA risk management action. While adequate cooking of food is the most important means of control, control during farming or processing is recognised as important for the reduction of exposure to the hazard by the consuming population.

The main objective of this work was to identify means that could be employed to minimise risk and reduce the burden of campylobacteriosis in the New Zealand population by reducing the numbers of *Campylobacter* on fresh poultry meat. An assessment was to be made of the effectiveness of temperature controls by freezing or chilling in the reduction of *Campylobacter* numbers achieved under standard industry practice, and under potential new chilling regimes.

The information from this project will feed into a risk model for *Campylobacter* in poultry meat and assist the assessment of risk management options undertaken using the quantitative risk model.

This report is supplied in two parts; firstly an overview of the work that was carried out, the New Zealand legislation concerning freezing and the effects of freezing on bacteria. Secondly there is a draft paper written in the style of the Journal of Food Protection. Since it is in a scientific paper format it is written in a concise manner, and so extra detailed data are included as appendices.

Project work was carried out as follows:

- A literature review was undertaken to define “freezing” under NZ law and in scientific terms; An assessment was made in regard to the NZ legislation concerning the definition of freezing and related to the scientific parameters concerning chilling and freezing (presented in the body of the report)

- A survey was conducted to measure the effect of current “crust freezing” techniques used by industry on surface numbers of *Campylobacter* (data presented in the scientific paper draft)

- Experiments were carried out to determine the effect of freezing temperatures on the reduction of *Campylobacter* numbers (data presented in the scientific paper draft)

## 2 ANALYSIS OF LEGISLATION AND LITERATURE

This review includes New Zealand legislation concerning the definition of freezing and regulations pertaining to chill conditions for meat storage. Scientific data are presented that describe the events that occur during the freezing of meat and how this relates to bacterial survival. Additional sections address the survival of *Campylobacter* during freezing and frozen storage, and also at chill temperatures.

### 2.1 New Zealand Regulations: Freezing and Chilling

The legal definitions of the temperatures involved in freezing and chilling are encompassed in the *Food Hygiene Regulations (1974).*

Under Part 2 (Conduct and maintenance of food premises), regarding food storage:

(d) All food to be sold by retail in a frozen condition shall, —

(i) Before being displayed for sale, have been maintained in a wholesome condition at or below a temperature of -18°C; and

(ii) While being displayed for sale, be maintained in a wholesome condition at or below a temperature of -12°C, —

and shall not at any time have been refrozen after thawing.

Part 8 (Meat and fish) regulates the storage of meat and fish in an unfrozen state:

> (44)(1)(a) All meat and fish shall be stored, as soon as practicable after delivery and when not being processed, at a temperature below 2°C in the room or cabinet required by regulation 14 of these regulations, and shall at all times be protected from contamination.
>
> (46)(2)(a) All meat or fish, when not being prepared or displayed for sale, shall be stored at a temperature below 2°C, or, in the case of shellfish in shells, below 10°C, in the room or cabinet required by regulation 14 of these regulations.
>
> (46)(2)(c) All meat or fish set out for individual selection by customers shall be pre-wrapped in suitable, durable wrappers of sufficient weight and strength to resist tearing and puncturing, so as to completely enclose the meat or fish, and to provide adequate protection from contamination, and shall be kept at a temperature below 2°C in the room or cabinet required by regulation 14 of these regulation.
>
> (46)(3) No person shall display or expose, or cause or permit to be displayed or exposed, any meat or fish for retail sale, for any period exceeding 12 hours, except in a refrigerated cabinet or display unit at the temperature not exceeding 13°C in the case of meat and 7°C in the case of fish.

In summary, frozen foods are required to be stored at -18°C, but may be stored at -12°C when being presented for sale. Fresh meat is required to be kept at 2°C, unless it is being displayed to retail sale (not for individual customer selection) in which case the storage temperature may be allowed to rise to 13°C.

## 2.2 Physical Effects of Freezing on Meat

During freezing, free water in foods is converted to ice. The temperature at which water starts to freeze depends on the concentration of solutes in the water, such as proteins and carbohydrates, that are associated with the food (Gill, 2002). Meat begins to freeze at –1.5°C and about half the liquid in meat is frozen at –2.5°C (Roberts *et al*., 1998). Usually there is a rapid increase in the ice fraction (the proportion of total water that has formed ice crystals) within a few degrees, but beyond this large decreases in temperature are needed to continue development of the ice fraction. For example, between 0 and -5°C the ice fraction in meat increases to 74%. At -10°C the ice fraction makes up 83%, and at -20°C it reaches 88% (Gill, 2002). At -40°C, meat is considered to be totally frozen, yet around 10% of the water still remains unfrozen and is usually associated with structural proteins (Farkas, 1997; Gill, 2002). Fresh meat has a water activity ($a_w$) of 0.99, and this declines with freezing as the ice fraction develops (Figure 1) (Ayres *et al*., 1980).

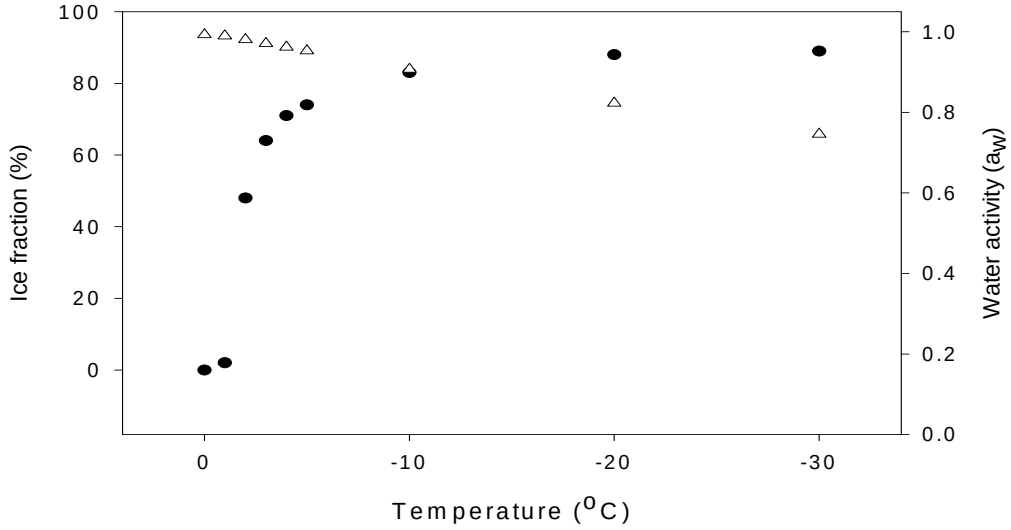



**Figure 1: Change in ice fraction of total water (circles) and water activity (triangles) in muscle tissue with decreasing temperature. Data from Riedel (1957) and Leistner *et al.* (1981)**

## 2.3 Effects of Freezing on Bacteria

While freezing and frozen storage has some impact on bacteria, frozen foods are not sterile and are not sterilised by prolonged freezing (Ayres *et al*., 1980). Survival of bacteria on meat is related to the rate of freezing. Slow freezing has been shown to be more lethal than rapid freezing (Ayres *et al*., 1980). During slow freezing (e.g. a reduction of 1°C/min), most microorganisms move into the unfrozen fraction of water in the food (Gill, 2002). As extracellular ice forms in this fraction, the solutes become more concentrated in the unfrozen water. This causes increased water loss from the bacterial cells and exposes them to osmotic stress over a prolonged period (Farkas, 1997). Osmotic stress causes a change in the intracellular pH and ionic strength, which inactivates enzymes, denatures other proteins, and subsequently interferes with metabolic processes. The membranes and membrane transport

systems may also be irreparably damaged, and the bacteria can become more sensitive to oxidative stress (Farkas, 1997).

An increase in the freezing rate can increase survival of microorganisms by reducing the period over which they are exposed to osmotic stress. Additionally, depending on the chemistry and concentration of solutes in unfrozen water, an increase in the freezing rate can cause the solutes to freeze with the water (i.e. freeze as a solution). This reduces the amount of osmotic stress microorganisms are exposed to from the remaining unfrozen water fraction. Very high rates of freezing (in excess of 10°C/min) decrease the survival of bacteria by inducing the formation of intracellular ice crystals that can cause mechanical damage to the cell. This rate of freezing is very difficult to achieve in food processing (Farkas, 1997; Gill, 2002). Mechanical damage by extracellular ice formation can occur independent of the rate of freezing, but the extent of bacterial inactivation mediated by this method appears to be limited (Gill, 2002).

Survival of bacteria during freezing may also be enhanced by the presence of cryogenic solutes such as glycerol or glycine, or by compounds that decrease the freezing point of foods, such as salt. Other compounds, such as lactic acid in meat, may increase freezing injury, particularly during slow freezing. The growth phase of the bacterium will also influence freezing survival. Bacteria in the exponential phase are more sensitive to freezing compared to those in the stationary phase (Gill, 2002).

The number of viable bacteria tends to decline with prolonged frozen storage, although there is usually some stabilisation after a few months where further reduction is minimal. The species of bacteria present in the frozen product depends on the initial population (Gill, 2002). Some are killed, while others are only sublethally damaged and can recover upon thawing, particularly if frozen storage is above -10°C (below -10°C sublethally damaged bacteria tend to die over time, hence the recommendation that frozen meat be stored at or near -18°C). Usually the process of freezing, rather than frozen storage, is more lethal to bacteria (Roberts *et al*., 1998).

