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ORIGINAL ARTICLE

Assessment of the hygienic performances of two beef carcass cooling processes from product temperature history data or enumeration of bacteria on carcass surfaces

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The carcass cooling processes at two beef slaughtering plants were examined. Temperature histories were collected from the deep leg, the aitch bone pocket surface and randomly selected surface sites of carcasses passing through each process. For each process, sets of 25 temperature histories were collected for each type of site, with a single history being collected from each of 75 randomly selected carcasses. A swab sample was obtained from a randomly selected site on each of 25 randomly selected carcasses entering and 25 leaving each process. Total aerobic counts, coliforms and Escherichia coli *were enumerated in each sample. Carcasses resided in the chiller at plant A for between 15·8 and 28·0 h, but for between 20·0 and 24·0 h at plant B. The ranges of minimum temperature attained at all three types of site were generally lower at plant B than at plant A. However, 1/25 carcasses at both plants had high minimum temperatures indicative of ineffective cooling. An* E. coli *proliferation value was calculated for each temperature history from a surface site. The sets of proliferation values for aitch bone pocket sites on carcasses passing through either process complied with three points of a four point criteria for acceptable carcass cooling, but one value in each exceeded the stipulated maximum value. Proliferation values for randomly selected sites indicated that if temperature alone controlled bacterial growth during cooling, then numbers of* E. coli *on cooling carcasses would on average increase by about 1 log unit at plant A but by only about 0·3 log units at plant B. However, enumeration of bacteria showed that cooling reduced the mean numbers of total counts, coliforms and* E. coli *on carcasses at plant A by <0·5 log units, while at plant B, cooling reduced the mean numbers of total counts by about 0·5 log units, and mean numbers of coliforms and* E. coli *by 2 log units. The findings indicate that microbiological data are required to properly assess the hygienic effects of carcass cooling processes, but that temperature history data may be conveniently used for monitoring the maintenance of standard operating procedures in such processes.*

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Introduction

During carcass dressing the surfaces of carcasses are contaminated with bacteria, which

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may include pathogenic species. While the surfaces of carcasses remain warm, growth of contaminating pathogens may occur. It is therefore hygienically desirable that carcass cooling processes be well controlled in order to restrict any possible proliferation of pathogens. Demonstration of such control would seem to be a necessary part of the Hazard Analysis Critical Control Point (HACCP) systems that slaughtering plants are being required to implement (USDA 1996). However, there is as yet no generally accepted means of assessing the hygienic performances of carcass cooling processes. This deficiency arises because factors other than temperature can also affect bacterial growth on carcass surfaces. As well as this, temperature and other air conditions in the vicinity of carcass surfaces can vary widely within a chiller.

Traditional carcass cooling involves the exposure of carcasses to a flow of cold air only. In such processes, evaporation of water from the carcasses tends to dry the carcass surfaces. Surface drying will inhibit the growth, and can even reduce the numbers of bacteria on carcasses. Thus, it has been shown, in experimental circumstances, that adjustments of the temperature, humidity and speed of the air to which cooling carcasses are exposed can result in increases, decreases and even no change in the total numbers of aerobic bacteria recoverable from the carcasses (Nottingham 1982).

Traditional cooling process practices result in loss of carcass weight (James and Bailey 1990). Consequently, meat packers in North America have widely adopted the practice of spraying carcasses with water during the first hours of chilling. Such spray-chilling is uncommon elsewhere, in part because of regulatory concerns that the intentional prevention of surface drying could allow the uncontrollable proliferation of pathogenic bacteria. However, industrial experience has not substantiated that concern, while experimental studies of spray-chilling have shown that, as with traditional chilling, the total numbers of bacteria on carcasses can be increased, decreased or maintained by adjustments of air temperatures and speeds, and of the frequencies and duration of spraying cycles (Greer et al. 1990, Strydom and Buys 1995).

