DOMESTIC FOOD PRACTICES IN NEW ZEALAND

QUANTIFYING THE REDUCTION OF *CAMPYLOBACTER JEJUNI* ON SKIN-ON CHICKEN BREASTS COMMERCIALLY FROZEN AND STORED FOR UP TO 10 WEEKS IN A DOMESTIC FREEZER

FINAL REPORT

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Dr Lynn McIntyre

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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

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Dr Stephen On
Food Safety Programme Leader

Dr Lynn McIntyre
Project Leader

Dr Andrew Hudson
Peer Reviewer

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SUMMARY

This project was initiated to quantify the reduction of two *Campylobacter jejuni* strains, STu48 and ST474, following commercial freezing (-30°C), commercial frozen storage (-21°C) for two weeks and domestic storage (-18°C) for a further eight weeks. Significant but variable reductions in *C. jejuni* numbers were observed over time for both strains, with reductions most rapid during the 14 days of commercial frozen storage. Overall, mean *C. jejuni* populations declined by approximately 1.8 to 3.5 $\log_{10}$ cfu over a 1 to 6 week storage period following commercial freezing, but were not completely eliminated. Under the specific conditions investigated, commercial freezing and short term storage (up to 28 days) produced significantly greater mean *C. jejuni* reductions versus domestic freezing. Similar but larger reductions were achieved by both processes over a longer storage period (up to 70 days), suggesting that frozen chicken should ideally be stored for at least 4 weeks to achieve maximum pathogen reductions. Clearly, while freezing has a significant impact on the reduction of *C. jejuni* on poultry, the results obtained from this work should be considered "best case scenario" given reports of the pathogen's lesser survival on poultry skin (vs. skinned/ cut muscle). Data also demonstrate that freezing is an inconsistent process subject to variability related to a number of processor and consumer factors. This information will be valuable in supporting risk management initiatives by the New Zealand Food Safety Authority to control *C. jejuni* through the food chain, and contribute additional data to ongoing pathogen risk model developments in this area.

1 INTRODUCTION

Campylobacteriosis is the most frequently reported gastrointestinal illness in New Zealand, with greater than 50% of cases being attributed to consumption of chicken (Eberhart-Phillips *et al.*, 1997). A recent commentary by Baker and colleagues (2006) on the rates of *Campylobacter* infection in New Zealand has proposed that all fresh poultry should be withdrawn from the food supply and replaced with frozen or processed alternatives until industry interventions can reliably reduce *Campylobacter* to acceptable levels. This suggestion, similar to interventions previously introduced in some European countries (Sandberg *et al.*, 2005; Georgsson *et al.*, 2006), is based on scientific evidence that freezing can reduce *Campylobacter* loads. Such an approach does not, however, correspond with New Zealand consumer preference for fresh poultry purchase. The domestic food handling survey conducted by ESR in 2005 reported that just 10% of respondents purchased only frozen chicken, while 71% purchased 50% or more of their poultry fresh. However, 66.3% of consumers freeze over half of this fresh poultry at home in domestic freezers (Gilbert *et al.*, 2005; Gilbert *et al.*, 2007). This demand for fresh poultry has been independently confirmed by both Tegel and Inghams, whose sales of (non-value-added) frozen poultry portions have been in decline for a number of years.

Freezing of foods is a common means of extending shelf life through the combined effects of low temperature and reduced water activity, although it is not, in practice, an absolute means of guaranteeing the safety of any food. It can however be combined with other decontamination methods such as hot water treatment to enhance the destruction of pathogens (James *et al.*, 2007). Injury and subsequent death of microorganisms during freezing is thought to be due mainly to osmotic stress and/or dehydration rather than mechanical damage via the production of ice crystals (Gill, 2002).

A previous literature review conducted by ESR (Turner, 2004) reported that *Campylobacter* levels can be reduced by 0.5 to >2.5 logs by freezing, depending on the rate and temperature of freezing, and the composition of the food in question. The majority of publications to date have used domestic freezing temperatures in the range -18 to -20°C. A recent publication (Ritz *et al.*, 2007) demonstrated that *C. jejuni* survives less well on chicken skin versus muscle tissue, and that the estimation of survival by laboratory methods can be confounded by the use of selective media and higher incubation temperatures that create greater stress conditions and, as a consequence, may underestimate survival.

Subsequent experimental work conducted by ESR investigated the effect of domestic freezing and frozen storage on survival of two *C. jejuni* strains of diferring genotype on skin-on chicken breast portions (McIntyre, 2008). Domestic freezing produced highly variable results with respect to *C. jejuni* reduction over time. Mean reductions ranging from 1 – 1.3 $\log_{10}$ cfu per sample occurred over the first two weeks of frozen storage, but the largest mean reduction of *C. jejuni* (3.5 $\log_{10}$ cfu) was achieved after six weeks of frozen storage.

In contrast to the use of domestic freezers at the consumer level, the poultry industry uses blast freezing processes. This method is favoured by the food industry as it maintains the quality of the product due to the formation of smaller ice crystals, preventing tissue damage and excessive drip upon thawing. According to literature, there are reported differences in kill for rapid freezing versus slower freezing, with slower freezing being more effective due to the extended exposure of microorganisms to osmotic shock and dehydration conditions

(Gill, 2002). This suggests that domestic freezing of poultry may be more effective in reducing *Campylobacter* than commercial blast freezing as currently practiced. Therefore the way in which chicken is frozen and the length of time that it is held at effective killing temperatures are important considerations regarding the possible application of freezing as an intervention strategy for *Campylobacter*.

To enable the New Zealand Food Safety Authority to be better informed regarding freezing of poultry as a potential intervention, experiments were conducted to quantify the reduction of two *C. jejuni* poultry strains on skin-on chicken breast portions after commercial freezing and domestic storage in a domestic fridge-freezer with a bottom-loading freezer compartment (identified in an NZFSA-commissioned survey as being the most commonly owned freezer type in New Zealand by McIntyre *et al*., 2007).

2 MATERIALS AND METHODS

2.1 Cultures

Campylobacter jejuni strains p145a and p110b [hereafter referred to as (sequence type) STu48 and ST474 respectively] were obtained from the culture collection of Professor Nigel French, Massey University. Both were isolated from whole chicken carcasses purchased in the Manawatu region and stored long term at -80°C. Strains were streaked onto Columbia Blood Agar (CBA) and incubated in a CO_2 incubator at 37°C for 48 hours prior to sub-culture in modified Exeter broth (Wong *et al*., 2004). Prior to experimentation, 48 hour old cultures previously incubated as described in modified Exeter broth were enumerated by serial dilution (using sterile 0.1% peptone water) and spread plating on modified charcoal cephoperazone desoxycholate agar (mCCDA; Oxoid). All plates were incubated at 37°C for 48 hours in a MACS VA5000 microaerophilic work station (81% N_2:3% O_2:6% H_2:10% CO_2 gas mix) to determine total counts. Enumeration was conducted twice and counts obtained were used to calculate suitable inoculum levels taking into account anticipated losses associated with the rinse recovery method (data not shown).

