final report

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Combining refrigeration and predictive models for beef carcases

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Summary

Simplified models of carcase chilling, that provide a prediction of temperature and water activity over the entire carcase surface, were developed in PRMS.043A: "Modelling of Beef Carcass Chilling". Similarly, models for microbial growth based on temperature and water activity have been developed, e.g. the *E. coli* model underlying the Refrigeration Index (RI). Models also exist for microorganisms responsible for spoilage of meat that could also be combined with models for prediction of temperature and water activity changes on carcasses.

The objectives of this project were to:

i) determine whether the RI, which makes a calculation of *E. coli* growth based on temperature data from the slowest cooling point on the carcase and for constant and conservative levels of water activity, pH and lactate leads to unnecessarily conservative estimates of RI;

ii) assess the reliability of the carcase chilling model; and

iii) compare predicted changes in *E. coli* and pseudomonad levels under different cooling regimes and at different sites on the carcase to determine whether there is scope for optimisation of cooling regimes that minimise levels of both organisms.

Carcase temperatures at the butt, flank and brisket for five sides of beef ('sides') in each of four plants, in duplicate, were provided by Food Science Australia. Periodic water activity data for those carcases were also available. Operating parameters for each of the four plants were provided and, using software generated in PRMS.043A, analogous predicted temperatures and water activities on the butt, flank and brisket sites of those sides were generated.

For both the observed and modelled data, the corresponding number of generations of *E. coli* were predicted using the *E .coli* model of Ross *et al.* (2003) and the Refrigeration Index. Growth of psychrotrophic pseudomonads was predicted using the validated model of Neumeyer *et al*. (1997). Additionally, simple metrics of cooling rates and desiccation (carcase drying) were developed and used to identify which, if any, sites on the carcase could be identified as slowest or fastest cooling, or 'driest' *cf.* 'wettest'.

The analyses suggested that the Pham-Trujillo model, developed in PRMS.043A, in its current form does not well describe observed carcase temperature and water activity changes during air cooling. The reason for the apparently poor predictions were not pursued in this study, but it is noted that the model relies on a relatively large number of inputs. The fidelity of the data used as inputs to the carcase chilling model was not assessed in this study. Similar results were obtained by Pham and Trujillo, however, when they used the same Food Science Australia data to assess the performance of the model. As such, some of the original project aims could not be completed because the Pham-Trujillo model could not be relied upon to generate accurate predictions of cooling and drying of carcase surfaces during air chilling.

A number of approaches were used to address the first objective, i.e. the potential over-estimation of *E. coli* growth by the RI as it is currently applied. It was found that, as expected, the butt cools significantly more slowly than either the brisket or flank. The butt was found to be generally drier than either of the other two sites. Inclusion of variable water activity during carcase cooling was found to lead to lower *E. coli* growth estimates on almost all cases. When both cooling and drying are taken into account for *E. coli* growth predictions, predicted *E. coli* growth is not significantly different at the butt, flank or brisket sites. The extent of the conservatism in the RI due to the assumption of water activity of 0.995, ranges from ~1.2 at the flank and brisket to 2.4 at the butt. Overall, the over-estimation of *E. coli* growth by the RI was estimated to be 1.6-fold

Analysis of the potential growth of *E. coli* and potential growth of psychrotrophic pseudomonads, responsible for aerobic spoilage of red meat, indicated that under all

commercial chilling regimes considered pseudomonads had greater growth potential than *E. coli*, and in most cases was many-fold higher. This is explained by the observation that, for the data used for the comparisons, temperatures were almost always in the rangein which pseudomonads are expected to have faster growth rate than *E. coli* (i.e. ≤ 15 - 20°C). It was noted, however, that at sites in which greater drying occurred, the difference between predicted growth of *E. coli* and psychrotrophic pseudomonads was significantly less. Thus, the butt had the lowest overall ratio of growth of psychrotrophic pseudomonads:growth of *E. coli*. Moreover, the butt site was found to support significantly less growth of Pseudomonads than the flank or brisket, despite that it is the slowest cooling site of the three considered.

Any process that decreases the overall temperature experienced by the carcase during chilling will reduce pseudomonad growth. The inhibition of growth appears to be greatly enhanced by reduction in water activity at the same time.

Acknowledgements

The assistance of Messrs Neil McPhail and Ian Eustace of Food Science Australia, Cannon Hill, Queensland, for timely provision of the datasets is gratefully acknowledged

Associate Professor Tuan Pham, University of New South Wales prepared and provided software to enable prediction of carcase surface water activity and temperature during air chilling.

The contributions of Messrs. Seven Rasmussen and Adam Teo in using the Pham model to generate predictions, and initial work to combine predictive microbiology models with the data from Food Science Australia and the Pham model, is gratefully acknowledged.

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Contents

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1. Introduction

Simplified models of carcase chilling, that provide a prediction of temperature and water activity over the entire carcase surface, were developed in PRMS.043A: Modelling of Beef Carcass Chilling". Similarly, models for microbial growth based on temperature and water activity have been developed in MLA–funded projects, e.g. the *E. coli* model underlying the Refrigeration Index (RI). Models also exist for microorganisms responsible for spoilage of meat.