Despite the evident complexity of the interactions between the environmental conditions within chillers and the behaviour of bacteria on cooling carcasses, practical considerations have resulted in proposals that the hygienic performance of carcass cooling processes be assessed on the basis of temperature data alone. The simplest forms of such proposals refer to the deep-leg temperatures of carcasses. That is, the proposals refer to the temperature at the slowest-cooling region of the carcass. There are various criteria for acceptable carcass cooling based on deep-leg temperatures. These may stipulate: a maximum time for the temperature to fall to 7°C, or to some other temperature regarded as being sufficiently low to preclude any further substantial growth of pathogens; an acceptable cooling curve; or two cooling curves that define a range of acceptable temperatures at any stage of the cooling process (Bailey 1986).

As such criteria relate only indirectly to surface conditions and take no account of the large effects of temperature on rates of bacterial growth, they would seem to be inherently flawed for assessing the possible growth of bacteria on carcass surfaces. It has, therefore, been suggested that carcass cooling processes be assessed by the collection of temperature histories from the slowest cooling area on the surface of each carcass in a set selected at random from the carcasses passing through a process, with calculation of the *Escherichia coli* proliferation that each temperature history would allow (Gill et al. 1991a). The hygienic performances of the process would then be assessed from the distribution of *E. coli* proliferation values.

Although such a procedure does allow some comparison of processes in terms of possible bacterial growth related to pathogen proliferation, it suggests increases in *E. coli* numbers of far larger magnitudes than are generally seen to occur, at least in part because no direct account is taken of the range of temperatures at different points on the surface of any cooling carcass. That deficiency might be remedied by collecting temperature histories from randomly selec-

ted sites rather than the warmest site on cooling carcass surfaces, but such a procedure has not yet been investigated.

Even if such a procedure was practicable, the data could be properly interpreted only if reliable relationships between such data and the actual behaviour of the indicator organism on carcasses cooling in commercial processes could be discerned. Obviously, the same caveat also applies to the procedures for assessing the hygienic performances of chilling processes from other temperature data.

A procedure for more directly assessing changes in the numbers of *E. coli* on carcasses as a result of processing has been lacking. However, such a procedure has recently been proposed. The procedure involves the collection of swab samples from randomly selected sites on randomly selected carcasses entering or leaving a process, or operation, with the enumeration of *E. coli* in each sample to a detection level of 1 cfu 100 cm^{-2} (Gill et al. 1996). With the assumption that the bacteria on carcasses are log normally distributed, the mean numbers of *E. coli* on the carcass population at entry to or exit from a process or operation can be estimated from a set of values for *E. coli* numbers from 25 samples. Comparison of the estimated mean numbers on carcasses at entry or exit allows a direct assessment of the hygienic effects with respect to the safety of the process or operation. This procedure, and temperature monitoring procedures were therefore applied for assessment of the hygienic performances of two spray-chilling processes for beef carcasses, to discern the views of the processes likely to arise from each assessment procedure.

Materials and Methods

The cooling processes

During winter months, the carcass cooling processes were concurrently examined at two beef packing plants that each slaughter approximately 2000 cattle during a daily work shift. The chillers at both plants are of modern design, although beef sides are manually spaced on the rails in the chiller at plant A but are automatically spaced in the chiller at plant B. In both chillers the beef sides are sprayed periodically with water of approximately 4°C, while refrigerated air at a temperature of 2°C is blown from the coils, during loading and for the first 8 h after completion of loading of each chiller. For the remainder of the chilling process spraying is discontinued, and the off-coil air temperature is reduced to about –5°C.

Collection of temperature histories

Temperature histories were collected using MIRINZ -Delphi temperature data loggers (True-Test, Auckland, New Zealand), each fitted with an external thermistor probe encased in a tapered Teflon sheath. The loggers were set to record temperatures between +40 and –20°C, with an accuracy of ±0·25°C and resolution of 0·25°C, at intervals of 1·875 min.

For recording deep temperature, the probe was inserted at the thickest point of the hind leg until the tip lay at the centre of the tissue mass in that region.

For recording surface temperature, a disc of stainless steel, (40 mm diameter) was held against the carcass surface by means of a plastic staple passed through holes at opposite sides of the disc. The probe was then inserted into a cone-shaped slot running across the diameter of the disc between the two holes for the retaining staple. The dimensions of the slot were such that its inner surfaces fitted tightly into the probe sheath when the temperature-sensitive probe tip lay at the centre of the disc. The discs for probe retention at meat surfaces are manufactured by the Meat Industry Research Institute of New Zealand, Hamilton, New Zealand.