2.2 Irradiation of chicken samples

Individual skin-on chicken breast samples of similar size and weight (mean 150 g with a standard deviation of 14 g; Brendon Hasson, Pers. Comm.) were obtained from Tegel Foods, Christchurch, and individually packaged in Whirlpak bags. Samples were then couriered in chilly bins to Schering-Plough Animal Health, Wellington and irradiated in bulk at 25kGy to provide *Campylobacter*-free samples for this work. Samples were returned by courier in chilly bins re-packed with ice packs and stored in a walk-in cooler prior to use.

2.3 Sample inoculation, freezing and temperature monitoring

Skins of irradiated chicken portions were surface-inoculated under contained laboratory conditions at ESR using 0.1 mL volumes of diluted suspensions of individual *C. jejuni* strains (at a level of ~10^5 cfu per portion), with triplicate samples prepared for each strain, thawing condition and sampling time. All inoculated samples and uninoculated data logger control samples (see below) were placed into one of two cardboard cartons (supplied by Tegel Foods) and chilled prior to transfer to the Tegel Hornby plant in Christchurch where samples were exposed to commercial blast freezing, in their cartons, for approximately 43 hours followed by frozen storage for two weeks. Unsampled portions were transferred back to ESR in chilly bins containing frozen ice packs and placed into the empty bottom-loading freezer compartment of a Fisher & Paykel *ActiveSmart*™ domestic fridge-freezer for a further 8 weeks. Triplicate samples for each sampling point were located in the same carton during freezing, but were split across top, middle and bottom shelves for domestic storage.

To determine freezing rates and monitor storage temperatures, two i-button data loggers programmed to record every 12 minutes were attached to each of four uninoculated chicken portions to record both internal and external temperatures (Figure 1). Two portions were then placed into each carton, one portion in the centre and one portion at the edge. Air temperatures were also recorded throughout commercial blast freezing and frozen storage using individual data loggers taped to the inside of carton lids. Data loggers were removed from samples after two weeks of commercial storage and data were downloaded.

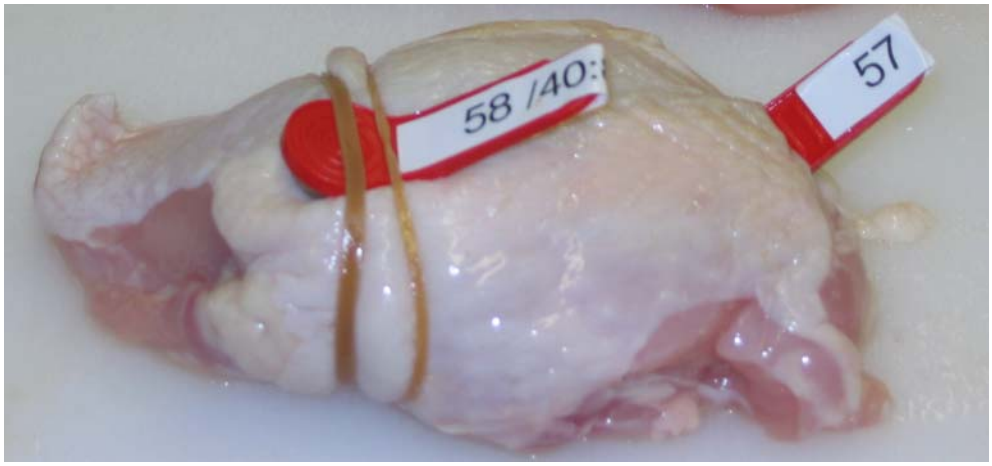

Figure 1: Placement of internal (57) and external (58) data loggers in a skin-on breast portion

Loggers were then re-attached to the control samples and placed into the domestic freezer on the top and bottom shelves (see Table 1 for a summary of data logger locations). Domestic freezer temperatures were also recorded using additional data loggers to ensure consistent storage conditions at ESR.

Table 1: Summary of information related to data logger placement and sample location during commercial freezing of skin-on chicken portions in cartons

Data Logger	Portion #	Carton #	Sample location in carton	Data logger placement	Sample location in domestic freezer
P1 C1 centre internal	1	1	Centre	Internal	Top shelf, centre
P1 C1 centre external	1	1	Centre	External	
P2 C1 edge internal	2	1	Edge	Internal	Top shelf, edge
P2 C1 edge external	2	1	Edge	External	
C1 Air	-	1	-	Inside Lid	-
P1 C2 centre internal	1	2	Centre	Internal	Bottom shelf, centre
P1 C2 centre external	1	2	Centre	External	
P2 C2 edge internal	2	2	Edge	Internal	Bottom shelf, edge
P2 C2 edge external	2	2	Edge	External	
C2 Air	-	2	-	Inside Lid	-

2.4 Microbiological analysis

2.4.1 Verification of irradiation

A small number of irradiated chicken portions were microbiologically tested for *Campylobacter* and other microbes to confirm the effectiveness of irradiation treatment using a presence-absence Exeter broth enrichment and mCCDA plating method. The rinse method

employed for enumeration (see section 2.4.2) was used to determine the presence of general microbial populations using CBA.

2.4.2 Bacterial enumeration

At defined intervals up to 70 days of storage, three samples per strain were removed from either the carton stored at Tegel Foods (days 1, 2, 7 and 14) or the domestic freezer at ESR (days 28, 42, 56 and 70). Samples were double-bagged using Whirl-Pak bags and either thawed overnight for 17 hours in a 20°C incubator or in the fridge compartment (4°C) of the fridge-freezer for 41 hours prior to enumeration. Thawed samples were then rinsed with 30 mL of 0.1% peptone water (reduced to 15 mL for day 42 - 70 samples) and the weight of recovered rinse water (including sample drip) was recorded. A fixed volume of rinse (dependent on total recovered rinse volume) was then removed and centrifuged at 1600 *x g* for 5 minutes (Heraeus Labofuge GL) to concentrate cells prior to plating. The pellet was re-suspended in 5 mL of sterile 0.1% peptone water, diluted as required, and plated onto mCCDA to determine *C. jejuni* counts. For samples stored short term, 0.1 mL volumes were spread plated in duplicate while subsequent testing of samples stored for longer periods employed 1 mL volumes spread over 3 plates. All plates were incubated at 37°C for 48 hours under microaerophilic conditions as previously described (section 2.1) prior to counting. Reduction data (calculated as described in Appendix 2 and expressed as $\log_{10}$ cfu per portion unless otherwise stated) were then graphed using GraphPad Prism® version 5.0 software.