Models that predict microbial growth on meat on the basis of temperatures and water activities on the carcase could be combined with models for prediction of temperature and water activity changes on carcasses. This could enable the optimisation of chilling processes, e.g. to optimise meat quality and minimise weight loss, while maintaining microbiological quality and safety, without the need for costly and time consuming microbiological challenge trials.

The Refrigeration Index is now a well-established tool in the export meat industry. In its current form, the RI is expected to be deliberately conservative, i.e. it is expected to predict more growth of *E. coli* than would actually occur, because deliberately conservative values of pH, lactic acid and water activity are used in the RI calculations. Using predictions from the PRMS.043A model it would be possible to begin to estimate the magnitude of overestimation of *E. coli* growth by the RI model by comparing RI values to growth estimates that include the effect of water activity changes that occur during air chilling.

Finally, for the purpose of demonstrating the potential value of this combination of predictive microbiology and carcase modelling, it was considered that it would also be valuable to examine the change in populations of Pseudomonas, the organisms primarily responsible for aerobic spoilage of meat, in comparison to predicted changes in *E. coli*, to be able to simultaneously compare the effects of alternate chilling regimes on microbiological safety (RI) and microbiological quality (predicted growth of Pseudomonads).

Many of the potential benefits proposed above rest on the assumption that the various models discussed above are reliable. The models for *E. coli* and pseudomonas have been evaluated and the limits of their reliability have been documented. There has been little opportunity, however, to assess the accuracy of the carcase chilling models.

The objectives of this project were to:

i) assess the reliability of the carcase chilling model;

ii) determine whether the RI, which makes a calculation of *E. coli* growth based on temperature data from the slowest cooling point on the carcase and for constant and conservative levels of water activity, pH and lactate, leads to unnecessarily conservative estimates of RI; and

iii) compare predicted changes in *E. coli* and pseudomonad levels under different cooling regimes and at different sites on the carcase to determine whether there is scope for optimisation of cooling regimes, i.e. those that minimise levels of both organisms.

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2. Methods and Materials

2.1 Overview and Data Sources

To address the first objective, measurements of water activity and temperature changes on carcases at various abattoirs, kindly provided by Mr. Neil McPhail of Food Science Australia, Cannon Hill. These included two datasets each from four abattoirs for each of five sides. For each data set and carcase ("side") characteristics of the side were available as were the chiller operating parameters. These values, shown in Appendix A, were used as inputs to generate the estimates of chilling and drying of each individual carcase using the PRMS.043Amodel. Thus a total of 120 chilling/drying profiles were available from which observed carcase temperature and water activity could be compared to those predicted by the model.

To address the second objectives of the project it was proposed that growth on a carcase should be calculated at the following points:

- the point where the RI temperature measurements are made;
- the three ESAM sites;
- the fastest and slowest cooling sites according to the carcase chilling model;
- the wettest and driest sites according to the carcase chilling model;
- other points that would assist in the development of understanding of growth across the whole carcase,

using both the RI model and a fuller version of the model that included time-dependent water activity changes in the predictions of *E. coli* growth.

To address the third objective, predictions of Pseudomonad growth were made using the same temperature and water activity data as were used for *E. coli* growth predictions. The predicted amount of growth was compared and analysed as a function of rate of cooling and drying to determine whether some chilling regimes led to low predicted growth of both groups of bacteria.

2.2 Temperature and Water Activity Data

As noted above, measured carcase surface temperatures and water activities for 120 sides of beef in 4 different abattoirs were provided by Mr. Neil McPhail of Food Science Australia, Cannon Hill. Temperatures were measured to 0.1°C resolution, and at six minute intervals, on three sites ("butt", "flank" and "brisket") as shown in Figure 1. Water activities were measured with an Aqualab CX-D dew-point water activity meter (0.001 resolution, but ± 0.003 accuracy) using thin sections (1 mm) of tissue excised from the carcass surface at the above-mentioned sites. Samples for water activity measurement were taken at 2 hourly intervals. Each data set contained from 100 to 150 data.

The data supplied included temperature measurements for approximately one hour before the nominal time zero. Calculations were made for the full temperature history and also for the temperature history commencing at the nominal time zero. For the purposes of this report, all comparisons of predicted growth are based on growth after the nominal time zero, though the other set of predictions and comparisons based on the full temperature history is available.

For each side of beef, chiller operating parameters and characteristics of the individual sides of beef were recorded. These parameters included: weight (kg), fat depth (mm), and time on floor (min) for each side of beef, air temperature (°C), relative humidity (%). chilling time (h) and air velocity, air temperature, air turbulence over the brisket, butt and flank. These data are detailed in Appendix 1.

The above data were used as inputs to the model of Associate Professor Tuan Pham of the School of Chemical Engineering and Industrial Chemistry, University of New South Wales, developed within MLA project PRMS.034. The model was incorporated into software by Professor Pham which was provided to this project and used to generate predictions of carcase surface temperature and water activity at the butt, brisket and flank for each side of beef.