For the collection of temperature histories from the slowest cooling area of the carcass surface, discs were placed within the aitchbone pocket, on the psoas major/minor muscle complex, as caudally and medially as possible. This region has been previously identified as the slowest cooling area of beef side surfaces (Scott and Vickery 1939), and the procedure for collecting the temperature history of that area has been fully described (Gill et al. 1991a). For the collection of tem-

perature history from other areas of the carcass surface, discs were placed at sites selected at random from a grid that specifies 126 areas of the beef side surface (Gill et al. 1996). After placement of each probe, the logger was attached to a convenient point on the beef side by means of a skewer passed through the unsealed lip of the plastic pouch in which each logger had been sealed.

At each plant, on each of 5 days, loggers were placed to record five deep, five slowest cooling surface and five random surface site temperature histories, with a single logger being placed with each of 15 carcasses selected at random from these entering the chiller. Each logger was recovered within 10 mins of the monitored beef side exiting the chiller.

Integration of surface temperature histories with respect to the growth of E. coli

Each surface temperature history was integrated with respect to a model describing the relationship between temperature and the rate of the aerobic growth of *E. coli*. The model has the form:

$y=(0{\cdot}0513x-0{\cdot}17)^2$ when x is between 7 and 30°C;
$y=(0{\cdot}027x+0{\cdot}55)^2$ when x is between 30 and 40°C;
$y=2{\cdot}66$ when x is between 40 and 47°C; and
$y=0$ when x is <7 or >47°C;

where y is the growth rate expressed as generations h^{-1} and x the temperature in °C (Gill et al. 1991a).

The model was applied in a computer program that interrogates a logger—it requests definitions of the times that a product temperature history recording begins and ends, calculates from the model proliferation during each record interval, and derives the total proliferation by summation of the incremental proliferations (Gill et al. 1988).

Microbiological sampling and analysis

At each plant, on each of 5 days, five samples were collected from beef sides selected at random from sides entering the chiller, and similarly, from sides exiting the chiller. The site for sampling each carcass was selected at random from the grid also used for the selection of random sites from which surface temperature histories were collected.

Each sample was obtained by swabbing an unlimited area of approximately 100 cm^2 with a 5×5 cm, 8 ply, sterile gauze swab (Curity gauze sponge; Kendall Canada Inc. Peterborough, ON, Canada) which had been moistened with 0·1% w/v peptone water. Each swab was placed in a separate Stomacher bag, which was then immersed in slush ice until each swab was processed within 3 h of being collected.

Each swab was stomached for 2 min, with 10 ml of 0·1% w/v peptone water, using a Colworth Stomacher 400 (Baxter Diagnostic Corp., Edmonton, AB, Canada). A 1 ml portion of each homogenate was used to prepare 10-fold dilutions to 10^{-3}, in 0·1% w/v peptone water. Portions of 0·1 ml of the homogenate and each dilution were spread on duplicate plates of plate count agar (PCA; Difco, Detroit, MI, USA). The plates were incubated for 2 days at 25°C. Flora numbers were preferably determined from plates bearing 20 to 200 colonies, when such numbers were available.

After the preparation of the spread plates, a 0·1 ml portion of each homogenate was diluted in 10 ml of ice-cold peptone water then stored on ice in a refrigerator. The remaining homogenate was filtered through the prefilter of an Iso-Grid^R filtration unit (QA Laboratories, Toronto, ON, Canada) and a hydrophobic-grid membrane filter (QA Laboratories) clamped in the unit. The swab within the Stomacher bag was squeezed to expel the homogenate that it otherwise retained. Each membrane filter was removed from the filtration unit and was placed on a plate of lactose monensin gluconurate agar (LMGA; QA Laboratories). The LMGA plates were incubated at 35°C for 24 h. The filters were examined under 5× magnification using a Model 101 Iso-Grid Line Counter (QA Laboratories), and the squares containing blue colonies were counted. The counts were converted to a most probable number (MPN) of coliforms by application of the formula $MPN=n\times \ln(n/(n-X))$, where n is the total number of squares on a filter, and X is the count of squares containing blue colonies (Entis and Boleszezuk 1990). Each filter was