In general, Gram-negative bacteria are more susceptible to freezing injury than Gram-positive organisms (Gill, 2002). *Campylobacter* is especially sensitive to freezing, though there appears to be some variation in freezing tolerance between strains of *C. jejuni* (Roberts *et al*., 1998; Archer, 2004). The freezing of carcasses from poultry flocks that test positive for *Campylobacter* is mandatory in some countries, and has been shown to reduce the risk of campylobacteriosis (Archer, 2004). *Salmonella* is reasonably tolerant of freezing, though may be reduced in number during frozen storage. *Salmonella* Typhimurium was stable when frozen on fish or meat around -20°C (Archer, 2004). *Escherichia coli* O157:H7, *Listeria monocytogenes* and the spores of *Clostridium perfringens* are all able to survive freezing (Roberts *et al*., 1998).

## 2.4 Survival of *Campylobacter* During Freezing

Many studies have demonstrated that freezing and frozen storage reduce the survival of *Campylobacter* on meat. Zhao *et al*. (2003) inoculated three isolates of *C. jejuni* on to chicken wings, which were frozen at -20°C or -30°C for 72 hours. Freezing chicken to -20°C and -30°C for 30 minutes had a minimal effect on *C. jejuni* survival, but over 72 hours both

treatments reduced the number of *C. jejuni* recovered (by 1.3 $\log_{10}$ CFU/g at -20°C and 1.8 $\log_{10}$ CFU/g at -30°C). Long-term freezing (52 weeks) of the inoculated chicken wings at -20°C decreased *C. jejuni* by 3 $\log_{10}$ CFU/g after 8 weeks. Beyond this the counts remained between 3 and 4 $\log_{10}$ CFU/g less than the original inoculum. Long-term freezing at -86°C caused only a small decrease of around 0.5 $\log_{10}$ CFU/g from the original inoculum. These results indicate that short-term freezing can reduce the loading of *C. jejuni*, and frozen storage for 8 weeks or longer at -20°C can increase inactivation. Freezing at -86°C seems to preserve *C. jejuni*. In a final experiment, inoculated chicken wings were super-chilled in temperatures between -80°C and -196°C until the internal portions of wings reached -3.3°C but were not frozen. Other than the treatment at -196°C, which involved immersion of the chicken (in bags) in liquid nitrogen, super-chilling did not induce a reduction in *C. jejuni* exceeding 1 $\log_{10}$. Super-chilling at -196°C caused a reduction of 2.4 $\log_{10}$ CFU/g (Zhao *et al*., 2003).

A similar reduction in viable bacteria was seen on raw irradiated chicken skin inoculated with *C. coli* and frozen at -20°C for 48 hours. Several other temperatures were also assessed, and the greatest reduction was seen at -20°C, declining by $1.70 \pm 0.15$ $\log_{10}$ CFU/cm$^2$ (Thurston Solow *et al*., 2003). Whole chicken carcases collected from a factory were halved and the *C. jejuni* enumerated on one half fresh, while the other half was frozen at -15°C for 14 days, then thawed at 5°C overnight followed by 3 hours at room temperature before enumeration. The freeze/thaw treatment reduced the viable *C. jejuni* in two lots of chickens by 70% and 80% (from a mean number of *C. jejuni* of 340 CFU per fresh carcass half to 2 CFU per treated carcass half, n=30). It was likely that the reduction was caused more by injured cells rather than by inactivation, as by including an enrichment step in the enumeration, smaller reductions of around 50% and 30% were determined for the two chicken lots. The enrichment step may allow the cells to repair and/or proliferate (Stern *et al*., 1985).

*C. jejuni* was inoculated into raw and cooked ground beef, ground chicken and ground cod, then frozen at -18°C. The reduction of *C. jejuni* was similar between the raw and cooked products and all three meats. A large decline was detected over the first 24 hours (1 to 1.5 $\log_{10}$ CFU/g), and the number of cells recovered levelled off at this concentration for the remaining four days of the experiment. The addition of salt at 1% or 2% did not have an effect at this temperature (Abram & Potter, 1984). When inoculated into ground beef, the concentration of two isolates of *C. jejuni* slowly declined over a period of 90 days when stored at -18°C. The samples were initially blast frozen before storage, so this may account for the slow decline, rather than a rapid decrease upon freezing as seen in other work. There was also some suggestion that incorporation of the bacteria among the cells of the ground beef offers some cryoprotection. The estimated rate of reduction per day was between 8.61 and 21.91%, with a total loss of between 4.4 and 5-$\log_{10}$ after 90 days (Grigoriadis *et al*., 1997).

Inoculation of *C. jejuni* on to beef strips and subsequent frozen storage at -18°C caused a rapid reduction of these bacteria by 1 to 2-log within the first week (Moorhead & Dykes, 2002) or two weeks (Gill & Harris, 1982), with little subsequent change over the following days or months. Increasing the pH of the beef increased survival of *C. jejuni*, causing a total reduction of around 1 $\log_{10}$ bacteria/cm$^2$ over 30 days frozen storage compared to a reduction of just over 2 $\log_{10}$ bacteria/cm$^2$ on meat at normal pH (Gill & Harris, 1982).

In summary, these studies indicate that freezing will reduce, but not eliminate *Campylobacter* from meat, and frozen storage can further reduce the concentration of bacteria. There is some evidence of variability between isolates of *Campylobacter* with regard to their ability to survive freezing. A number of *C. jejuni* isolates from poultry and human clinical samples were tested for survival in various media at -20°C by Chan *et al*. (2001). The recovery of all isolates reduced upon freezing and subsequent frozen storage, with the extent dependent on the media used, but there were differences in the rate of decline between isolates in the same medium. Some isolates had a slow rate of decline over time, and at the other extreme one isolate was no longer detectable after 18 days. The clinical isolates tended to decline the slowest and also showed more resilience to cold when stored at 4°C. Potentially, the cooling of poultry might select for those that are more cold tolerant, and these may constitute the majority of the inoculum that reaches consumers and so relevant to human exposure (Chan et al., 2001).

## 2.5 *Campylobacter* Survival Under Chilling

There is convincing evidence that *C. coli* and *C. jejuni* survive better on meat under refrigeration temperatures than at ambient temperatures. Various isolates of *C. jejuni* inoculated into ground chicken declined to a greater extent over 17 days when stored at 23°C (reduction of 2.5-5 $\log_{10}$ CFU/g) compared to storage at 4°C (1-2 $\log_{10}$ CFU/g) (Blankenship & Craven, 1982). Survival on chicken is increased by the introduction of a micro-aerobic atmosphere (Blankenship & Craven, 1982; Grigoriadis *et al*., 1997). Both *C. jejuni* and *C. coli* survived better on chicken skin when stored over 48 hours at 4°C than at higher temperatures (25, 37 and 42°C) (Solow *et al*., 2003). There is large variability in cold tolerance between isolates of *C. jejuni*, and there is some evidence that clinical isolates tend to survive refrigeration temperatures better than those obtained from poultry (Chan *et al*., 2001).

## 2.6 Conclusions

Freezing does not sterilise meat products, but reduces the initial bacterial loading. Bacteria are inactivated during freezing primarily by osmotic stress, and to a lesser extent by the formation of extracellular ice crystals. Faster freezing rates tend to increase bacterial survival, with the exception of very rapid freezing, which may cause formation of intracellular ice crystals. Most reduction of *Campylobacter* occurs during the freezing process, but during frozen storage the decline in viable bacteria continues, although a stable level may be reached. *Campylobacter* appear to be reasonably tolerant of chilling temperatures, though there is marked variability in cold-tolerance between isolates which is evident during both chilling and freezing.

Greater reduction or control of *Campylobacter* during poultry processing operations could be achieved through optimisation of the freezing temperature and rate.

## 2.7 References


Abram, D.D. & N.N. Potter (1984) Survival of *Campylobacter jejuni* at different temperatures in broth, beef, chicken and cold supplemented with sodium chloride. *Journal of Food Protection* 47: 795-800.

Archer, D.L. (2004) Freezing: an underutilized food safety technology? *International Journal of Food Microbiology* 90: 127-138.

Ayres, J.C., Mundt J.O. & Sandine W.E. (1980) *Microbiology of foods*. San Francisco: W.H. Freeman and Company.

Chan, K.F., H.L. Tran, R.Y. Kanenaka & S. Kathariou (2001) Survival of clinical and poultry-derived strains of *Campylobacter jejuni* at a low temperature (4°C). *Applied and Environmental Microbiology* 67: 4186-4191.

Farkas, J. (1997) Physical methods of food preservation. In M.P. Doyle, L.R. Beuchat & T.J. Montville, *Food Microbiology: Fundamentals and frontiers*. Washington D.C: ASM Press, pp. 497-519.

Gill, C.O. (2002) Microbial control with cold temperatures. In V.K. Juneja & J.N. Sofos, *Control of foodborne microorganisms*. New York: Marcel Dekker, Inc. pp. 55-74.

Gill, C.O. & L.M. Harris (1982) Survival and growth of *Campylobacter fetus* subsp. *jejuni* on meat and in cooked foods. *Applied and Environmental Microbiology* 44: 259-263.

Grigoriadis, S.G., P.A. Koidis, K.P. Vareltzis & C.A. Batzios (1997) Survival of *Campylobacter jejuni* inoculated in fresh and frozen beef hamburgers stored under various temperatures and atmospheres. *Journal of Food Protection* 60: 903-907.

Leistner, L., W. Rodel & K. Krispien (1981) Microbiology of meat and meat products in high and intermediate moisture ranges. In L.B. Rockland & G.F. Stewart (eds.), *Water activity: Influences on food quality*. London: Academic Press, pp. 885-916.

Moorhead, S.M. & G.A. Dykes (2002) Survival of *Campylobacter jejuni* on beef trimmings during freezing and frozen storage. *Letters in Applied Microbiology* 34: 72-76.

Riedel, L. (1957) Kalorimetrische untersuchungen über das gefrieren von fleisch. *Kaltetechnik* 9: 38-40.