2.4.3 *C. jejuni* confirmation

The identities of randomly selected presumptive *C. jejuni* colonies isolated from Day 0, Day 1 and Day 70 plates during the experiment were confirmed by PCR using the method published by Wong *et al*. (2004). The identity of atypical or non-*Campylobacter* colony morphologies was noted and investigated as required.

2.5 Statistical analysis

One-way ANOVA and Tukey-Kramer multiple comparisons tests were used to investigate the significance of differences in *C. iejuni* $\log_{10}$ counts between days using SAS System V.9.1. A two-sample (unequal variance) t-test was employed to compare domestic and commercial freezing data using Microsoft Excel. Results below detectable limits were given absolute values in order to include them in analyses. A p-value of <0.05 was taken to be statistically significant in all cases.

3 RESULTS AND DISCUSSION

3.1 Temperature profiles during commercial freezing and storage

Data loggers recording air and internal/external chicken portion temperatures were removed from uninoculated portions after approximately two weeks of frozen storage in cartons (representing the termination of commercial freezing and storage, and the beginning of domestic freezer storage at ESR). Data were downloaded and exported to Microsoft Excel, and data loggers were returned to samples for further measurement of long term domestic storage. Figure 2 shows the temperature profiles for the ten data loggers employed to measure the internal and external temperatures of four skin-on chicken portions blast frozen in either the centre or to the outer edge of two cartons containing inoculated portions destined for long term frozen storage. Blast freezing ultimately achieved a sample temperature of -29 to -29.5°C, with an air temperature of -29.5°C, after which samples were relocated to a freezer (also at Tegel Hornby) operating at an average temperature of -21°C.

Figure 3 illustrates the temperature profiles for the blast freezing segment of the process only. The air temperature loggers C1 and C2 demonstrated a rapid decline in air temperature as the cartons were moved into the blast freezer. Both portions located at the edge of the cartons (P2 C1 and P2 C2) showed similar freezing profiles, while portions in the centre of the cartons (P1 C1 and P1 C2) took longer to decline in temperature initially, with temperature plateaus particularly notable for P1 C1. An uneven distribution of samples between the two cartons may have contributed to the variation in freezing profiles noted for centrally located portions – carton 1 contained 58 samples while 40 samples were frozen in carton 2.

The temperature changes occurring during blast freezing are summarised in Table 2. Overall, all portions reached -29 to -29.5°C within 37.4 to 40.6 hours but, as previously mentioned in relation to Figure 3, portions located in the centre of the cartons took longer to freeze down to -10°C than those close to the edge. This supports the view expressed in the domestic freezing report (McIntyre, 2008) that 'hot spots', in the centre of freezer compartments and in this particular situation cartons, may subject portions to variable freezing conditions. The differences between internal and external sample temperatures over time were small.

Table 2: Summary of times and air temperatures related to the commercial freezing of chicken portions

Logger location:	Time (hrs) required to reduce portion temperature from:			Lowest temp (°C)
	0 to -10°C	0 to -20°C	0 to lowest temp.	
P1 C1 centre internal	14.9	17	37.4	-29
P1 C1 centre external	16.4	19.2	40	-29.5
P2 C1 edge internal	6.2	14.6	40.6	-29.5
P2 C1 edge external	4.2	9.8	40.4	-29.5
C1 air	-	-	-	-29.5
P1 C2 centre internal	10	14	38.8	-29
P1 C2 centre external	10.8	14.3	38.6	-29.5
P2 C2 edge internal	4.6	8	39.8	-29.5
P2 C2 edge external	5.9	10.7	39.7	-29.5
C2 air	-	-	-	-29.5