then transferred to a plate of buffered 4-methylumbelliferyl-β-D-glucuronide agar (BMA; QA Laboratories). The BMA plates were incubated at 35°C for 3 h before being examined under magnification as for the LMGA plates, but with the BMA plates illuminated with long wave-length ultraviolet light from a UVL-56 Blak-Ray[R] lamp (UVP Inc., San Gabriel, CA, USA). Squares containing large, blue-white, fluorescent colonies were counted, and MPN values for *E. coli* were calculated as in the estimation of coliforms numbers.

When *E. coli* and/or coliform numbers in the undiluted homogenate were too numerous to count (log number >4 100 cm^{-2}), the stored, diluted portion of the homogenate was treated as was the undiluted homogenate to obtain coliform and *E. coli* counts.

Analysis of microbiological data

All bacterial counts were transformed to $\log_{10}$ values. Values for the mean log (X) and standard deviation (s.d.) of each set of $\log_{10}$ values were calculated on the assumption that the $\log_{10}$ values were normally distributed (Brown and Baird-Parker 1982). In the calculation of X and s.d. for sets of $\log_{10}$ coliform and *E. coli* counts, values of –0·5 100 cm^{-2} were assumed for samples in which coliforms or *E. coli* were not detected at the level of 1 coliform of *E. coli* 100 cm^{-2}. A value for the $\log_{10}$ of the arithmetic mean (log A) was calculated from the formula $\log A = X + \ln 10\ \text{s.d.}^2/2$ (Kilsby and Pugh 1981). All calculations were performed with Microsoft Excel (Version 4, statistical functions; Microsoft Corp., Redmond, WA, USA).

Results

At plant A, the carcasses resided in the chiller for between 15·8 and 28·0 h, and averaged 21·7 h, with 64% of the residence times being between 21 and 24 h, and 27% of the times being <21 h (Fig. 1). At plant B, the residence times ranged from 20·0 to 24·0 h, and averaged 22·6 h with 96% of the times being between 21 and 24 h.

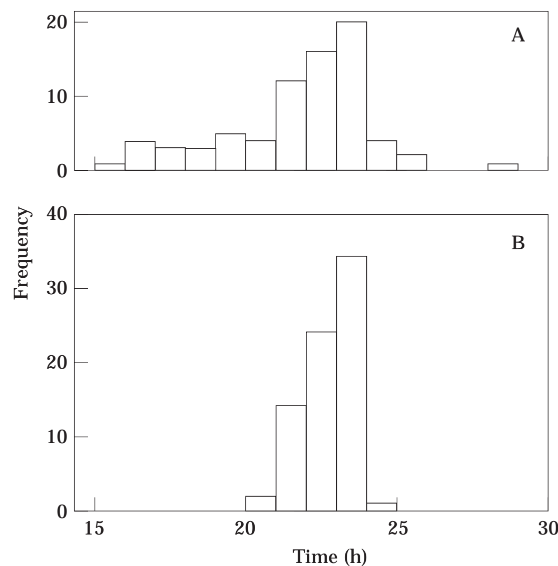

Figure 1. The frequency distributions of the times of residence of 75 carcasses in the chiller at each of two beef slaughtering plants.

Deep leg cooling curves were typically smooth, with initial temperatures between 35 and 40°C. At plant A, the minimum deep leg temperature attained by 24 of the 25 carcasses ranged from 3·5 to 14·0°C, with the remaining carcass attaining a minimum deep leg temperature of 19·0°C (Fig. 2). At plant B, the minimum deep leg temperature attained by 24 of the 25 carcasses ranged from 1·5 to 7·5°C with the remaining carcass attaining a minimum deep leg temperature of 20·3°C.

Cooling curves for surface temperatures within the aitch bone pocket typically showed several periods of an hour or more during which temperatures rose to peak 2 or 3°C above the temperature at the start of each such period, as well as smaller and shorter temperature fluctuations at other times. Initial temperatures were generally between 15 and 25°C. At plant A, the minimum temperatures attained at the aitch bone pocket site ranged from –4·3 to 9·8°C with 17 of those temperatures being <3°C (Fig. 3). At plant B, the minimum temperatures attained at the aitch bone pocket site for 24 of the 25 carcasses ranged from –3·0 to 2·8°C with the equivalent temperature for the remaining carcasses being 11·3°C.