Roberts, T.A., J.I. Pitt, J. Farkas & F.H. Grau (eds.) (1998) *Microorganisms in foods. 6: Microbial ecology of food commodities*. International Commission on Microbiological Specifications for Foods. London: Blackie Academic & Professional.

Stern, N.J., P.J. Rothenberg & J.M. Stone (1985) Enumeration and reduction of *Campylobacter jejuni* in poultry and red meats. *Journal of Food Protection* 48: 606-610.

Thurston Solow, B., O.M. Cloak & P.M. Fratamico (2003) Effect of temperature on viability of *Campylobacter jejuni* and *Campylobacter coli* on raw chicken or pork skin. Journal of Food Protection 66: 2023-2031.

Zhao, T., G.O.I. Ezeike, M.P. Doyle, Y.-C. Hung & R.S. Howell (2003) Reduction of *Campylobacter jejuni* on poultry by low-temperature treatment. *Journal of Food Protection* 66: 652-655.

## 3 DRAFT SCIENTIFIC PAPER

TITLE OF PAPER: The effect of low temperature on *Campylobacter* on poultry meat

AUTHORS: R. J. Whyte[1], J. A. Hudson[1], N. J. Turner[1] and R. L. Cook[2]

[1]Food Safety Programme
Institute of Environmental Science and Research Limited
ESR Christchurch Science Centre
27 Creyke Road, PO Box 29-181, Ilam, Christchurch, New Zealand
[2] New Zealand Food Safety Authority
PO Box 2835, Wellington, New Zealand

RUNNING HEAD: Low temperature effect on Campylobacter
KEY WORDS: *Campylobacter*, freezing, poultry

[*] Author for correspondence: Tel. +64-3-351-6019; Fax +64-3-351-0010; E-mail: andrew.hudson@esr.cri.nz

ABSTRACT

The effect of poultry crust freezing (lowering the temperature to –2°C, holding for 150 minutes and then equilibrating to 2°C) on the survival of *Campylobacter* was assessed. Naturally-occurring *Campylobacter* was measured on chicken portions obtained prior to, and following crust freezing. The results showed no significant change in the levels of *Campylobacter* through the crust freezing process practiced by two companies, although the rejection of some data exceeding the maximum level estimated by the MPN meant that the dataset available for analysis was small.

Nine isolates of *Campylobacter jejuni* were chilled in sterile chicken drip to five final temperatures ranging from –2 to –10°C. No significant differences were noted between the nine isolates; no change in numbers occurred with chilling and there was no evidence for cellular injury. A cocktail of three *C. jejuni* isolates was inoculated onto the skin of chicken portions, and chilled to –2 or –10ºC under two different cooling profiles. The final count on chicken portions chilled to –2°C did not differ from the pre-cooling count. When chilled to –10°C an approximate 1 $\log_{10}$ difference in counts could be measured, with the most likely reason being the time for which the samples were frozen (around 19 h compared to 4 h at –2°C).

Crust freezing as currently practiced is not reducing the number of *Campylobacter* on fresh poultry. There is potential for manipulation of chilling conditions to achieve such an aim but legal and practical reasons would currently prevent this from occurring.

There is potential for using freezing as a means of reducing the numbers of *Campylobacter* on chicken meat. However, for this to be an available option for industry it is necessary to understand the behaviour of *Campylobacter* under such conditions.

Many studies have reported the effect of freezing and frozen storage on the survival of *Campylobacter* on meat. When inoculated onto chicken wings and frozen at -20°C or -30°C for 72 h the numbers of *C. jejuni* were reduced by 1.3 $\log_{10}$ CFU $g^{-1}$ and 1.8 $\log_{10}$ CFU $g^{-1}$ respectively *(18)*. Freezing for 52 weeks at -20°C resulted in a decrease of 3 $\log_{10}$ CFU $g^{-1}$ over the first 8 weeks, but thereafter the counts remained between 3 and 4 $\log_{10}$ CFU $g^{-1}$ less than the inoculum. In a final experiment, inoculated chicken wings were super-chilled in temperatures between -80°C and -196°C until the internal temperature reached -3.3°C but were not frozen. Other than the treatment at -196°C which resulted in a reduction of 2.4 $\log_{10}$ CFU $g^{-1}$, super-chilling did not result in a reduction exceeding 1 $\log_{10}$.

A similar reduction in counts was seen on raw irradiated chicken skin inoculated with *C. coli* and frozen at -20°C for 48 hours. Several other temperatures were also assessed, and the greatest reduction was seen at -20°C, declining by 1.70 ± 0.15 $\log_{10}$ CFU/$cm^2$ *(15)*. Whole chicken carcasses were halved and *C. jejuni* enumerated on one half, while the other half was frozen at -15°C for 14 days, then thawed at 5°C overnight followed by 3 hours at room temperature before enumeration. The freeze/thaw treatment reduced the prevalence of viable *C. jejuni* in two lots of chickens by 70% and 80%, and the mean number reduced from 340 CFU to 2 CFU per treated carcass half (n=30). It is likely that the reduction was caused more by injury to cells rather than by inactivation, as by including enrichment in the enumeration, smaller reductions of around 50% and 30% resulted. The enrichment step may allow the cells to repair and/or proliferate *(16)*.

*C. jejuni* was inoculated into raw and cooked ground beef, ground chicken and ground cod, then frozen at -18°C. The reduction of *C. jejuni* was similar in each case, with most of the decline occurring over the first 24 h (1 to 1.5 $\log_{10}$ CFU/g), and the number remaining at this concentration for the next four days *(1)*. A difference has been noted in the reduction of numbers mediated by freezing at -20°C on chicken skin compared to ground chicken *(2)*, with around twice the reduction occurring in ground chicken (1.38-3.39 $\log_{10}$) compared to chicken skin (0.56-1.57 $\log_{10}$).

When inoculated into ground beef, the concentration of two isolates of *C. jejuni* slowly declined over a period of 90 days when stored at -18°C. The samples were initially blast frozen before storage, so this may account for the slow decline, rather than a rapid decrease upon freezing as seen in other work. The estimated rate of reduction per day was between 8.6 and 21.9%, with a total loss of between 4.4 and 5-$\log_{10}$ after 90 days *(9)*.

Inoculation of *C. jejuni* on to beef strips and subsequent frozen storage at -18°C caused a rapid reduction in numbers of 1 to 2-log within the first week *(12)* or two weeks *(8)*, with little subsequent change. On high pH beef, survival of *C. jejuni* was increased, with a reduction of around 1 $\log_{10}$ $cm^{-2}$ over 30 days compared to a reduction of just over 2 $\log_{10}$ $cm^{-2}$ on meat at normal pH *(8)*.

A recent survey showed there was no initial decrease in *Campylobacter* on chicken portions after 2 days of freezing to –20°C while numbers had reduced by 1-log after 10 days and 2-logs after 21 days (*13*). After 21 days freezing, 80% of samples were still positive by qualitative culture.

There is some evidence of variability between *Campylobacter* isolates in regard to their ability to survive freezing. A number of *C. jejuni* isolates from poultry and human clinical samples were tested for survival in various media at -20°C *(4)*. The number of all isolates reduced, with the extent dependent on the media used, but there were differences in the rate of decline between isolates in the same medium. The clinical isolates tended to decline the slowest and also showed more resilience to cold when stored at 4°C. Potentially, the cooling of poultry might select for strains that are more cold tolerant, and these may constitute the majority of the inoculum that reaches the consumer.

These studies indicate that freezing will reduce, but not eliminate, *Campylobacter* from meat, and frozen storage can further reduce the concentration of bacteria. The work described here sought to determine the effect of "crust freezing", a process currently being used by the New Zealand poultry industry, on numbers of *Campylobacter* present on chicken and to examine the effects of freezing to temperatures somewhat lower than those currently used in this process.

The current industry practice of "crust freezing" uses a reduction from 0 to -2°C over 110 min, followed by holding for 150 min. Product is then allowed to come up to 2°C over the following 24 h. This is applied to boxed chicken pieces and so there is a gradient of temperatures within the box that can range from 0 to -5°C, depending on the location of the portion within the box. The practice was developed to extend shelf life rather than for the purpose of reducing numbers of *Campylobacter*.

## MATERIALS AND METHODS

**Survey of "Crust Freezing".** This survey measured the effect of "crust freezing" techniques used by industry on surface numbers of *Campylobacter* on chicken portions at two different poultry processors (companies A and B), one in the North Island, and the other in the South island. Portions of fresh thigh meat with skin on and bone in were sampled on 5 different occasions from each of two poultry processors (100 samples in total). On each sampling occasion 5 portions were taken before they entered the crust-freezing phase and 5 portions taken the following day after crust freezing, storage and equilibration back to chilled storage temperature.

Each sample was placed into a sterile Whirlpak bag and 100 ml of Exeter broth added *(11)* which had been modified *(17)*. After massaging the sample to dislodge bacterial cells adhering to the surface, portions of broth were removed to set up a 3 row MPN series in the same medium. The first batch of samples was tested using 10 ml, 1 ml and 0.1 ml inoculum volumes, but this was adjusted after the first sampling week to 1 ml, 0.1 ml and 0.01 ml inoculum volumes to increase the sensitivity of the enumeration. Tubes were incubated under 10% $CO_2$ for 48 h (4 h at 37°C then 42°C) and streaked to Exeter agar (incubated at 42°C in 10% $CO_2$ for 48 h). Isolates were identified using PCR as previously described *(17)*.

The Whirlpak bag containing the remaining broth and chicken thigh sample was incubated and streaked to m-Exeter agar as above. One presumptive *Campylobacter* colony was picked from the m-Exeter agar plate from each sample, purified on Columbia Blood (CB) agar and confirmed using PCR.