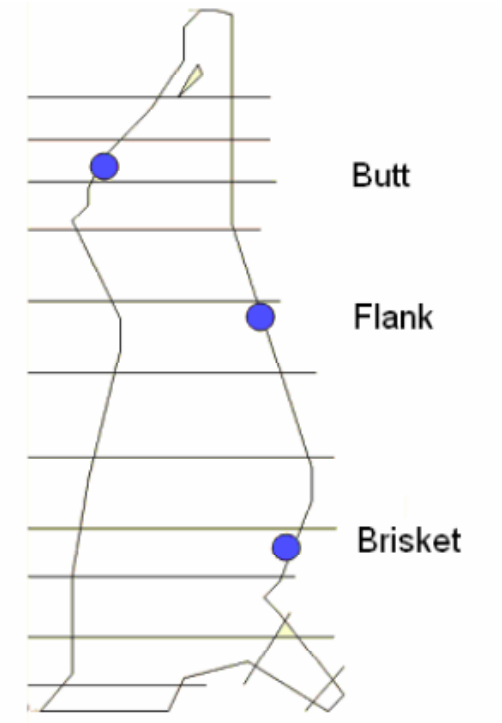

Figure 1 Diagram of a beef carcase indicating the 13 transverse slices *as per* Davey and Pham (2000) delineating the sections used by the model of Pham and Trujillo (2005). The diagram also shows the location of temperature and water activity sampling points on the carcase utilised in other studies undertaken by Food Science Australia and complementing the current project. (*Reproduced from* Pham and Trujillo, 2005).

2.3 Slowest and Fastest Cooling, and Wettest and Driest Sites

An objective measure of the fastest and slowest cooling sites and 'wettest' and 'driest' sites on the carcasses was required to be able to compare predictions of growth at these sites and to assess the combined influence of temperature and water activity on microbial growth at specific sites. While predicted microbial growth might have been used as a proxy to characterise these conditions, it does not allow differentiation of the effects of temperature and water activity or for identification of warmest/coolest and wettest/driest sites.

E. coli does not grow at temperatures below ~7°C, nor at water activities below ~0.95. On the assumption that temperature always decreases continuously during a cooling cycle, the integral of temperature over time (i.e. the area below the time-temperature graph) provides a simple measure of relative rates of cooling, i.e. the time-temperature graph with the lowest integral indicates the 'fastest' cooling. Thus, the calculation of relative cooling rate was determined by calculating the sum of the product of the temperature above 7°C and the time interval. This value was termed the temperature index. The relative dryness of the site ("Drying Index") was calculated by taking the sum of the product of the water activity above 0.95 and the time interval. Where temperature was less than or equal to 7°C, or the water activity less than or equal to 0.95, the growth over that interval was taken as 0.

The carcase cooling/drying data, described in Section 2.2 above, involved monitoring sides of beef for different lengths of time in different plants. Thus, identification of slowest cooling or driest sites is confounded and results from different plants cannot be combined for statistical analysis unless the effects of specific cooling times are included.

Instead, for each plant, the average of the Drying Index and average of the Cooling Index was calculated for all three sites on all five carcases (i.e. fifteen data). The Drying Index and Cooling Index for each of the fifteen carcase/sample site combination was then divided by the mean Drying Index and Cooling Index for each plant to generate, for each sample site and carcase position, a relative drying and relative Cooling Index. In effect, the Drying Indices and Cooling Indices were normalised for each plant to enable comparison between plants. The normalised values were used to identify whether any of the three samples sites had consistently higher or lower drying, or cooling, indices.

2.4 Growth predictions

For each set of measured temperature and water activity data at the three sites on each side and for each corresponding set of *predicted* temperatures and water activities (from the Pham-Trujillo model), the growth of *E. coli* was calculated at six minute intervals during the cooling process.

In the FSA data, water activity was determined at two-hourly intervals. To estimate the water activity at each six minute interval corresponding to each temperature record, water activity was assumed to change linearly between measured values. Thus, the difference between successive water activity measurements was divided by 20, and this value used as the increment of water activity change *per* six minutes between those successive water activity measurements.

For each six-minute interval during the chilling process bacterial growth rate was calculated based on:

i) the measured temperature and measured, or inferred, water activity at the beginning of that interval

ii) .The growth rate (generations *per* hour) was multiplied by 0.1 h to estimate the number of generations of growth in the interval. The estimated total amount of growth over the chilling cycle was determined as the sum of growth over all six minute intervals.

E. coli growth was estimated using the model of Ross *et al*. (2003), the model underlying the Refrigeration Index. pH and lactic acid data were not available, thus, calculations were based on assuming that the pH of the meat was 6.2 and that the lactic acid concentration was 51.7 mM. These are the default values used in the RI. For calculations of the RI, the water activity was assumed to be 0.995, which is the default value specified in the RI.

Pseudomonad growth was predicted using the model of Neumeyer *et al*. (1997) at six minute intervals for measured temperature and water activity values only. The Neumeyer *et al*. (1997) model does not include pH or lactic acid terms.

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3. Results and Discussion

3.1 Comparison of the Pham-Trujillo Model and Observed Temperature and Water Activity

Predicted *E. coli* growth was used to assess the predictions of the Pham-Trujillo model with corresponding observed water activity and temperature changes during chilling. This approach was chosen because it incorporates the combined effects of temperature and water activity into a single, practically relevant, measure and facilitates comparison. For this reason it is a more useful metric for comparison that the Drying or Cooling Indices.