The *E. coli* proliferation values calculated from 23 of the temperature histories from

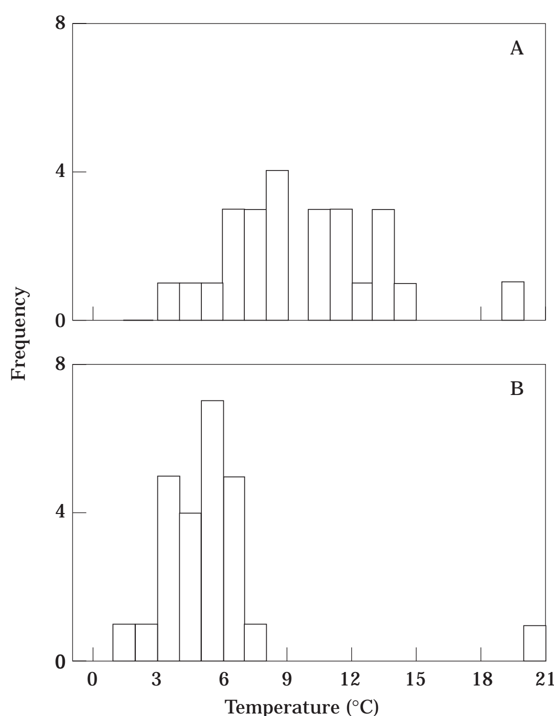

Figure 2. The frequency distributions of the minimum temperatures attained in the deep legs of 25 carcasses on their exit from the chiller at each of two beef slaughtering plants.

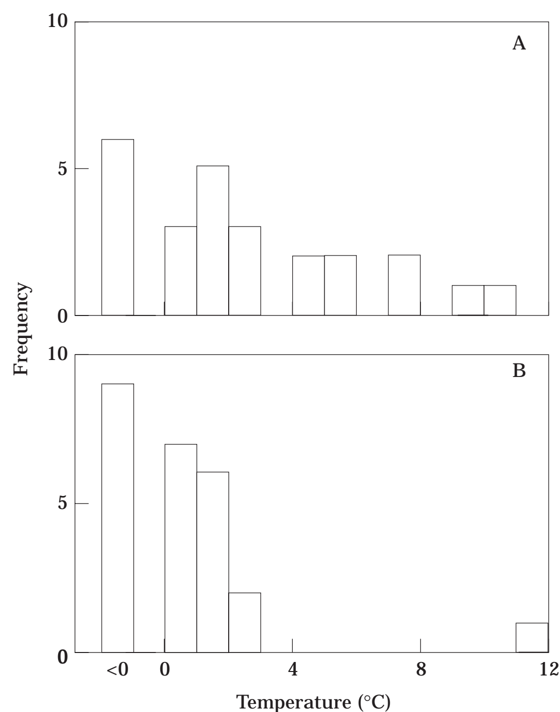

Figure 3. The frequency distributions of the minimum temperatures attained at the aitch bone pocket surface site of 25 carcasses on their exit from the chiller at each of two beef slaughtering plants.

plant A were between 0·7 and 5·9 generations, with the remaining values being 8·3 and 15·3 generations (Fig. 4). The *E. coli* proliferation values calculated from 24 of the temperature histories from plant B were between 0·2 and 4·4 generations, with the remaining value being 24·7 generations. The average proliferation values for plants A and B were 3·8 and 2·9 generations, respectively.

Cooling curves for surface temperature at randomly selected sites were of similar erratic form to those for aitch bone pocket surface sites but with the temperatures often falling to 10°C or below within the first hour. At plant A, the minimum temperatures ranged from –4 to 14·8°C, with 20 of those temperatures being <3°C (Fig. 5). At plant B, 23 of the 25 minimum temperatures ranged from –3·5 to 2°C, with the minimum temperature for the remaining randomly selected sites being 4·8 and 16·5°C.