**Effect of Freezing Temperature on Survival *in vitro*.** Laboratory experiments were conducted to assess the effects of freezing on *C. jejuni* over temperatures in the range of -2 to -10°C. Nine *Campylobacter* isolates (three from chickens, three from human cases and three of types isolated from human cases and chickens) were grown in m-Exeter broth *(17)* for 48 h, harvested and re-suspended in sterile chicken drip *(3)*. Prior to conducting cooling experiments, the viable counts of the stock cultures were determined, and volumes of appropriate chicken drip dilutions pipetted into a series of sterile tubes which were placed in a programmable refrigerated incubator. Samples were exposed to a range of time/temperature combinations to determine the effect of freezing temperature on *Campylobacter* numbers, and the temperature of the samples monitored with data loggers (ThermoChron iButtons DS1921L-F52, temperature range -20°C to +85°C). After incubation, samples were serially diluted and enumerated on modified Exeter agar *(17)* and CB agar containing 5% sheep blood. CB agar was used to determine the proportion of injured cells following chilling. While these experiments were not replicated the results obtained for the nine isolates at five temperatures indicate that the observations are repeatable.

**Effect of Freezing to –2 and –10°C on *C. jejuni* on chicken.** Additional experiments were performed on chicken breasts chilled at rates of -0.018°C $min^{-1}$ (to mimic industry conditions) and -0.009°C $min^{-1}$ to -2 and -10°C. Three *C. jejuni* isolates were grown in m-Exeter broth, combined into a cocktail and diluted in sterile peptone and a count of the inoculum made on CB agar plates. Chicken breasts with skin on were purchased and two 2x2 $cm^2$ areas marked using sterile pins prior to chilling at 2°C for at least 2 h. Following this a data logger was placed under the skin of the chicken breast and one square was inoculated with 50µl *C. jejuni* while the other had a similar volume of sterile peptone applied. The chicken portions were held for an additional 30 minutes at 0°C to allow bacterial attachment. In each experiment three chicken breasts were removed at this point and enumeration of *C. jejuni* performed on the marked areas by excising the marked skin, adding it to 5 ml of peptone in a sterile bag and homogenising for 1 minute in a Colworth 400 stomacher (A.J. Seward, London, England). The remaining samples were frozen under the conditions described above and *C. jejuni* present on excised skin samples enumerated as described above. These experiments were performed in triplicate.

**Statistical Analysis.** To assess whether the data from companies A and B could be combined, an exploratory data approach was undertaken. Initial analysis revealed a difference in variance within the groups. The T-test and F-test were applied to the data groups, both as individual groups and as grouped pairs, i.e. pre-chill and post-chill. The mean and variance of each group of 5 data points was calculated and the bias corrected Bartlett's test for equality of variances applied. The test is very sensitive to departures from normality. The assumption was made that the means of the observations (data groups) are normally distributed.

RESULTS

**The Effect of Crust Freezing.** Of the one hundred chicken thigh samples tested, all (100%) were positive for *Campylobacter* (Table 1). Using PCR to identify presumptive *Campylobacter* isolates, 99 were confirmed as *C. jejuni* and one as *C. coli*. The MPN values obtained for pre-crust freezing portions ranged from <30 to >11,000 MPN *Campylobacter*

per sample. Nine samples (18%) had counts greater than or equal to 11,000 MPN/sample, sixteen (32%) contained 1000 to 11,000 MPN/sample, eighteen (36%) 100 to 1000 MPN/sample and seven (14%) had less than 100 MPN/sample. Portions tested after crust-chilling had counts that ranged from 40 to >11,000 MPN *Campylobacter*/sample. Three post-chill samples (6%) had counts of greater than or equal to 11,000 MPN/sample, thirteen (26%) contained 1000 to 11,000 MPN/sample, twenty five (50%) had 100 to 1000 MPN/sample and nine (18%) had less than 100 MPN/sample.

Data recorded as "<" or ">" were a problem as the approach of halving or doubling the values respectively skews the results. For example values of >11,000, assumed to be 22,000 included in analyses involving otherwise much smaller numbers would result in a disproportionate contribution to the mean of any data set. The ">11,000" values were therefore excluded from the analysis.

Bartlett's test showed that the individual data subsets could not be combined as they are drawn from different distributions, i.e. not normal distributions. However the F and T tests give an indication of the probability that the data from samples obtained prior to and after crust freezing are different, and therefore by implication changed by the process. These data are shown in Tables 2 and 3.

The values obtained in the T test indicate that for company A the values between the pre and post chill samples were not likely to have been different overall, whereas the generally lower values for company B indicates that the data sets may have been different (i.e. there was an effect). However, in two from three instances the direction was an increase in numbers from pre to post crust freezing. For the F tests the probabilities were low indicating that there was no significant affect on numbers mediated by the crust freezing process.

***In vitro.*** Only when the final temperature achieved was -8 or -10°C did freezing of the chicken drip occur. No significant effect could be measured on any of the nine isolates when they were brought from 2°C to -2, -4, -6, -8 or -10°C, held for 2.5 h at this temperature and then re-equilibrated to 2°C over a further 2 h. Example data for freezing to -10°C are shown in Figure 1. In addition there were no differences in the counts obtained on m-Exeter or CB agars, indicating that cellular injury had not occurred.

**Inactivation on chicken breast portions**. When chilled to -2°C there was no significant difference noted between the counts made before and after freezing for samples cooled at the industry rate or half of that rate. However, the surface temperatures of the chicken portions were very similar and close to the desired slower cooling rate of -0.009°C min-1 at sub-zero temperatures (data not shown). Freezing did not occur.

When the final temperature achieved was -10°C, however, differences of approximately 1 $\log_{10}$ unit between the pre- and post-freezing populations were noted (Figure 2). The difference in numbers was similar for portions cooled at either rate. However, the actual rate of cooling achieved (Figure 3) at the surface of the chicken portions was not close to the desired cooling curves. These samples did freeze at the surface.

## DISCUSSION

Campylobacteriosis remains a significant enteric disease for New Zealand, resulting in a high proportion of the costs of enteric disease for the country *(14)*. The work presented here forms part of a co-ordinated programme of work aimed at identifying transmission routes of the disease alongside their relative importance to allow interventions to be prioritised. A component of this programme is the production of a quantitative risk model for *Campylobacter* in poultry, the construction of which has identified some data gaps that need to be filled so that the model is able to represent the farm to fork continuum as best it can. Two data gaps identified in the modelling process include determining the numbers of *Campylobacter* on chickens arriving at the slaughter plant, which will be reported elsewhere, and the effect of crust freezing applied after spin chilling and prior to retail distribution.

Chicken portions tested in this survey were obtained from two poultry processing plants, one in the North and the other in the South Island of New Zealand. All portions were found to be contaminated externally with *Campylobacter*, with counts of ranging from <30 MPN/portion to >11,000 MPN/portion prior to crust freezing. Most of the isolates were identified as *C. jejuni*, which is a similar finding to other surveys of New Zealand chicken *(5, 10)*.

The range of MPN values obtained was a problem for the statistical analyses. Resourcing constraints meant that a limited range of dilutions could be prepared for MPN determination. Ideally a wider range of dilutions, especially to account for high numbers should be used, although MPN determinations are expensive and laborious. Possibly a combination of MPN determinations and plate counting over a series of dilutions could provide the sensitivity and range required for such work.

The statistical analyses applied to the data for crust freezing were not able to demonstrate a consistent trend, i.e. the crust freezing process does not seem to result in a statistically significant reduction in *Campylobacter* numbers. Sampling of 5 portions before and after crust freezing was in anticipation that any detrimental effect from crust freezing should be manifested over a suitably large number of samples. The fact that, in some cases, the mean number rose after crust freezing indicates that any effect there may be is not detectable above the natural variability in numbers of *Campylobacter* on the portions.

Alternative approaches would be to sample half a portion prior to, and the other half of the portion after crust freezing, or to inoculate portions with known numbers of *Campylobacter*. In the former case there would be an assumption that any *Campylobacter* present on a portion is uniformly distributed over the portion, and the fact that only a half portion gets frozen may influence the rate of freezing because there is only half the mass present. In the latter case, laboratory-grown inocula may not behave in the same way as natural contaminants which may have adapted to, or been selected for, survival under chilled conditions. All approaches therefore have drawbacks, and possibly any re-assessment of new freezing processes needs to use more samples from the same batch.

*In vitro* freezing experiments failed to show differences in survival between isolates at the same temperature, or between the same isolates at different temperatures in that no changes in numbers could be measured after chilling/freezing in chicken drip. No information describing the survival at temperatures in the range of 0 to -10°C could be located for

*Campylobacter*, but with other bacteria it is the rate of cooling that influences survival rather than the final temperature reached. During slow freezing (e.g. a reduction of 1°C $min^{-1}$), most microorganisms move into the unfrozen fraction of water in the food *(7)*. As extracellular ice forms in this fraction, the solutes become more concentrated in the unfrozen water and this causes increased water loss from bacterial cells and exposes them to osmotic stress. Osmotic stress causes a change in the intracellular pH and ionic strength, and subsequent denaturation of proteins. The membranes and membrane transport systems may also be irreparably damaged, and the bacteria can become more sensitive to oxidative stress *(6)*.

Additionally, if the freezing rate is increased, the solutes also freeze simultaneously with the water (i.e. freeze as a solution) and this reduces osmotic stress microorganisms are exposed to from the remaining unfrozen water fraction. This tends to favour survival of microorganisms by reducing the period over which they are exposed to osmotic stress.

The freezing rates used in the *in vitro* experiments described here were of the order of -0.2°C $min^{-1}$ and would therefore constitute a slow freezing rate and so this should have resulted in more damage than if the rate had been faster. It is possible that the time period over which the samples were frozen was too short for significant damage to have occurred, but the time periods used were selected to mimic those used by industry.