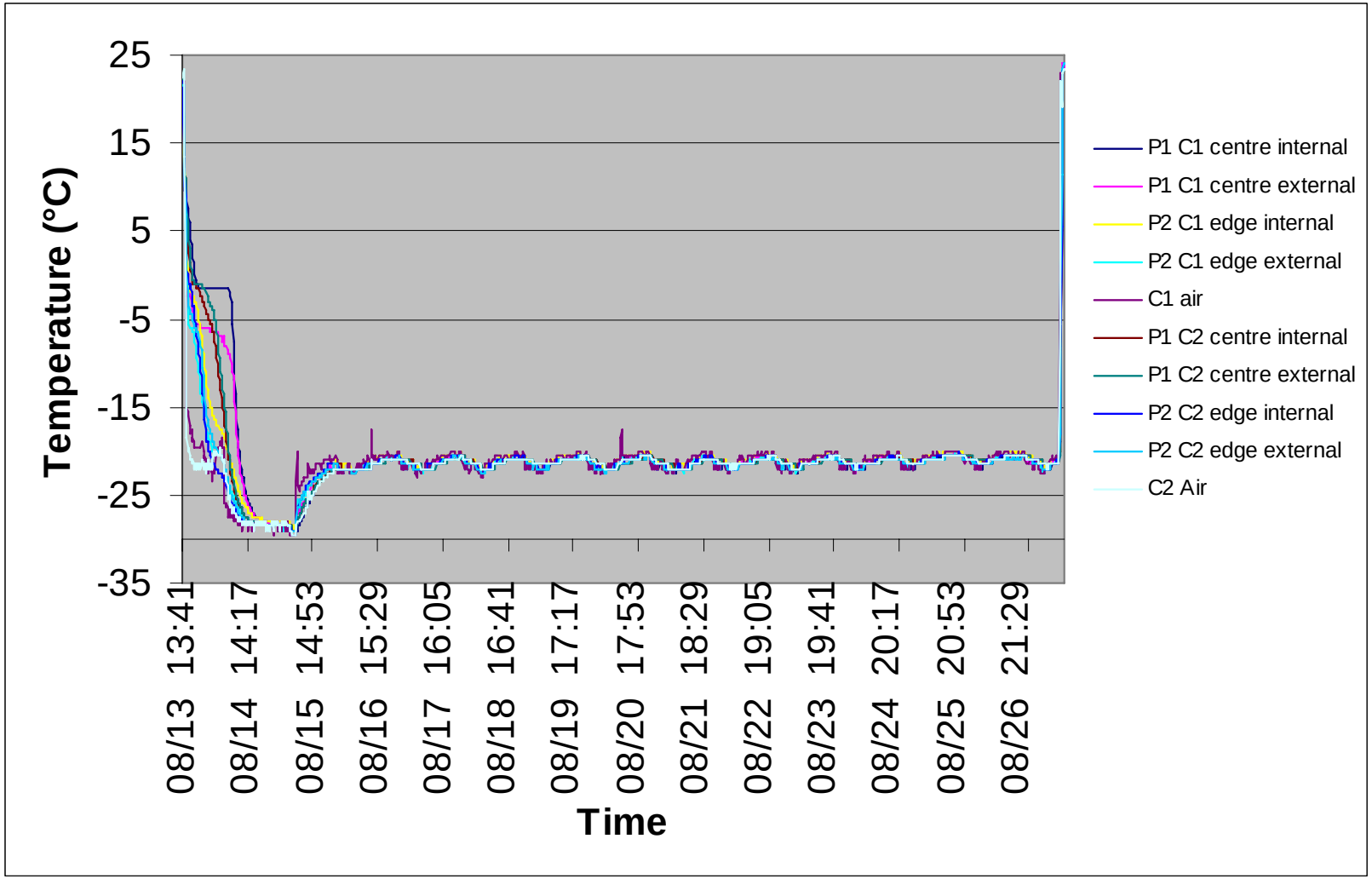

Figure 2: **Temperature profiles for commercial blast freezing and 2 weeks of frozen storage of skin-on chicken portions (P = portion #; C = carton #; centre/edge = portion location within carton; internal/external = logger location within portion)**

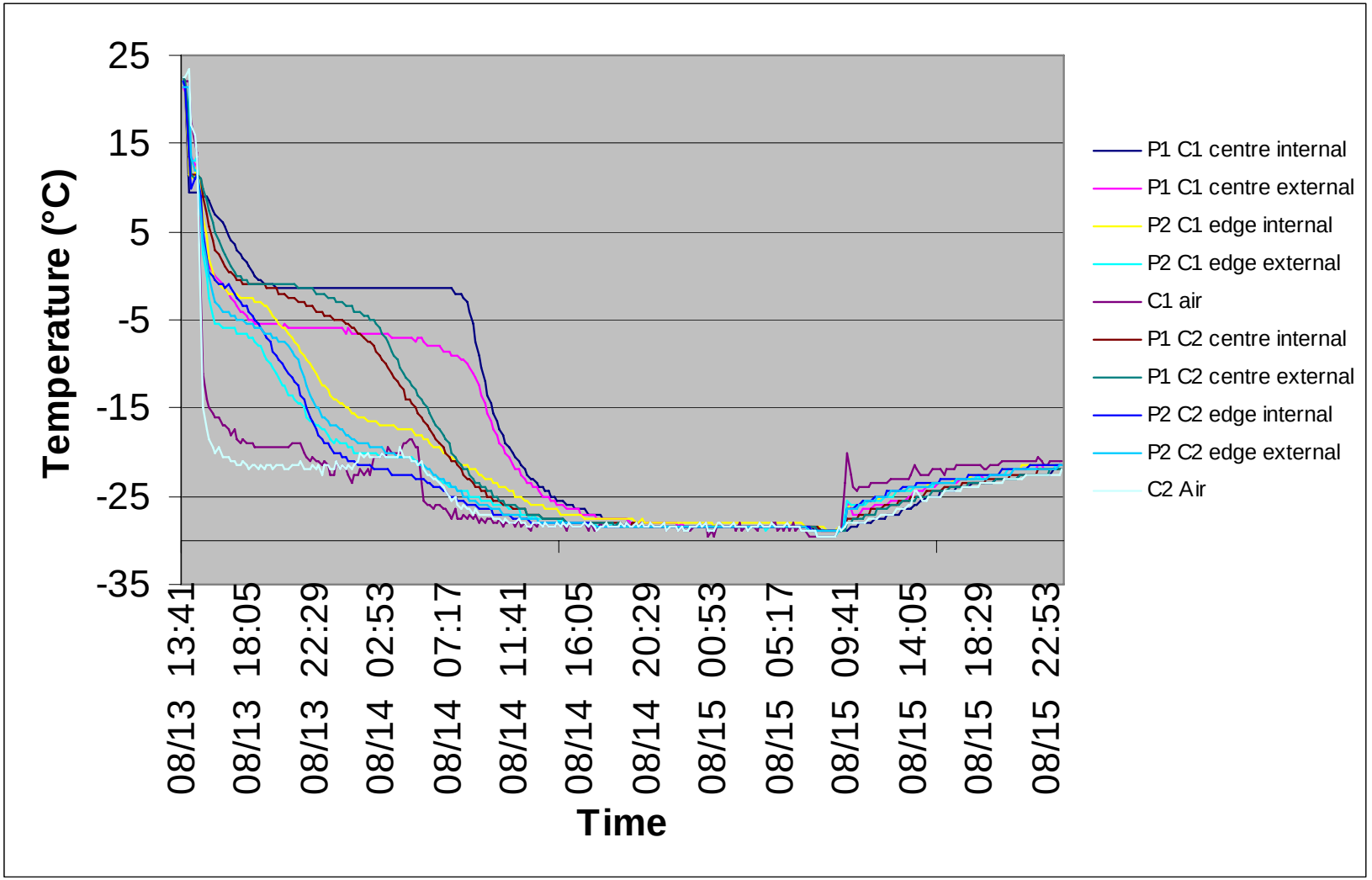

Figure 3: Temperature profiles for commercial blast freezing of skin-on chicken portions (P = portion #; C = carton #; centre/edge = portion location within carton; internal/external = logger location within portion)

In terms of differences between commercial and domestic freezing, the time required to reduce the temperature of the external sample surfaces to -10°C by commercial freezing was approximately 4 to 5 hours shorter in duration (4.2 – 16.4 hours) than domestic freezing (9.7 – 20.8 hours). To reduce the external temperature to -20°C took 9.8 to 19.2 hours by commercial freezing, while domestic freezing times ranged from 24 to 332 hours to reach -18°C (McIntyre, 2008). The larger range of times noted for domestic freezing is unsurprising given that the domestic and commercial blast freezers operated at different temperatures (-18 versus -30°C) and the rate of change in temperature as it nears the ambient temperature of the freezer slows dramatically. However, it appears that the initial stages of freezing are not greatly different in terms of time, and that external surfaces would be less affected by differences in freezing rate than internal tissues.