The *E. coli* proliferation values calculated from 23 of the temperature histories from plant A were between 0·4 and 5·8 generations, with the remaining values being 9·9 and 20·3 generations (Fig. 6). The *E. coli* proliferation values calculated from 24 of the temperature histories from plant B were between 0·0 and 2·0 generations, with the remaining value being 14·5 generations. The average proliferation values for plants A and B were 3·2 and 1·1 generations, respectively.

At plant A, total counts were recovered from all samples from carcasses entering or leaving the chiller, but coliforms and *E. coli* were recovered from fewer carcasses leaving than from those entering the chiller (Table 1). The estimated values for log mean numbers (log A) and the log total numbers recovered (*n*) indicated that numbers of total counts, coliforms and *E. coli* on carcasses were similarly reduced by the chilling process, by <0·5 log units. At plant B, total counts were recovered from all samples for carcasses entering or leaving the chiller, and coliforms and *E. coli* were recovered from 24 of the 25 carcasses entering the chiller. However,

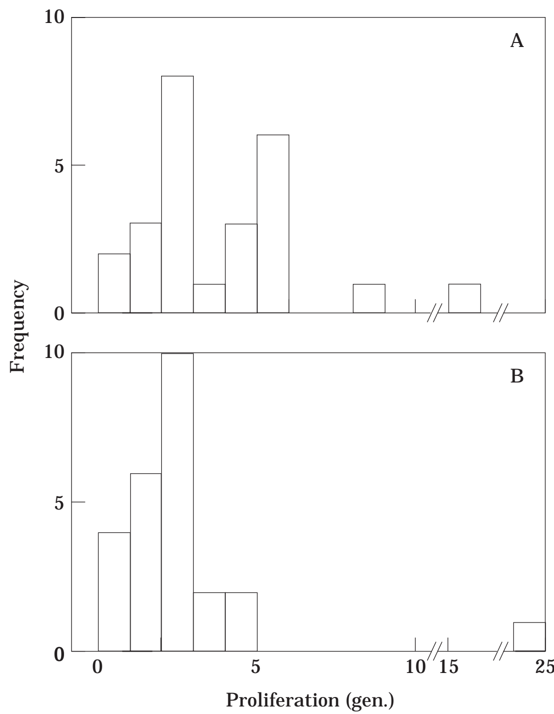

Figure 4. The frequency distributions of the *Escherichia coli* proliferations calculated from temperature histories from the aitch bone surface site of 25 carcasses passing through the chiller at each of two beef slaughtering plants.

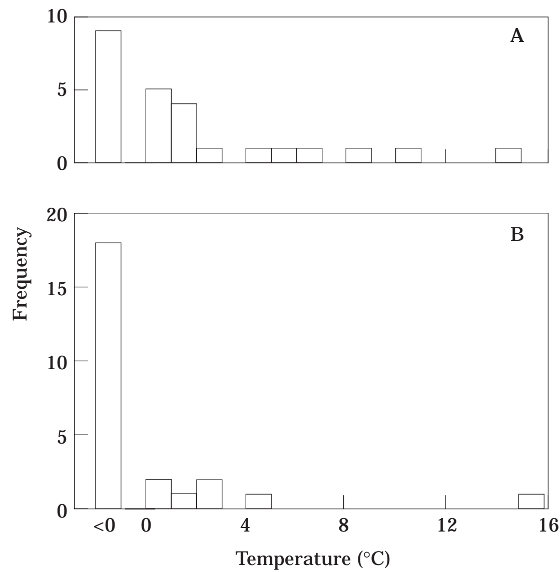

Figure 5. The frequency distributions of the minimum temperatures attained at randomly selected sites on the surfaces of 25 carcasses on their exist from the chiller at each of two beef Slaughtering plants.