Figure 2 compares the *E. coli* growth predicted from the temperature and water activity data predicted by the model of Pham and Trujillo with growth predicted from the measured temperature and water activity data.

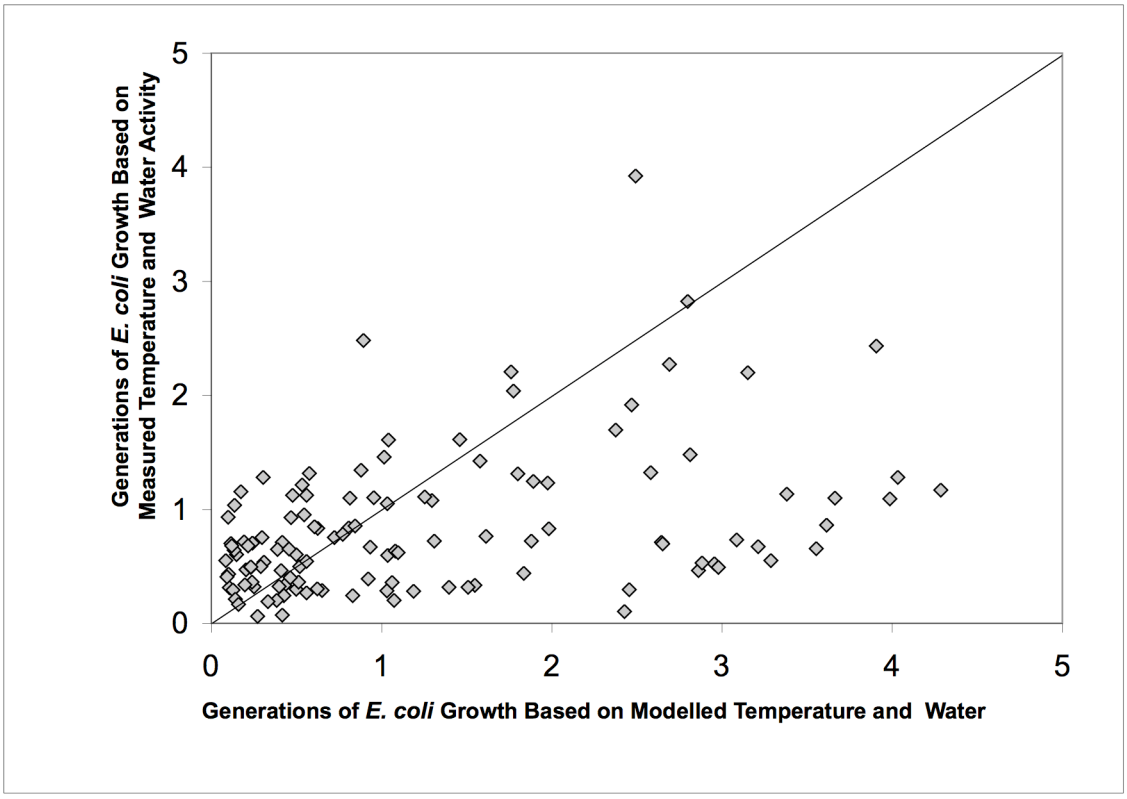

Figure 2. Comparison of predicted *E. coli* growth on carcases during air chilling based on measured temperature and water activity data and water activity and temperature predicted by the Tuan-Trujillo model. The line shown is the 'line of equivalence'.

The figure shows that correlation between growth estimates is poor (R^2 = 0.161), and that the modelled temperature and water activity data, in general, lead to much higher predictions of *E. coli* growth. The data were sorted by carcase sample-site and by processing plant and the correlation between growth predictions for these sub-sets of data also assessed. No significant correlations were found (data not shown).

In general, slopes of regression lines through the data were significantly less than one. The ratio of the predicted growth using the modelled temperature and water activity values to that predicted using the measured temperature and water activity was determined. The mean ratio

was 2.0 (SD ± 2.6), reinforcing that the modelled temperature and water activity values would usually lead to overestimation of growth.

Reasons for the overestimation using the modelled temperature and water activity estimates were not investigated in detail but it was noted that modelled temperatures at 'time zero' were often significantly higher than those actually observed. It was also observed that the measured water activities seemed to fluctuate widely compared to those predicted by the Pham-Trujillo model.

Similar results were obtained by Pham and Trujillo (2005), however, when they used the same Food Science Australia data to assess the performance of the model. They noted that agreement was poor, but observed that there was qualitative agreement between predictions of the model and the Food Science Australia data also used in this study.

Given that the agreement between the Pham-Trujillo model and observed temperatures and water activities was unreliable, the remaining objectives of the project were addressed using the measured temperature and water activity data. Additionally, no attempt was made to determine the fastest and slowest cooling sites, or the wettest and driest sites, from the predictions of the carcase chilling model. Instead, the analysis was limited to the measured data for the three sites (butt, flank, brisket) contained in the data provided by Food Science Australia.