Work with chicken portions similarly was not able to measure inactivation of *Campylobacter* cells when the temperature was reduced to -2°C, though some inactivation occurred when the temperature was reduced to -10°C. However there was no difference in the inactivation caused by the two freezing rates. Inspection of the temperature at the skin surface (Figure 3) shows that, despite the programmed rate of chilling in the incubator, the rates of chilling from -2 to -10°C were actually very similar for both sets of samples. The difference between the two lay in the time that the two sets of samples remained at -2°C.

When the final temperature achieved was -10°C the chilling rate approximated 1°C in 30 minutes, while at -2°C it was closer to 1°C in 130 minutes, both of which could be regarded as slow chilling and slower than in the *in vitro* experiments. Given the results for the *in vitro* work it is possible that the extra time that the portions were subjected to freezing at -10°C (19 h 40 min) compared to that needed to achieve -2°C (4 h 20 min) had more of an influence on survival of *Campylobacter* than either the rate of cooling or the final temperature reached.

It can be concluded that crust freezing, as currently used by the New Zealand poultry industry, is not significantly altering the numbers of *Campylobacter* present on the surface of fresh chicken portions. While the potential for freezing to be used as a means of reducing *Campylobacter* numbers has been shown in this work, the freezing rates and temperatures achieved are likely to lie in a range that i) currently would not be legally permissible and ii) would require a significant investment from industry in order to move away from its current practices.

ACKNOWLEDGEMENTS

We would like to thank the members of the New Zealand poultry industry for their co-operation with this work. The New Zealand Food Safety Authority is thanked for its financial support.

REFERENCES

(1) Abram, D. D. and N. N. Potter. 1984. Survival of *Campylobacter jejuni* at different temperatures in broth, beef, chicken and cod supplemented with sodium chloride. *Journal of Food Protection*. 47:795-800.

(2) Bhaduri, S. and B. Cottrell. 2004. Survival of cold-stressed *Campylobacter jejuni* on ground chicken and chicken skin during frozen storage. *Applied and Environmental Microbiology*. 70:7103-7109.

(3) Birk, T., H. Ingmer, M. T. Anderson, K. Jørgensen and L. Brøndsted. 2004. Chicken juice, a food-based model system suitable to study survival of *Campylobacter jejuni*. *Letters in Applied Microbiology*. 38:66-71.

(4) Chan, K. F., H. L. Tran, R. Y. Kanenaka and S. Kathariou. 2001. Survival of clinical and poultry-derived isolates of *Campylobacter jeuni* at low temperature (4$^{o}$C). *Applied and Environmental Microbiology*. 67:4186-4191.

(5) Devane, M., C. Nicol, A. Ball, J. D. Klena, P. Scholes, J. A. Hudson, M. G. Baker, B. J. Gilpin, N. Garrett and M. G. Savill. 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. *Journal of Applied Microbiology*. In Press.

(6) Farkas, J. 1997. Physical methods of food preparation, p.497-519. In (ed.), Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington, D.C.

(7) Gill, C. O. 2002. Microbial control with low temperatures, p.55-74. In Juneja, V. K. and Sofos, J. N. (ed.), Control of foodborne microorganisms. Marcel Dekker, New York.

(8) Gill, C. O. and L. M. Harris. 1982. Survival and growth of *Campylobacter fetus* subsp. *jejuni* on meat and in cooked foods. *Applied and Environmental Microbiology*. 44:259-263.

(9) Grigoriadis, S. G., P. A. Koidis, K. P. Vareltzis and C. A. Batzios. 1997. Survival of Campylobacter jejuni inoculated in fresh and frozen beef hamburgers stored under various temperatures and atmospheres. *Journal of Food Protection*. 60:903-907.

(10) Hudson, J. A., C. Nicol, J. Wright, R. Whyte and S. K. Hasell. 1999. Seasonal variation of *Campylobacter* types from human cases, veterinary cases, raw chicken, milk and water. *Journal of Applied Microbiology*. 87:115-124.

(11) Humphrey, T., M. Mason and K. Martin. 1995. The isolation of *Campylobacter jejuni* from contaminated surfaces and its survival in diluents. *International Journal of Food Microbiology*. 26:295-303.

(12) Moorhead, S. M. and G. A. Dykes. 2002. Survival of Campylobacter jejuni on beef trimmings during freezing and frozen storage. *Letters in Applied Microbiology*. 34:72-76.

(13) Sandberg, M., M. Hofshagen, Ø. Østensvik, E. Skjerve and G. Innocent. 2005. Survival of *Campylobacter* on frozen broiler carcasses as a function of time. *Journal of Food Protection*. 68:1600-1605.

(14) Scott, W. G., H. M. Scott, R. J. Lake and M. G. Baker. 2000. Economic cost to New Zealand of foodborne infectious disease. *The New Zealand Medical Journal*. 113:281-284.

(15) Solow, B. T., O. M. Cloak and P. M. Fratamico. 2003. Effect of temperatutre on viability of *Campylobacter jejuni* and *Campylobacter coli* on raw chicken and pork skin. *Journal of Food Protection*. 66:2023-2031.
(16) Stern, N. J., P. J. Rothenberg and J. M. Stone. 1985. Enumeration and reduction of *Campylobacter jejuni* in poultry and red meats. *Journal of Food Protection*. 48:606-610.
(17) Wong, T. L., M. Devane, J. A. Hudson, P. Scholes, M. Savill and J. Klena. 2004. Validation of a PCR method for *Campylobacter* detection on poultry packs. *British Food Journal*. 106:642-650.
(18) Zhao, T., G. O. I. Ezeike, M. P. Doyle, Y.-C. Hung and R. S. Howell. 2003. Reduction of *Campylobacter jejuni* on poultry by low-temperature treatment. *Journal of Food Protection*. 66:652-655.

**Table 1:** ***Campylobacter*** **presence and most probable number (MPN) in retail chicken liver samples (internal and external). All samples were positive in the presence/absence test**

| Date | Company | Pre-chill (MPN/ portion) | Post-chill (MPN/ portion) |
|---|---|---|---|
| 21-Dec-04 | A | >1100 | >1100 |
| 21-Dec-04 | A | >1100 | >1100 |
| 21-Dec-04 | A | >1100 | >1100 |
| 21-Dec-04 | A | >1100 | >1100 |
| 21-Dec-04 | A | >1100 | >1100 |
| 17-Jan-05 | A | 430 | 150 |
| 17-Jan-05 | A | 750 | 390 |
| 17-Jan-05 | A | 430 | 2400 |
| 17-Jan-05 | A | 230 | 150 |
| 17-Jan-05 | A | 930 | 140 |
| 25-Jan-05 | A | 4600 | 11000 |
| 25-Jan-05 | A | >11000 | 4600 |
| 25-Jan-05 | A | >11000 | >11000 |
| 25-Jan-05 | A | >11000 | 4600 |
| 25-Jan-05 | A | 4600 | 930 |
| 31-Jan-05 | A | 1500 | 430 |
| 31-Jan-05 | A | 930 | 230 |
| 31-Jan-05 | A | 930 | 11000 |

| | | | |
|---|---|---|---|
| 31-Jan-05 | A | 430 | 930 |
| 31-Jan-05 | A | 430 | 230 |
| 7-Feb-05 | A | 1500 | 430 |
| 7-Feb-05 | A | 930 | 2400 |
| 7-Feb-05 | A | 2400 | 4600 |
| 7-Feb-05 | A | 4600 | 390 |
| 7-Feb-05 | A | 430 | 430 |
| 15-Feb-05 | B | 2400 | 430 |
| 15-Feb-05 | B | 430 | 230 |
| 15-Feb-05 | B | 430 | 430 |
| 15-Feb-05 | B | 90 | 90 |
| 15-Feb-05 | B | 90 | 230 |
| 22-Feb-05 | B | 40 | 40 |
| 22-Feb-05 | B | <30 | 40 |
| 22-Feb-05 | B | <30 | 40 |
| 22-Feb-05 | B | 90 | 70 |
| 22-Feb-05 | B | 40 | 930 |
| 16-Mar-05 | B | >11000 | 430 |
| 16-Mar-05 | B | 4600 | 230 |
| 16-Mar-05 | B | 4600 | 90 |
| 16-Mar-05 | B | 1500 | 4600 |
| 16-Mar-05 | B | 4600 | 230 |
| 22-Mar-05 | B | >11000 | 40 |
| 22-Mar-05 | B | >11000 | 430 |

| | | | |
|---|---|---|---|
| 22-Mar-05 | B | 11000 | 90 |
| 22-Mar-05 | B | >11000 | 230 |
| 22-Mar-05 | B | >11000 | 90 |
| 31-Mar-05 | B | 230 | 230 |
| 31-Mar-05 | B | 230 | 750 |
| 31-Mar-05 | B | 230 | 4600 |
| 31-Mar-05 | B | 200 | 430 |
| 31-Mar-05 | B | 230 | 1500 |

Table 2, top ↑

**Table 2: T-test of consecutive, sampling from the same processing batch, the data sets indicating the probability of whether the two samples are not significantly different**

| Pre:Post crust freezing | Data set | T value* | Direction of change |
|---|---|---|---|
| Company A | 1 | 0.85 | increase |
| | 2 | 0.44 | increase |
| | 3 | 0.78 | decrease |
| Company B | 1 | 0.38 | decrease |
| | 2 | 0.33 | increase |
| | 3 | 0.15 | increase |

** T-test of the data sets indicate the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean.*

Table 3, top ↑

**Table 3: F-test of consecutive, sampling from the same processing batch, the data sets indicating the probability of whether the two samples are not significantly different**

| Pre:Post crust freezing | Data set | F value* | Direction of change |
|---|---|---|---|
| Company A | 1 | 0.03 | increase |
| | 2 | $4.56 \times 10^{-4}$ | increase |
| | 3 | 0.81 | decrease |
| Company B | 1 | $2.93 \times 10^{-3}$ | decrease |
| | 2 | $2.13 \times 10^{-4}$ | increase |
| | 3 | $1.86 \times 10^{-8}$ | increase |