Table 1 Statistics for sets of 25 total aerobic counts (No cm^{-2}), coliform counts (No 100 cm^{-2}) or *Escherichia coli* counts (No 100 cm^{-2}) obtained from randomly selected sites on randomly selected carcasses entering or leaving the chillers at two beef slaughtering plants

Plant	Stage of the Process	Count	Statistics				
			X	s	no	log A	*n*
A	Entry	Total	3·06	0·92	0	4·03	5·19[a]
		Coliform	1·25	0·70	0	1·81	3·11[b]
		E. coli	0·02	0·55	11	0·37	1·72[b]
	Exit	Total	3·04	0·68	0	3·58	4·91[a]
		Coliform	0·46	0·89	8	1·37	2·96[b]
		E. coli	0·27	0·43	18	0·06	1·34[b]
B	Entry	Total	2·35	0·82	0	3·12	4·55[a]
		Coliform	1·13	0·88	1	2·02	4·02[b]
		E. coli	1·08	0·90	1	2·01	3·87[b]
	Exit	Total	1·87	0·73	0	2·48	3·76[a]
		Coliform	–0·23	0·39	15	–0·06	1·34[b]
		E. coli	–0·26	0·32	15	–0·14	1·15[b]

X = mean log
s.d. = standard deviation
no = number of samples from which bacteria were not recovered
log A = estimated log of the arithmetic mean
n = log total number recovered from [a]25 cm^2 or [b]2500 cm^2.

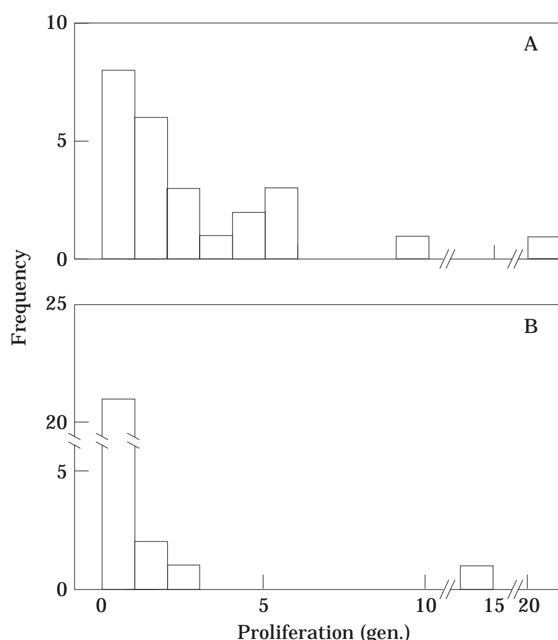

Figure 6. The frequency distributions of the *Escherichia coli* proliferations calculated from temperature histories from randomly selected surface sites on 25 carcasses passing through the chiller at each of two beef slaughtering plants.

coliforms and *E. coli* were recovered from only 10 of the carcasses leaving the chiller. The estimated values for log mean numbers and the log total numbers recovered indicated that numbers of total counts were reduced by the chilling process by about 0·5 log units, but that the numbers of coliforms and *E. coli* were both reduced by 2 log units.

Discussion

Collection of temperature histories of any types from carcasses passing through the cooling processes would show that the process at plant B was well controlled with respect to the time of carcass cooling whereas the process at plant A was not. Carcass cooling processes have traditionally been assessed by reference to the times required to reduce deep temperatures to 10 or 7°C (James and Bailey 1989). This method of assessment could be readily applied to the process at plant B, as the deep temperatures of most carcasses leaving the chiller were < 7°C, while the much higher, minimum deep temperature in one of the 25 carcasses would indicate that 4% of the carcasses were not being cooled effectively. However, the method could only be applied to the process at plant A by extrapolation of half the cooling curves to obtain estimates of the times required to reach the stipulated temperatures. While such a procedure is possible it is comparatively tedious (Gill et al. 1991b), and so not well suited for routine evaluation of chiller performance. Consideration of the minimum deep temperatures achieved at plant A would indicate a highly variable process performance, but would not necessarily suggest ineffective cooling of some carcasses as opposed to the cooling process being applied to some carcasses for too short a time.

Assessment of the processes on the basis of time and temperature data from temperature histories collected from a site on the surface within the aitch bone pocket would indicate process characteristics, and differences between the processes similar to those indicated by deep leg temperature histories. Both processes could be readily assessed on the basis of time taken to cool to 10°C, as most minimum temperatures for carcasses from plant A as well as for those from plant B were below 10°C, although times for attaining that temperature would sometimes be uncertain because of fluctuations in the cooling curves. Consideration of the minimum temperatures would again indicate generally superior cooling of carcasses at plant B, but with 4% of the carcasses at the plant being ineffectively cooled, while the variable performances of cooling at plant A could well be ascribed to variable times of the cooling process.