Following blast freezing and storage, samples were relocated to a domestic fridge-freezer. The temperature profiles for controls over 8 weeks of domestic storage are shown in Figure 4. External mean temperatures for samples placed in the centre and edge of the top freezer compartment respectively (P1 C1 and P2 C1) were -17.4 and -18.1°C, while samples placed into the centre and edge of the bottom compartment (P1 C2 and P2 C2) registered respective mean external temperatures of -18.2 and -17.0°C. The top and bottom shelf mean air temperatures were -17.9 and -17.5°C respectively. Several temperature spikes (below -5°C) representing automatic defrost cycles are apparent. One large spike in air temperature, reaching between 4 and 5.5°C, also occurred on day 42 (half way through domestic storage). While the cause of this temperature increase cannot be confirmed, it coincided with the removal of samples for day 42 of testing. It is likely that the freezer door was not closed properly at the time of sample removal, or alternatively that it opened when the fridge door was subsequently closed (after samples were placed into the fridge for thawing). Analysis of control temperatures revealed that the freezer door was closed at some point during the following hour and samples remained frozen despite the brief air temperature rise, thus this event is unlikely to have influenced subsequent data for samples tested on days 56 and 70.

3.2 Decline in *C. jejuni* numbers following commercial freezing and storage

The effect of commercial freezing and frozen storage on numbers of *C. jejuni* on skin-on chicken breast portions is shown in Figure 5 (see Appendix 2 and 3 for individual counts). Portions were surface inoculated with diluted suspensions of STu48 and ST474 at mean starting levels of 4.59 and 4.89 $\log_{10}$ cfu respectively prior to freezing. *C. jejuni* was enumerated using a rinse method followed by plating on mCCDA incubated at 37°C for 48 hours prior to counting. Multiplex PCR testing confirmed that randomly selected strains from day 0, 1 and 70 enumeration plates were *C. jejuni* (see Appendix 1).

A significant reduction ($p<0.05$) in mean *C. jejuni* numbers was observed following 2 days of frozen storage at -21°C (see Appendix 4 for statistical analysis). However, reductions then decreased after 7 days, followed by a more gradual increase over the following 35 days. Mean reductions between days 14 and 28 were significantly different ($p<0.05$). However, beyond 28 days of frozen storage, reductions generally levelled off as previously observed in the domestic freezing experiments and no statistically significant differences were noted ($p>0.05$). The greatest individual *C. jejuni* reduction (of almost 4 $\log_{10}$ cfu) was observed for strain STu48 on chicken thawed at 4°C after 42 days of frozen storage. The initial spiking effect after 1 to 2 days of freezing was not deemed of particular significance overall given

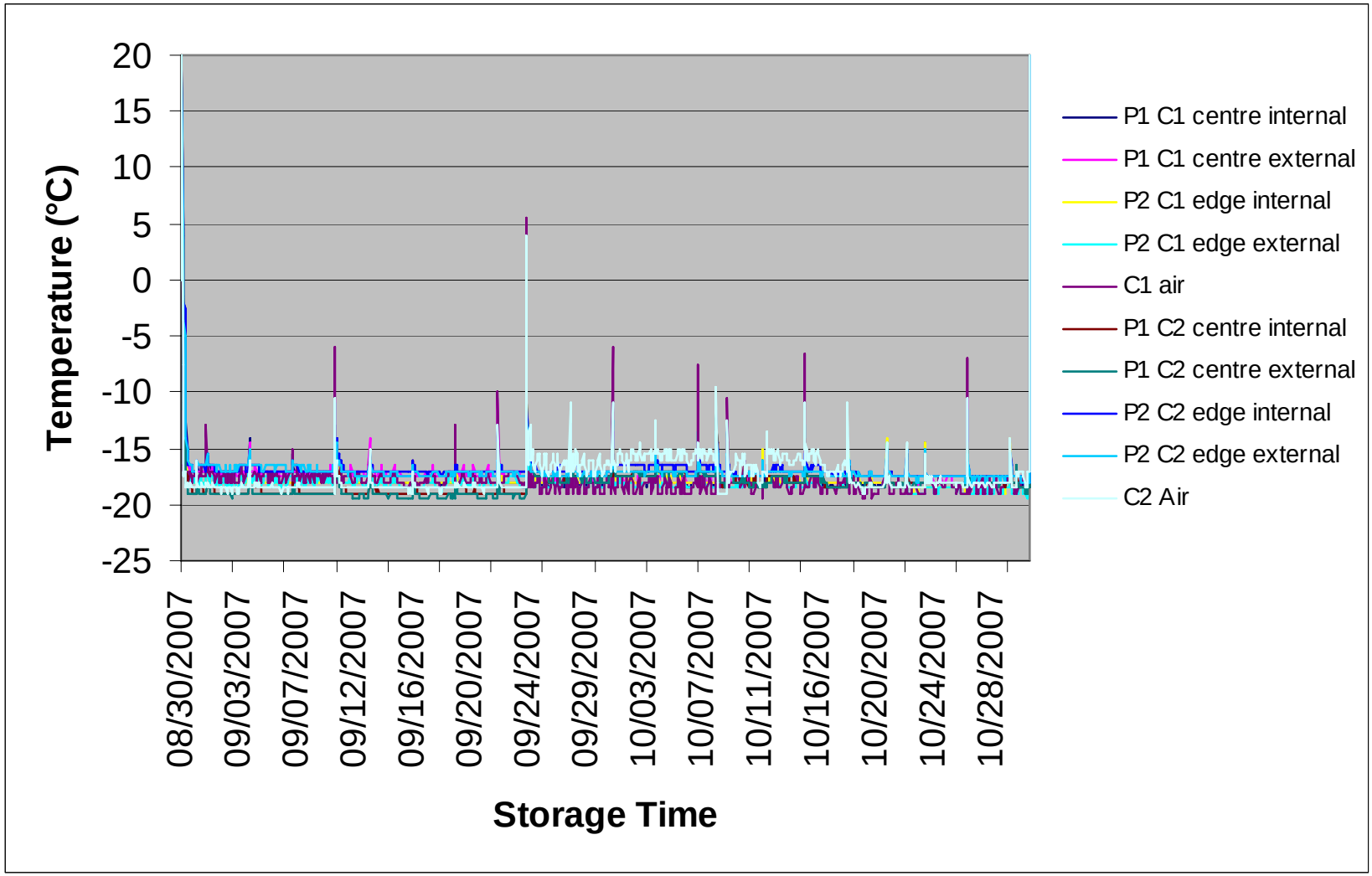

Figure 4: **Temperature profiles for 8 weeks of domestic frozen storage of skin-on chicken portions (P = portion #; C = carton #; centre/edge = portion location within carton; internal/external = logger location within portion)**