3.2 Identification of Slowest Cooling and Driest/Wettest Sites.

Relative Drying Indices and relative Cooling Indices (*see* Section 2.3) were calculated for each sampling site (butt, flank, brisket) for each of the five sides of beef in eight trials, conducted in four abattoirs. In four abattoirs trials involving five sides of beef were conducted twice. Subsequent to the above analyses an additional data-set from a fifth abattoir became available and was included in the analysis described in this section and Sections 3.3 and 3.4. Thus, 135 values for relative Drying Index and relative Cooling Index were used in the analysis.

The relative Cooling Index values were sorted according to their magnitude. A higher relative Cooling Index indicates a site that has experienced warmer temperatures, on average, than other sites on that side of beef during cooling. Once the data were ranked, it was apparent that samples from "butt" sites were correlated with higher temperature indices. The statistical significance of this correlation was assessed by calculating the mean and standard deviation of all relative Cooling Index values for all butt samples, all flank samples, and all brisket samples. The results are presented in Table 1.

Table 1: Differences in Mean Relative Cooling Index by Carcase Sample Site

Sample Site	Number of samples	Relative Cooling Index		Range	
		mean	SD	minimum	maximum
brisket	45	0.67	0.37	0.05	1.73
flank	45	0.61	0.33	0.16	1.52
butt	45	1.57	0.51	0.51	2.63

Using Student's *t*-test, the differences between relative rate of cooling at the brisket or the flank sites are not highly significant ($p > 0.1$) while relative rate of cooling at the butt *is* highly significantly less ($p \ll 0.001$) than either the flank or brisket sites. Thus, the butt was confirmed as the slowest cooling site on most carcases under most chilling regimes, and would

be expected to permit more microbial growth, or a higher RI value, than either the brisket or the flank.

For the relative Drying Index a similar approach was taken, and with similar outcomes except that the butt was identified as the *driest* site (lowest relative Drying Index), meaning that less growth would be expected to be possible on the butt, in comparison to the brisket or flank for a given carcase and chilling regime. Summary results are presented in Table 2. Using Student's *t*-test, the differences between relative dryness at the brisket and flank sites are not highly significant ($p > 0.1$), while the relative dryness of the butt *is* highly significantly greater ($p \ll 0.001$) than either the flank or brisket site.

Thus, it appears that while there are significant differences at the butt in terms of cooling and dryness, these effects would be expected to counteract each other. This prediction can be tested by evaluation of the average RI for each site compared to the predicted generations of growth when using an *E. coli* growth model that includes the effect of changing water activity.

Table 2: Differences in mean Relative Drying Index by Carcase Sample Site

Sample Site	Number of samples	Relative Cooling Index		Range	
		mean	SD	minimum	maximum
brisket	45	1.24	0.36	0.22	1.69
flank	45	1.15	0.25	0.33	1.61
butt	45	0.64	0.52	0.03	2.37

Using an approach analogous to that described in Section 2.3, the average and standard deviation of all RI values (5 sides X 3 sample sites) for each of the nine chiller trials was calculated. Similarly, the average and standard deviation of all corresponding predictions of generations of growth of *E. coli* using the model of Ross *et al.* (2003) ("Full Model") was calculated for each of the nine chiller trials. The difference in the two sets of estimates is that the RI model assumes that water activity is 0.995, while the Full Model accommodates the effect of carcase surface water activity changes in the growth calculations. Thus, the RI would be expected to reflect the differences identified by the Cooling Index analysis. Conversely, analysis of differences in growth on different carcase sites using the Full Model would be expected to show less difference, due to the counteracting effect of water activity changes identified in the Drying Index analysis.

The relevant data are presented in Table 3, for both the RI and the Full Model, using the same approach as described for the Cooling Index and Drying Index.

Statistical analysis of differences in the average RI and averages of the Full Model predictions of growth was undertaken using Student's t-test. For the RI approach, the mean RI at the butt was shown to be statistically significantly higher ($p \ll 0.001$) than either the mean RI determined from temperatures at the flank or at the brisket. Mean RIs calculated at the brisket and flank sites are not highly significantly different ($p > 0.1$) to each other.

Conversely, when mean relative Full Model predictions are compared there is no significant difference ($p > 0.1$) between the mean *E. coli* growth predicted at any of the three samples sites. Despite this, when the average *E. coli* growth is calculated for each sample site across all sides and cooling regimes, a significantly lower overall predicted growth is noted for the flank sites. Mean absolute generations of growth for all 45 sides is 0.98 (± 0.78, SD) at the brisket, 0.91 (± 0.64, SD) at the butt, and 0.64 (± 0.33, SD) at the flank.

Table 3: Mean Relative Refrigeration Index and Mean Relative "Full Model" Predictions by Carcase Sample Site

Sample Site	Number of samples	Relative Cooling Index		Range	
		mean	SD	minimum	maximum
Relative Refrigeration Index					
brisket	45	0.876	0.508	0.015	2.245
flank	45	0.694	0.437	0.188	2.245
butt	45	1.472	0.650	0.245	2.37
Relative Full Model *E. coli* growth predictions					
brisket	45	0.932	0.506	0.255	2.499
flank	45	1.130	0.645	0.048	3.187
butt	45	0.938	0.686	0.021	2.940

3.3 Quantification of Conservatism in the Application of the Refrigeration Index

The analysis in Section 3.2, above, has confirmed that the butt is the slowest cooling site of the three assessed and that it leads to conservative ('worst case') predictions of the extent of *E. coli* growth when the Refrigeration Index model, which assumes a constant and high water activity (0.995), is used. If water activity were included in the calculations, then sample site has no significant influence on predicted *E. coli* growth.