However, assessments of the processes from *E. coli* proliferation values would suggest their similarity. A four point criterion for definition of an acceptable carcass cooling process on the basis of *E. coli* proliferation values has been proposed. The criterion states that for proliferation values, calculated from aitch bone pocket surface site temperature histories, from a random sample of >20 carcasses passing through a chilling process, 80% of proliferation values should be <10 generations, none should exceed 14 generations, and that the average proliferation

should be ≦7 generations (Reichel et al. 1991). Both processes would similarly exceed that criterion with respect to three of the points, and fail the criterion on the point of the maximum tolerable proliferation. That point of failure would identify ineffective cooling of 4% of the carcasses at plant A as well as at plant B.

It is recognized that the *E. coli* proliferation criteria would refer only to the maximum possible growth of *E. coli* at some site on each carcass when temperature alone determined the rates of *E. coli* proliferation (Gill et al. 1991a). Temperature alone would then be expected to restrict overall proliferation to a greater degree than would be implied by the proliferation values used with respect to the criterion. Temperature histories from randomly selected sites on carcass surfaces supported this view, as the minimum temperatures recorded from those sites were generally lower than those for the aitch bone pocket site, and *E. coli* proliferation values estimated from randomly selected sites were lower than those estimated for aitch bone pocket sites. The comparative assessments of the two processes would be similar for the data sets from temperature histories from either type of site, but the proliferation values for randomly selected sites would suggest that *E. coli* numbers could, on average, increase by about 1 log unit on carcasses cooling at plant A, but that the average increase at plant B could be no more than 0·3 log units, and so would be difficult to detect. However, extensive proliferation of *E. coli* on 4% of the carcasses at both plants is obviously indicated.

Temperature history data can take no account of factors other than temperature that affect bacterial growth. The *E. coli* proliferation criterion was based on the performance of a carcass cooling process that complied with accepted 'Good Manufacturing Practice', during which, the literature indicates, any changes in the bacterial numbers on carcasses would be likely to be small (Greer and Dilts 1988). Consequently, the small, overall, decreases in bacterial numbers observed for the carcasses cooling at plant A would not conflict with current understanding of the hygienic effects of cooling processes. This view could also encompass the somewhat larger decrease in total counts resulting from the cooling of carcasses at plant B. However, no previous study of the hygienic effects of carcass cooling would suggest the large decreases in the numbers of coliforms and *E. coli* on cooling carcass observed at plant B. In the absence of any other direct information on the matter, any attempt to account for those decreases must be speculative. Even so, the difference in the reductions of total counts and coliforms or *E. coli* suggest that Gram negative bacteria are more susceptible than Gram positive organisms to the decontaminating effects of the cooling process, and thus that the freezing of sprayed water on the carcass surface may be an important factor (Lowry and Gill 1985). Certainly, the temperature history from randomly selected sites showed that >70% of the surface of carcasses at plant B was reduced below 0°C, whereas only half that area of carcass surface fell to those temperatures at plant A.

Whatever the explanation it is apparent that the hygienic effects of a carcass cooling process cannot be assessed with any certainty from temperature history data alone. Instead, the hygienic conditions of carcasses entering and leaving a cooling process must be assessed from appropriate microbiological data. None-the-less, the collection and evaluation of temperature history data remains a valid and convenient means of monitoring the maintenance of Standard Operating Procedures for a carcass cooling process being operated under a Hazard Analysis: Critical Control Point system (NAC 1993). Moreover, temperature history data would be needed to identify ineffective cooling, which would not necessarily be apparent from microbiological data alone.

The findings of this study also imply that regulatory definition of acceptable carcass cooling practices by reference to product temperatures alone is inherently flawed. In the present state of knowledge on the matter, it would instead seem appropriate that regulation require that there be no increase during carcass cooling in the mean numbers of *E. coli* on carcass, as determined by an appropriate method such as that used in this study.

Acknowledgements

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