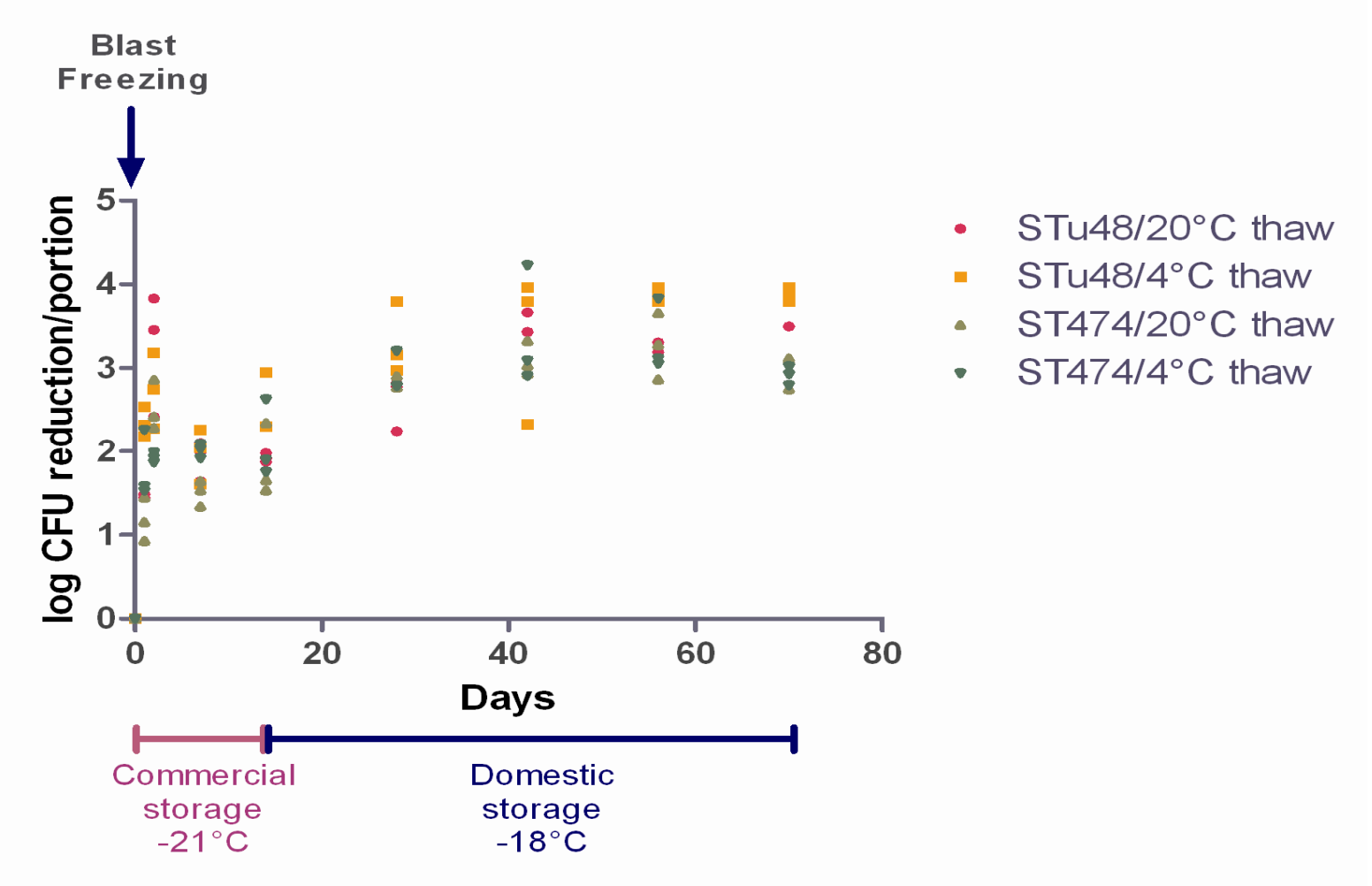

Figure 5: **Effect of commercial blast freezing (-30°C) and frozen storage on the reduction of *C. jejuni* strains STu48 and ST474 on skin-on chicken breast portions thawed at either 4 or 20°C.**

that frozen portions are typically held at -21°C for at least a week prior to distribution to supermarkets for purchase by consumers (Brendon Hasson, Tegel Foods, Pers. Comm.). Nonetheless, it is interesting to speculate on the cause of this data spike. Based on analysis of temperature data, samples removed from commercial frozen storage 1 day after blast freezing were increasing in temperature (ranging from -26 to -24.5°C) at the time of sampling, while the temperature of subsequent samples collected a day later (-22°C) would have been closer to the ambient air temperature of frozen storage. Given that this spiking effect was not observed for domestically frozen samples, it is possible that it occurred as a consequence of increased freeze-thaw stress related to the blast freezing process although, as previously mentioned, faster freezing is not typically as detrimental to bacteria as slower rates of freezing. However, given that *Campylobacter* is acknowledged to be more susceptible to freezing than other pathogens, it is hypothesised that some sub-lethal injury occurred under the more severe blast freezing conditions which, in combination with thawing, manifested in reduced counts early in experimentation. However, this cannot be experimentally confirmed as non-selective agar was not used for enumeration.

The effect of commercial freezing, frozen storage and thawing temperature on reductions of the two strains is further detailed in Table 3. The reduction in *C. jejuni* counts was most rapid during the first 14 days of frozen storage, with a mean reduction of 2 $\log_{10}$ cfu. Additional smaller reductions (of up to 0.9 - 1.5 $\log_{10}$ cfu) occurred over longer term storage. This trend is in agreement with other publications including Georgsson *et al.* (2006) who investigated the influence of freezing and duration of storage on *Campylobacter* in broiler carcasses. These carcasses were also commercially frozen at a poultry plant, but processing conditions were not described, making further comparisons difficult.

Table 3: Mean reduction of *C. jejuni* strains STu48 and ST474 after commercial freezing (-30°C), frozen storage (-21°C for 2 weeks followed by -18°C for 8 weeks) and thawing at either 4 or 20°C

Days of storage (carton #)	*C. jejuni* reductions ($\log_{10}$ cfu per portion)							
	STu48 20°C thaw / 17 h		STu48 4°C thaw / 41 h		ST474 20°C thaw / 17 h		ST474 4°C thaw / 41 h	
	Reduction	S.D.	Reduction	S.D.	Reduction	S.D.	Reduction	S.D.
Frozen commercial storage (-21°C)								
1 (C1)	1.470	0.023	2.347	0.178	1.175	0.260	1.790	0.408
2 (C1)	>3.233	0.734	2.737	0.456	2.521	0.301	1.924	0.061
7 (C1)	1.909	0.241	1.967	0.334	1.502	0.151	2.012	0.089
14 (C1)	1.920	0.054	2.622	0.456	1.841	0.436	2.093	0.468
Frozen domestic storage (bottom-loading fridge-freezer; -18°C)								
28 (C1&2)	2.613	0.320	>3.308	0.434	2.813	0.076	2.927	0.242
42 (C2)	3.631	0.185	3.364	0.900	3.092	0.206	3.408	0.715
56 (C2)	3.431	0.323	3.891	0.086	3.265	0.395	3.332	0.433
70 (C2)	3.790	0.256	3.891	0.086	2.985	0.204	2.914	0.112