Given that the RI is currently used for export operations, it is useful to quantify the extent of the over-prediction due to the conservative assumptions. The Food Science Australia dataset enables the effect of the conservative water activity assumption, at least, to be quantified.

Predictions for all sites on all carcases were made using the RI model and the Full Model. A direct comparison of all matching predictions is shown in Figure 3a. A direct comparison of matching predictions, but identified by carcase sampling site, is shown in Figure 3b. It is apparent that the RI almost invariably is greater than the generations of growth predicted by the Full model, i.e. when the effect of carcase surface drying is taken into account in *E. coli* growth calculations.

To better understand the influence of sample site on the expected over-prediction of growth by the RI, the ratio of generations of growth predicted by the RI to that predicted using the Full Model, was determined. Means of this ratio were determined for the full data set as well as sub-sets of the data according to carcase sampling site. Summary statistics, shown in Table 4, reinforce that the influence of water activity changes at the butt site leads to a greater than two-fold over-prediction of potential for *E. coli* growth (noting that specific pH and lactic acid influences are also ignored), while the effects at the brisket and flank sites are far less pronounced.

To assess the conservatism of use of the butt as the sample site for RI determinations, the ratio of the RI at the butt to the RI determined at the flank and brisket was determined for the 45 data sets. The ratio RI_{butt}: RI_{flank} was 2.82 (SD ± 1.66) while RI_{butt}: $RI_{brisket}$ was 2.21 (SD ± 1.62). These ratios were found not to be significantly different.

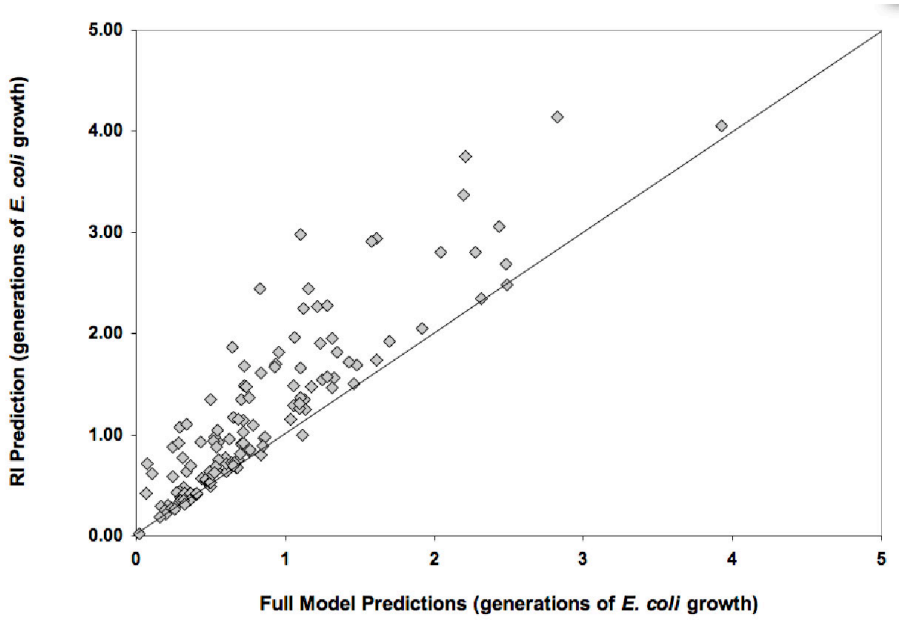

Figure 3a. Comparison of predictions of growth of *E. coli* using the Refrigeration Index with those of a model encompassing the effect of varying water activity, and showing that the omission of water activity usually leads to over-prediction of growth by the RI. The correlation between the two sets of predictions is significant (R^2 = 0.80). The line shown is the 'line of equivalence'.