** F-test of the data sets indicate the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean.*

## FIGURE LEGENDS

**Figure 2:** **Comparison of counts before and after freezing to –10°C in sterile chicken drip for nine *Campylobacter* isolates. Black bars represent counts on *m*-Exeter agar prior to cooling, white bars counts after cooling, and grey bars counts on CB agar after cooling**

**Figure 3:** **Changes in *C. jejuni* numbers inoculated onto chicken breast portions. Black bars show counts prior to freezing, and white bars show counts post freezing. Error bars represent the standard deviation of the count**

**Figure 4:** **Comparison of temperature profiles of chicken breast portions cooling under industry and slow conditions. O = target temperatures for industry cooling, ◊ = target temperatures for slow cooling, Light lines = air temperature and skin temperature of a chicken portion under industry cooling conditions, dark lines = air temperature and skin temperature of a chicken portion under slow cooling conditions**

Fig. 1, top ↑

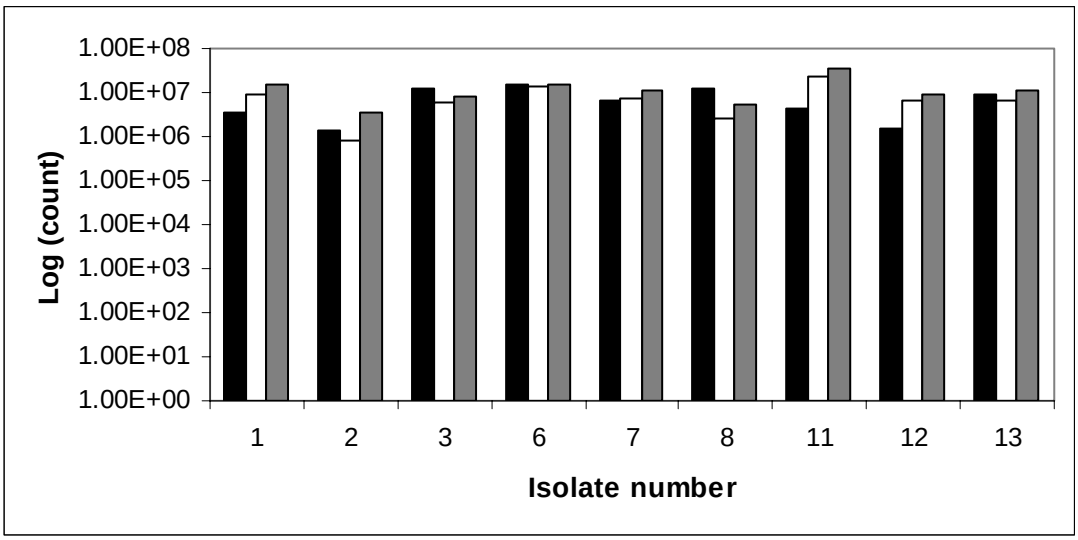

1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Log (count)
1
2
3
6
7
8
11
12
13
Isolate number

Fig. 2, top ↑

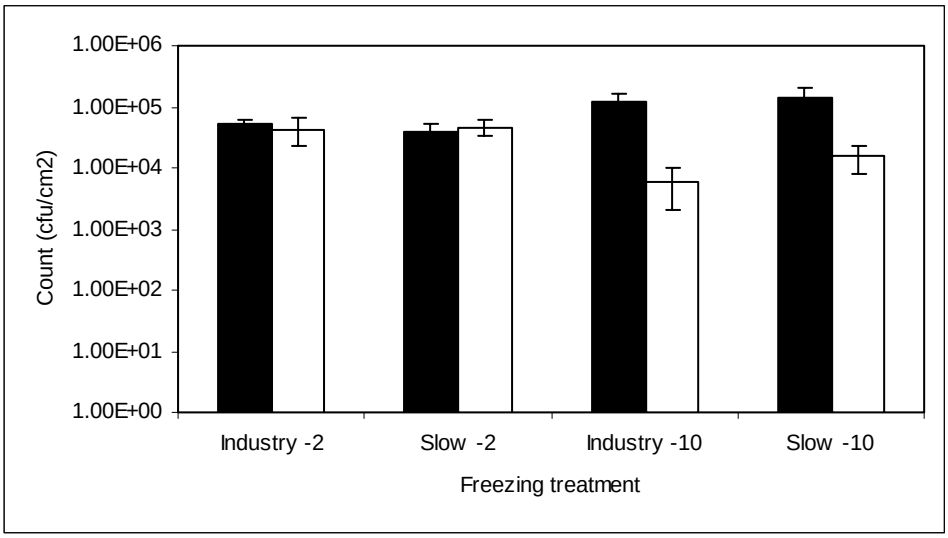
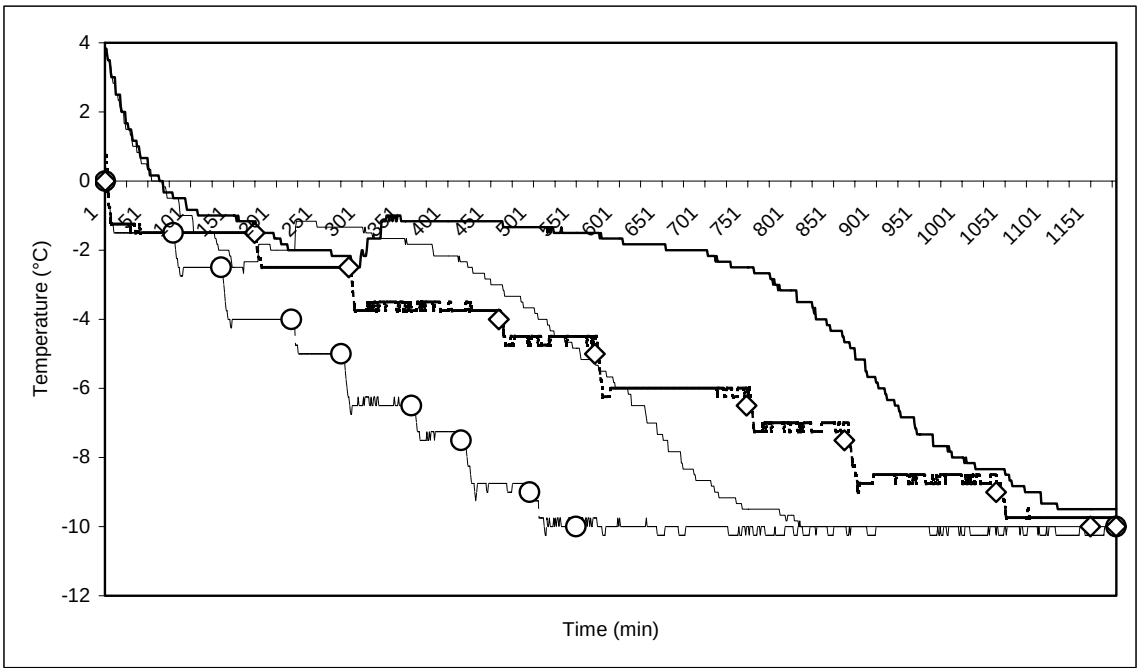

1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Count (cfu/cm2)
Industry -2
Slow -2
Industry -10
Slow -10
Freezing treatment

Fig. 3, top ↑


Temperature (°C)
4
2
0
-2
-4
-6
-8
-10
-12
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
751
801
851
901
951
1001
1051
1101
1151
Time (min)

## APPENDIX 1 STATISTICAL ANALYSIS

### Company survey

**Table 1: Company A. T-test of the data sets indicating the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean**

| | | |
|---|---|---|
| **Pre-chill** | 17-Jan:31-Jan | 0.25 |
| | 17-Jan:7-Feb | 0.18 |
| | 31-Jan:7-Feb | 0.09 |
| **Post-chill** | 18-Jan:1-Feb | 0.40 |
| | 18-Jan:8-Feb | 0.70 |
| | 1-Feb:8-Feb | 0.32 |

**Table 2: Company B. T-test of the data sets indicating the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean**

| | | |
|---|---|---|
| Pre-chill | 17-Jan:31-Jan | 0.40 |
| | 17-Jan:7-Feb | 0.03 |
| | 31-Jan:7-Feb | 4.76E-03 |
| **Post-chill** | 18-Jan:1-Feb | 0.01 |
| | 18-Jan:8-Feb | 0.10 |
| | 1-Feb:8-Feb | 0.25 |

**Table 3: Company A. F-test of the data sets indicating the probability of whether the two samples are not significantly different**

| | | |
|---|---|---|
| **Pre-chill** | 15-Feb:22-Feb | 0.17 |
| | 15-Feb:date_3 | 0.32 |
| | 22-Feb:date_3 | 1.77E-06 |
| **Post-chill** | 15-Feb:22-Feb | 0.77 |
| | 15-Feb:16-Mar | 0.37 |
| | 15-Feb:date_2 | 0.30 |
| | 15-Feb:date_4 | 0.17 |
| | 22-Feb:16-Mar | 0.35 |
| | 22-Feb:date_2 | 0.81 |
| | 22-Feb:date_4 | 0.16 |
| | 16-Mar:date_2 | 0.31 |
| | 16-Mar:date_4 | 0.75 |
| | date_2:date_4 | 0.14 |

**Table 4: Company B. F-test of the data sets indicating the probability of whether the two samples are not significantly different**

| | | |
|---|---|---|
| **Pre-chill** | 15-Feb:22-Feb | 5.89E-06 |
| | 15-Feb:date_3 | 2.18E-07 |
| | 22-Feb:date_3 | 0.14 |
| **Post-chill** | 15-Feb:22-Feb | 0.08 |
| | 15-Feb:16-Mar | 0.00 |
| | 15-Feb:date_2 | 0.88 |
| | 15-Feb:date_4 | 0.00 |
| | 22-Feb:16-Mar | 0.01 |
| | 22-Feb:date_2 | 0.11 |
| | 22-Feb:date_4 | 0.01 |
| | 16-Mar:date_2 | 2.58E-04 |
| | 16-Mar:date_4 | 0.88 |
| | date_2:date_4 | 0.00 |

Data groups that did not contain an entry of > were selected and paired thereby giving pre-chill and post–chill data for the same batch number (presumably flock). These groups were compared as given in Tables 5, 6, 7, and 8. The term *increase* and *decrease* refers to the change in *Campylobacter* levels (MPN counts) for the process, i.e. the term *decrease* is used when the levels of Campylobacter have reduced from pre-chill to post-chill.