Maximum mean reductions varied between 42 and 70 days depending on the strain and thawing temperature, and further data comparisons are prohibited by the variability of individual reductions obtained. However, overall, these commercial freezing data show mean *C. jejuni* reductions increasing from 1.8 to 3.5 $\log_{10}$ cfu over a 1 to 6 week storage period following commercial freezing, which is in agreement with published data previously reviewed for the domestic freezing report (Sandberg *et al*., 2003; Zhao *et al*., 2003; Bhaduri

& Cottrell, 2004; Georgsson *et al*., 2006; El-Shibiny *et al*., 2007; Ritz *et al*., 2007). It should however be noted that (i) these are "best case scenario" reductions given that *C. jejuni* survives less well on chicken skin as opposed to muscle (Ritz *et al*., 2007), and (ii) commercial freezing and subsequent storage did not completely eliminate either of the *C. jejuni* strains, with levels of survival ranging from 0.7 – 1 $\log_{10}$ cfu STu48 and 1.7 – 2.2 $\log_{10}$ cfu ST474 per portion after 70 days of frozen storage.

Mean reductions in *C. jejuni* following commercial freezing and storage at timepoints up to 28 days were statistically different ($p<0.05$) to those achieved using domestic freezing and identical storage periods, with reductions up to 0.8 $\log_{10}$ cfu higher under commercial freezing conditions as compared with the results from Part 1 of the domestic freezing work (see Appendix 5a for statistical analysis). However, beyond 28 days of storage, no significant differences in reductions between the two freezing processes were observed, with the exception of day 56 data which were unusually low in the domestic freezing trial, and therefore significantly different when compared to the commercial freezing data for the same time point. Similar statistical outcomes (Appendix 5b) were obtained when part 2 data from the domestic freezing work were included in the overall analysis, although in this case day 70 data were also deemed significantly different. However, it should be noted that most of the colony counts for samples tested at 42 days and beyond were below the generally accepted lower limit of 30 colonies for plating, and would be considered less reliable as a consequence.

Clearly, while freezing has a significant impact on the reduction of *C. jejuni* on poultry, the results of this work and the previous domestic freezing project suggest that freezing is an inconsistent process subject to variability related to a number of important factors; e.g. the chicken types being processed (skin-on versus skin-off for example), different processor practices, different consumer freezer types and consumer habits regarding duration of storage of frozen foods prior to use. Commercial freezing information was unavailable at the time of writing, so the degree of variability between poultry processors is unknown. At the consumer level, given that only one domestic freezer type was employed in this research project the results obtained may not necessarily reflect the situation using other freezer types. However, this may be a more important consideration when evaluating the process of freezing as opposed to frozen storage as was the case in this situation. Finally, the effect of freezing on different poultry strains remains unclear and additional testing of further poultry strains under identified variations in commercial conditions would be required to confirm this.

4 CONCLUSIONS

The results of this study demonstrate that commercial freezing at -30°C followed by frozen storage at -21°C for two weeks followed by -18°C domestic storage for an additional 8 weeks significantly reduces, but does not entirely eliminate, *C. jejuni* on skin-on chicken portions. Commercial freezing and storage for up to 28 days had a more significant impact on mean reductions of *C. jejuni* than previously reported for domestic freezing, but longer term storage (up to 70 days) under either condition produces similar overall reductions. This suggests that consumers should store chicken frozen for at least 4 weeks to achieve maximum pathogen reductions. Given reports of the pathogen's lesser survival on poultry skin (vs. skinned/cut muscle) the results presented here should be considered best case scenario. Overall, results obtained suggest that freezing is an inconsistent process and the magnitude of C. *jejuni* reductions is subject to variability associated with a number of processor and consumer factors.

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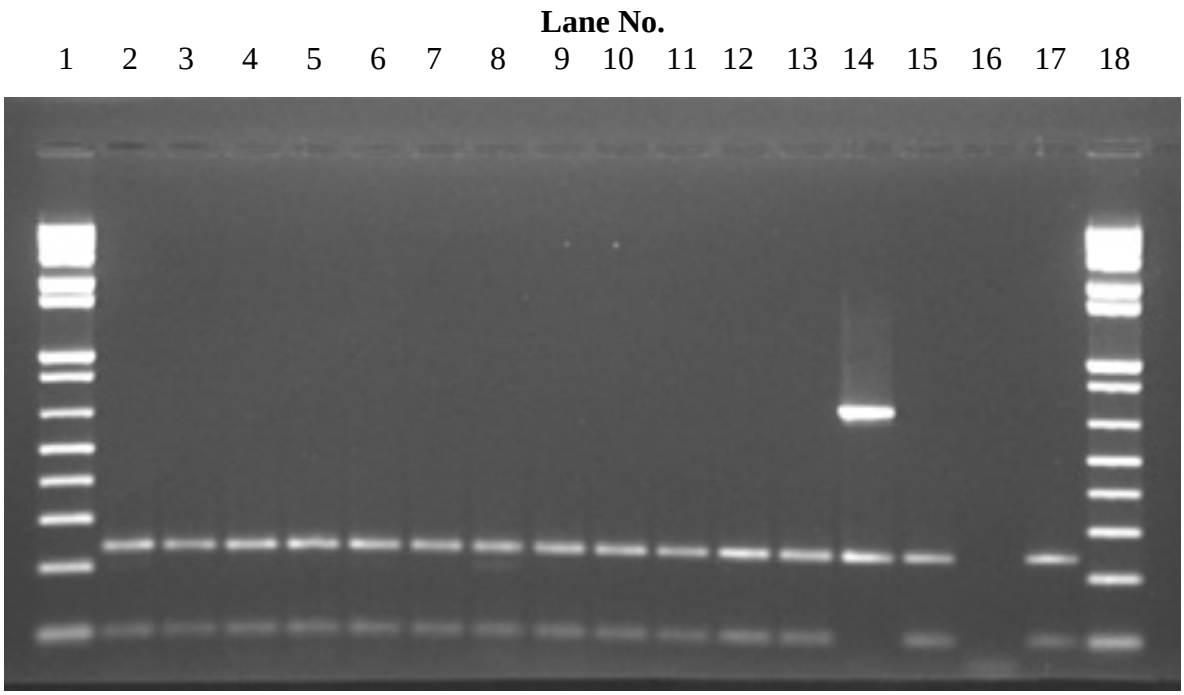
APPENDIX 1: PCR CONFIRMATION OF PRESUMPTIVE COLONIES AS *C. JEJUNI*



Gel electrophoresis of multiplex PCR products. Lanes 1 & 18: molecular weight marker (1 kb Plus DNA); Lane 15: *C. jejuni* control; Lane 14: *C. coli* control; Lane 17: PCR +ve; Lane 16: PCR –ve; Lanes 2 – 5 and 12 – 13: STu48 and ST474 strains from commercial freezing experiment.