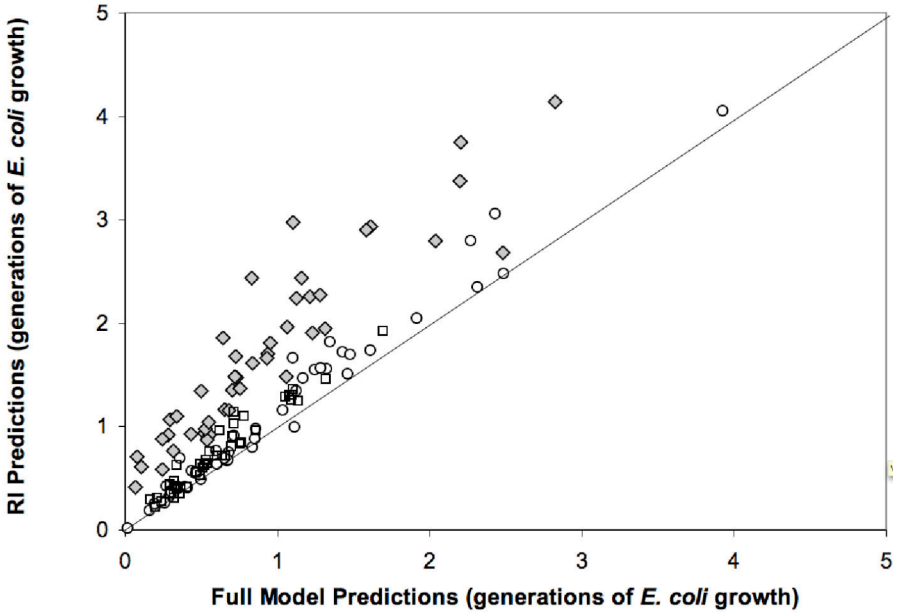

Figure 3b. Comparison of predictions of growth of *E. coli* using the Refrigeration Index with those of a model encompassing the effect of variable water activity, and also showing the influence of carcase sampling site on over-prediction of growth by the RI. Grey diamonds represent butt samples; open circles represent brisket samples; open squares represent flank samples.

Table 4: Comparison of Growth Predicted by the RI and the "Full Model", including the Effect of Sample Site

Site	average ratio (RI/Full Model)	SD	*n*
Brisket	1.16	0.18	45
Flank	1.24	0.19	45
Butt	2.41	1.50	45
Total Dataset	1.61	1.05	135

From the results presented in Table 4 it can be seen that the net effect of use of the RI is a 1.66-fold over-prediction of *E. coli* growth compared to predicted growth taking carcase surface drying into account. Due to the greater drying that is expected (on the basis of the preceding analyses) to occur on the butt, the level of conservatism is even greater, being estimated as 2.4. fold.

3.4 Comparison of E. coli *and* Pseudomonas *Growth*

To address the third objective of this study, growth of *E. coli* was estimated using the Full Model for all (three) carcase sample sites on the 45 carcase samples and compared to potential growth of Pseudomonads at the same sites. Figure 4 presents the results, showing comparisons of growth on butt, flank and brisket discretely by different plot symbols.

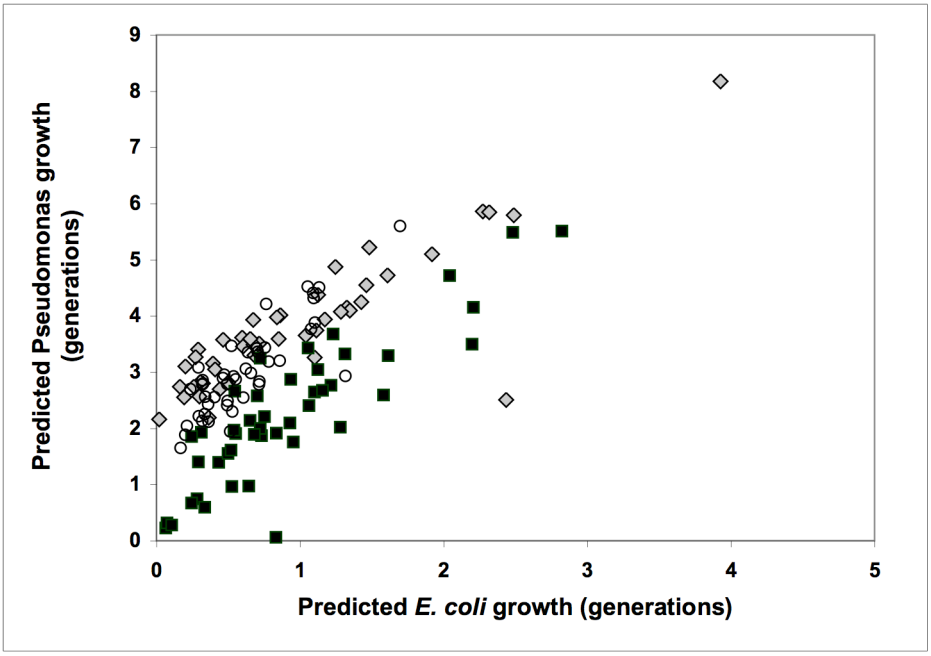

Figure 4. Comparison of the predicted extent of growth of *E. coli* and psychrotrophic pseudomonads at different sites on sides of beef during air chilling. Solid squares represent butt samples; shaded diamonds are brisket samples while open circles are predictions based on data collected at the flank site.

As expected, due to their adaptation to growth at chill temperatures, predicted growth of pseudomonads exceeds that of *E. coli* under almost all conditions - typically by a factor of two-

to five-fold. It is noted that predicted growth of *E. coli* and psychrotrophic pseudomonads is more similar at the butt site, than at either the flank or brisket. In other words, relatively *less* growth of Pseudomonads is predicted to occur at the butt than either the flank or brisket.

Given the differences apparent in the ratio of predicted pseudomonad growth and predicted *E. coli* growth at different sites, a preliminary analysis was undertaken to investigate factors that may be reliable predictors of potential Pseudomonas growth relative to the potential for *E. coli* growth.

Figures 5a, b show the relationship of the Drying and Cooling Indices to the ratio of predicted growth of Pseudomonads:predicted growth of *E. coli*.