**Table 5: Company A. T-test of consecutive, sampling from the same processing batch, the data sets indicating the probability of whether the two samples are not significantly different. T-test of the data sets indicating the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean**

| | | | |
|---|---|---|---|
| **Pre-chill:Postchill** | 17-Jan:18-Jan | 0.85 | increase |
| | 31-Jan:1-Feb | 0.44 | increase |
| | 7-Feb:8-Feb | 0.78 | decrease |

**Table 6: Company B. T-test of consecutive, sampling from the same processing batch, the data sets indicating the probability of whether the two samples are not significantly different. T-test of the data sets indicating the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean**

| | | | |
|---|---|---|---|
| **Pre-chill:Postchill** | 15-Feb:15-Feb | 0.38 | decrease |
| | 22-Feb:22-Feb | 0.33 | increase |
| | date_3:date_4 | 0.15 | increase |

**Table 7:** **Company B. F-test of consecutive, sampling from the same processing batch, the data sets indicating the probability of whether the two samples are not significantly different. T-test of the data sets indicating the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean.**

| | | | | |
|---|---|---|---|---|
| **Pre-chill:Postchill** | 17-Jan:18-Jan | 0.03 | NS | increase |
| | 31-Jan:1-Feb | 4.56E-04 | NS | increase |
| | 8-Feb:8-Feb | 0.81 | | increase |

**Table 8:** **Company B. F-test of consecutive, sampling from the same processing batch, the data sets indicating the probability of whether the two samples are not significantly different. T-test of the data sets indicating the probability of whether the two samples are likely to have come from the same two underlying populations that have the same mean.**

| | | | |
|---|---|---|---|
| **Pre-chill:Postchill** | 15-Feb:22-Feb | 2.93E-03 | Decrease |
| | 15-Feb:date_3 | 2.13E-04 | increase |
| | 22-Feb:date_3 | 1.86E-08 | increase |

## Combining data sets

Comparison of the data sets using the bias corrected Bartlett's test revealed the only Company A pre-chill data sets had the same variance, refer Table 9 below.

**Table 9:** **Results of the analysis of the pre and post chill data.**

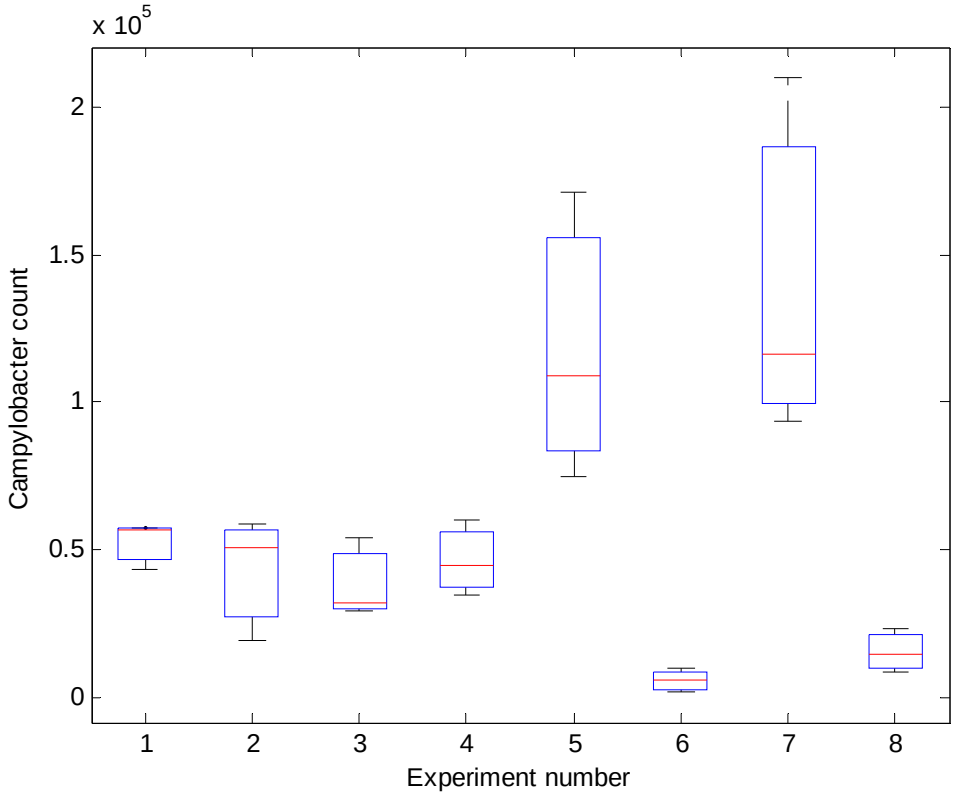
| | Bartlett's test | 95% confidence | Variances equal |
|---|---|---|---|
| **Company A** | | | |
| Pre-chill | 21.09 | 26.3 | yes |
| Post-chill | 55.54 | 26.3 | no |
| **Company B** | | | |
| Pre-chill | 43.88 | 26.3 | no |
| Post-chill | 31.71 | 26.3 | no |
| **Company A & B combined** | | | |
| Pre-chill | 74.77 | 26.3 | no |
| Post-chill | 43.87 | 26.3 | no |

The results in Table 9 indicate the data sets have different variances, with the exception of pre-chill, Company A. Therefore these data cannot be combined together into a larger data set as they come from different distributions, i.e. not normal distributions.

### *In vivo* experiments - chicken breast sampling

Hypothesis testing for the difference in means of two samples was applied using a two tailed T-test with $\alpha$=0.05 (5%). Both experiments at –2°C showed no statistically significant difference between the pre and post measures, 0.5005 industry and 0.4939 for slow with the 95% confidence intervals of (-0.2615 x $10^5$, 0.4515 x $10^5$) and (-0.3774 x $10^5$, 0.2165 x $10^5$) respectively. The experiments at –10°C exhibited a significant statistical difference between the pre and post results, 0.0165 for industry and 0.0255 for slow with the 95% confidence intervals of (0.3393 x $10^5$,1.909 x $10^5$) and (0.2502 x $10^5$, 2.2415 x $10^5$) respectively.

**Figure 1:** **Whisker-box plot of the pre and post cooling data. The lower and upper lines of the blue "box" are the 25th and 75th percentiles of the sample. The distance between the top and bottom of the box is the inter-quartile range. The red line in the middle of the box is the sample median. If the median is not centred in the box, that is an indication of skewness. X-axis key 1=Pre Industry –2°C; 2=Post Industry –2°C; 3= Pre slow –2°C; 4=Post slow –2°C; 5=Pre industry –10°C; 6=Post industry –10°C; 7=Pre slow –10°C; 8=Post slow –10°C.**



## APPENDIX 2 TEMPERATURE PROFILES

The following profiles provide examples of the cooling rates observed in both the inoculated chicken drip and inoculated chicken breast laboratory experiments.

### Chicken drip

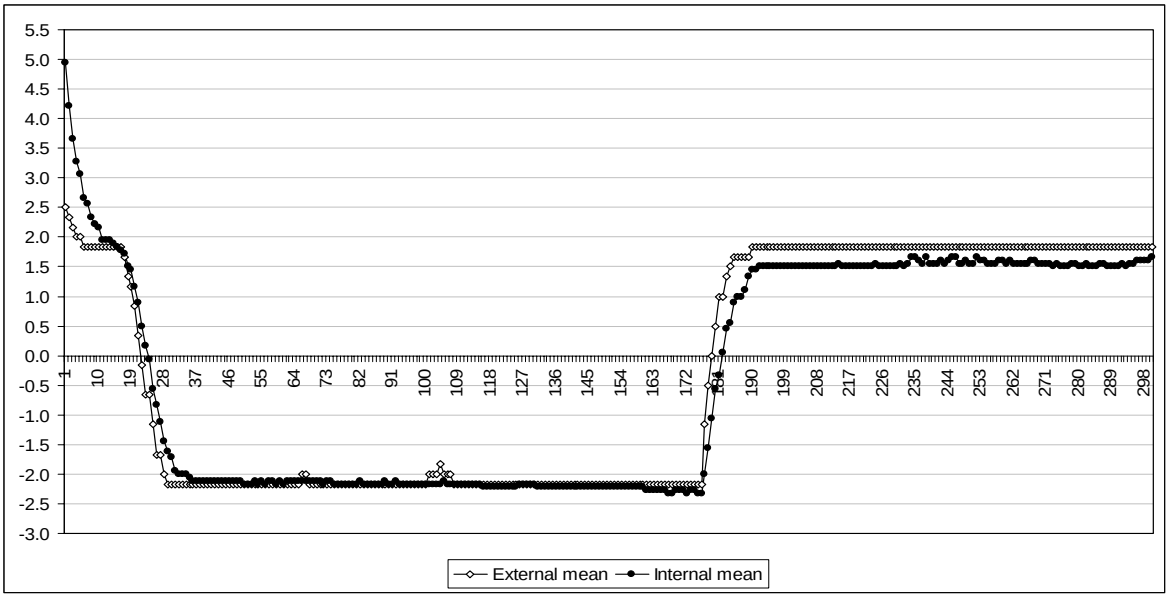



**Figure 1: Mean external (= incubator) temperature and mean internal (=chicken drip) temperature observed in inoculated chicken drip experiment where target temperature was –2ºC**