APPENDIX 2: RAW DATA FOR REDUCTION OF *C. JEJUNI* STu48

Day	Thaw	log_{10} cfu	Reduction[a]	Day	Thaw	log_{10} cfu	Reduction
0	N/A	4.496238	0	0	N/A	4.496238	0
0	N/A	4.664642	0	0	N/A	4.664642	0
0	N/A	4.607857	0	0	N/A	4.607857	0
1	20	3.010111	1.486126	1	4	2.176091	2.320146
1	20	3.184407	1.480234	1	4	2.480546	2.184096
1	20	3.165013	1.442844	1	4	2.070243	2.537614
2	20	2.082785	2.413452	2	4	2.221740	2.274497
2	20	1.207500	3.457142	2	4	1.915324	2.749318
2	20	<0.778	>3.829857	2	4	1.422074	3.185783
7	20	2.856528	1.63971	7	4	2.894731	1.601506
7	20	2.679768	1.984874	7	4	2.620945	2.043697
7	20	2.505150	2.102707	7	4	2.351968	2.255889
14	20	2.518514	1.977724	14	4	2.197281	2.298957
14	20	2.792392	1.87225	14	4	1.720159	2.944483
14	20	2.698970	1.908887	14	4	NR	NR
28	20	2.251638	2.244599	28	4	<0.69897	>3.797268
28	20	1.845098	2.819544	28	4	1.508530	3.156112
28	20	1.831799	2.776058	28	4	1.637776	2.970081
42	20	0.698970	3.797268	42	4	0.698970	3.797268
42	20	1.000000	3.664642	42	4	0.698970	3.965672
42	20	1.176091	3.431766	42	4	2.278754	2.329103
56	20	0.698970	3.797268	56	4	0.698970	3.797268
56	20	1.477121	3.187521	56	4	0.698970	3.965672
56	20	1.301030	3.306827	56	4	0.698970	3.908887
70	20	1.000000	3.496238	70	4	0.698970	3.797268
70	20	0.698970	3.965672	70	4	0.698970	3.965672
70	20	0.698970	3.908887	70	4	0.698970	3.908887

[a] Reduction calculated by subtracting individual portion counts after freezing and thawing from corresponding time zero count

N/A: Not applicable

NR: No result

APPENDIX 3: RAW DATA FOR REDUCTION OF *C. JEJUNI* ST474

Day	Thaw	log_{10} cfu	Reduction[a]	Day	Thaw	log_{10} cfu	Reduction
0	N/A	4.830375	0	0	N/A	4.830375	0
0	N/A	4.928396	0	0	N/A	4.928396	0
0	N/A	4.925656	0	0	N/A	4.925656	0
1	20	3.384712	1.445663	1	4	3.247482	1.582893
1	20	4.000434	0.927962	1	4	2.668735	2.259661
1	20	3.774517	1.151139	1	4	3.399097	1.526559
2	20	2.546543	2.283832	2	4	2.844166	1.986208
2	20	2.508530	2.419866	2	4	3.064030	1.864366
2	20	2.066699	2.858957	2	4	3.003645	1.922011
7	20	3.300921	1.529453	7	4	2.914989	1.915386
7	20	3.290727	1.637669	7	4	2.896832	2.031564
7	20	3.586489	1.339167	7	4	2.835127	2.090529
14	20	3.177536	1.652838	14	4	2.932410	1.897964
14	20	3.397940	1.530456	14	4	3.175004	1.753392
14	20	2.586996	2.33866	14	4	2.298580	2.627076
28	20	2.062582	2.767793	28	4	1.623249	3.207125
28	20	2.028029	2.900367	28	4	2.144574	2.783822
28	20	2.155673	2.769982	28	4	2.135133	2.790523
42	20	1.903090	2.927285	42	4	1.740363	3.090012
42	20	1.903090	3.025306	42	4	2.021189	2.907207
42	20	1.602060	3.323596	42	4	0.698970	4.226686
56	20	1.176091	3.654284	56	4	1.000000	3.830375
56	20	1.653213	3.275183	56	4	1.812913	3.115482
56	20	2.060698	2.864958	56	4	1.875061	3.050595
70	20	1.740363	3.090012	70	4	1.812913	3.017461
70	20	1.812913	3.115482	70	4	2.000000	2.928396
70	20	2.176091	2.749565	70	4	2.130334	2.795322

[a] Reduction calculated by subtracting individual portion counts after freezing and thawing from corresponding time zero count

APPENDIX 4: STATISTICAL ANALYSIS OF *C. JEJUNI* DATA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	120.8766	15.109575	54.52	<.0001
Error	98	27.158495	0.2771275		
Corrected Total	106	148.0351			

Least Squares Means for effect Day
Pr > |t| for H0: LSMean(i)=LSMean(j)
Adjustment for Multiple Comparisons: Tukey-Kramer
Dependent Variable: log10 cfu

i/j	Day 0	Day 1	Day 2	Day 7	Day 14	Day 28	Day 42	Day 56	Day 70
Day 0		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Day 1			0.0017	0.9986	0.7673	<.0001	<.0001	<.0001	<.0001
Day 2				0.0183	0.259	0.8754	0.0149	0.0029	0.0109
Day 7					0.9878	<.0001	<.0001	<.0001	<.0001
Day 14						0.0055	<.0001	<.0001	<.0001
Day 28							0.4571	0.1897	0.3939
Day 42								0.9999	1.0000
Day 56									1.0000
Day 70									

APPENDIX 5: STATISTICAL ANALYSES OF *C. JEJUNI* REDUCTION DATA FOR COMMERCIAL VERSUS DOMESTIC FREEZING (MICROSOFT EXCEL)

a. T-test results comparing commercial and domestic freezing data (part 1)

commercial v domestic	p-value
Day 1	<0.0001
Day 2	<0.0001
Day 7	<0.0001
Day 14	0.0003
Day 28	0.0011
Day 42	0.6967
Day 56	<0.0001
Day 70	0.0570

b. T-test results comparing commercial and domestic freezing data (parts 1& 2 combined)

commercial v domestic	p-value
Day 1	<0.0001
Day 2	<0.0001
Day 7	<0.0001
Day 14	0.0003
Day 28	0.0011
Day 42	0.1792
Day 56	<0.0001
Day 70	0.0352