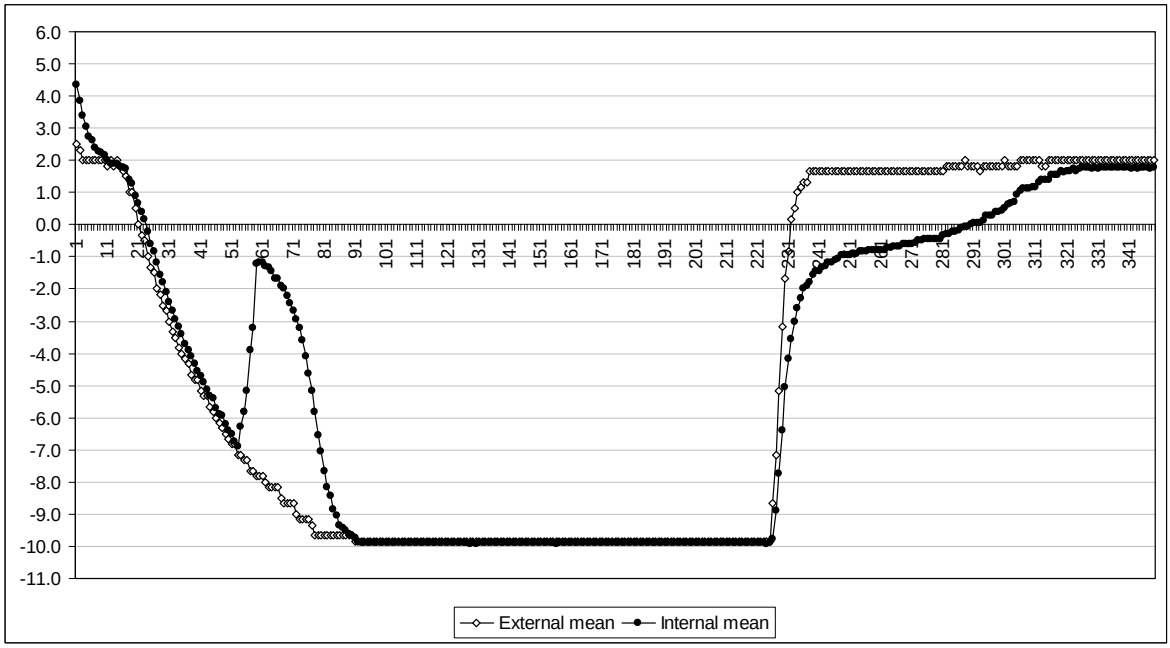



**Figure 2: Mean external (= incubator) temperature and mean internal (=chicken drip) temperature observed in inoculated chicken drip experiment where target temperature was –10ºC**

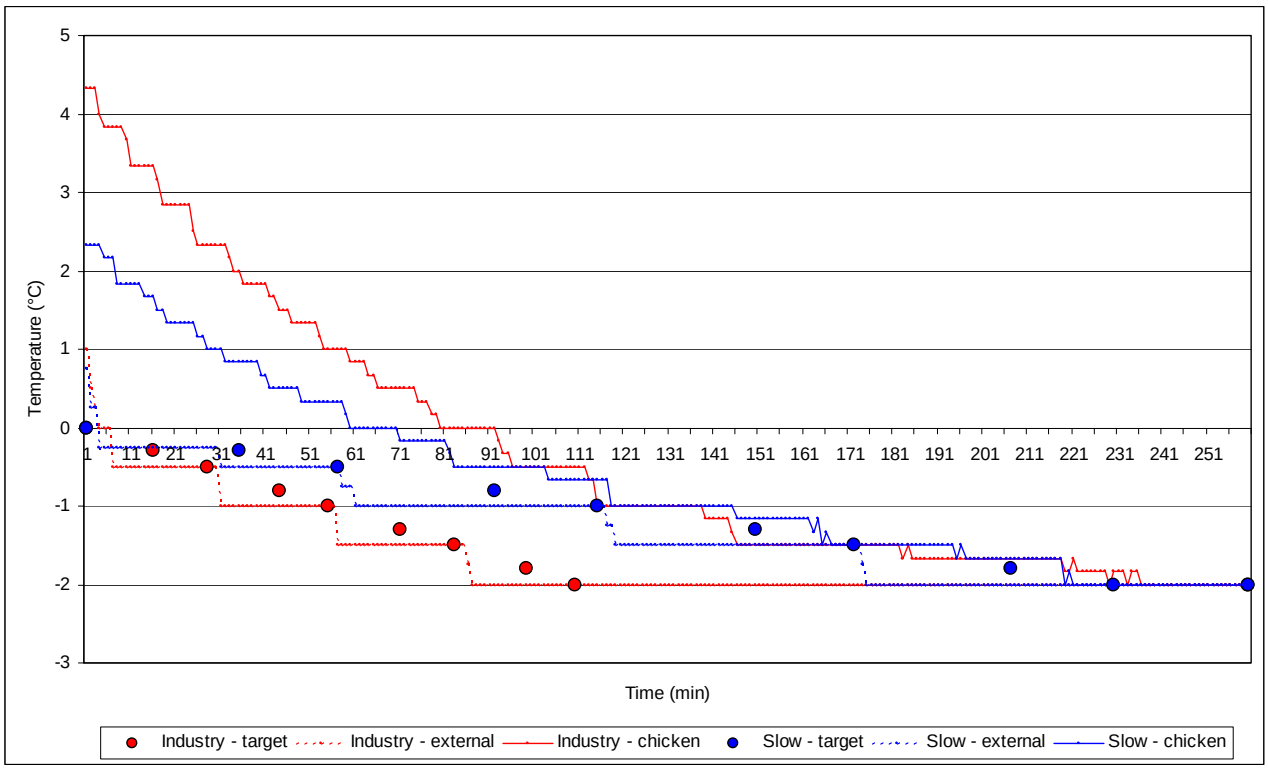



**Figure 3:** **Target cooling rate (○), mean incubator cooling rate (dotted lines) and mean cooling rate of chicken breasts (solid lines) for the industry-based cooling rate of 0.018ºC/min (red) and the slower cooling rate of 0.009ºC/min (blue) to the target temperature of –2ºC**

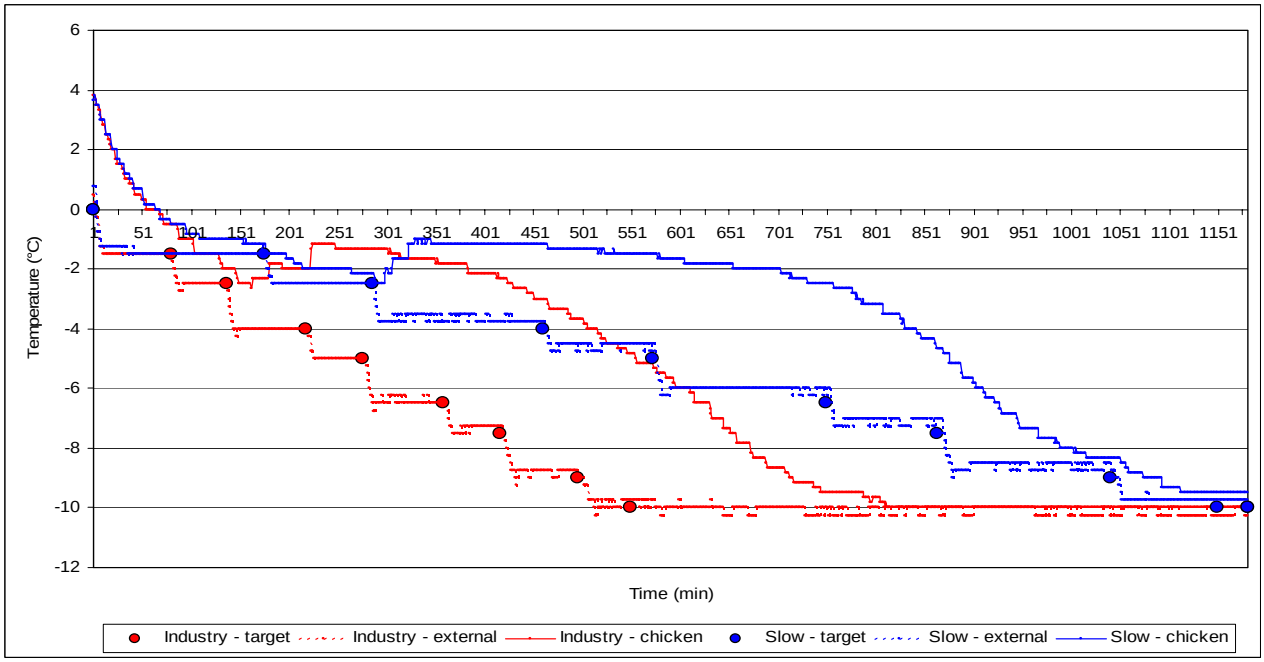



**Figure 4:** **Target cooling rate (○), mean incubator cooling rate (dotted lines) and mean cooling rate of chicken breasts (solid lines) for the industry-based cooling rate of 0.018ºC/min (red) and the slower cooling rate of 0.009ºC/min (blue) to the target temperature of –10ºC**