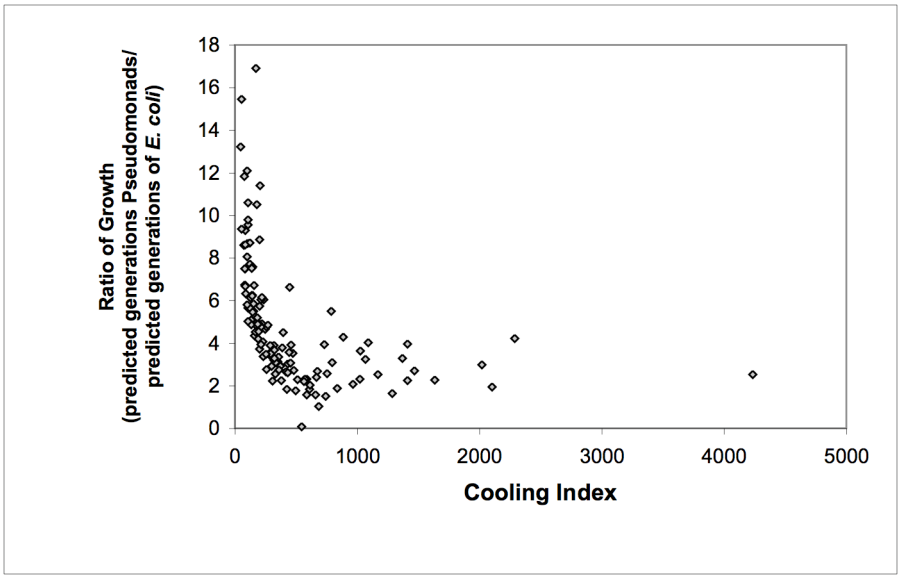

Figure 5a. Evaluation of the Cooling Index as a predictor of relative growth of Pseudomonads compared to *E. coli* on beef sides during air chilling.

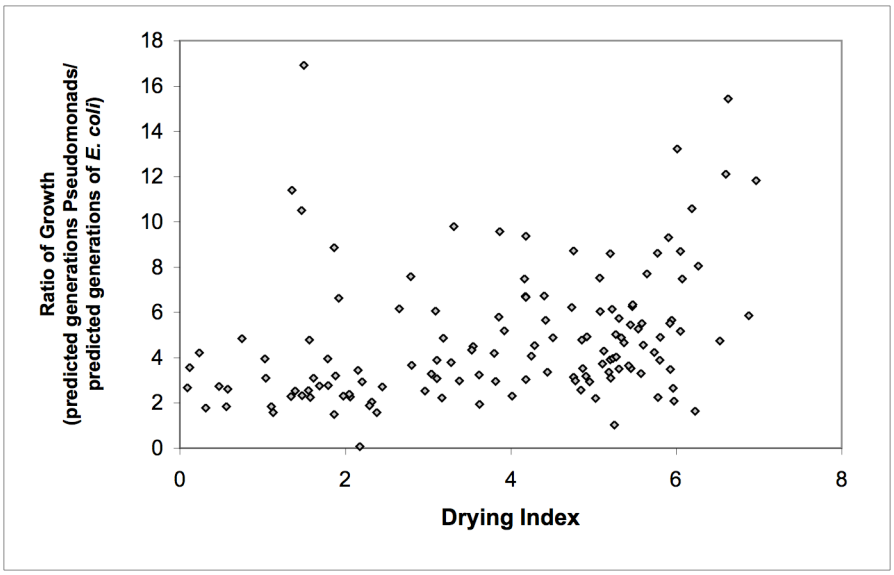

Figure 5b. Evaluation of the Drying Index as a predictor of relative growth of Pseudomonads compared to *E. coli* on beef sides during air chilling.

From Figure 5a it is apparent that very low Cooling Indices indicate much greater potential for Pseudomonas growth. This is to be expected because Pseudomonads, unlike *E. coli*, can grow at temperature less than 7°C, and even as low as 0°C. This plot is potentially misleading because, at very low Cooling Index values, i.e. when only a very small amount of *E. coli* growth is predicted, the use of a ratio leads to a very large value of the response variable.

The Cooling Index is based on the integral of time and temperatures above 7°C. As the Cooling Index increases, however, the difference in *E. coli a*nd pseudomonad growth is less pronounced (i.e. smaller ratio), presumably because a greater proportion of the cooling time allows growth of *both* pseudomonads and *E. coli*.

At temperatures up to ~15°C, growth of psychrotrophic pseudomonads will be faster than growth of *E. coli.* Commercial carcase cooling regimes would, however, be expected to be at temperatures less than 15°C for most of the time. This is confirmed by examination of the measured cooling data, which show that temperatures were below 15°C within 60 – 90 minutes of commencement of chilling. *E. coli* and psychrotrophic pseudomonads, however, have very similar lower water activity limits for growth (i.e. ~ 0.95). Accordingly, growth of psychrotrophic pseudomonads would almost always be expected to exceed that of *E. coli* during carcase chilling.

Due to the similarity of the lower water activity limits for growth, the ratio of predicted *E. coli* and pseudomonad growth might be expected to be less sensitive to the Drying Index. This prediction seems to be borne out by the data, as shown in Figure 5b.

The above interpretations may be confounded if faster cooling (i.e. lower Cooling Indices) are correlated with greater drying of the carcase surface, represented by lower Drying Index values. To investigate this possibility Drying Index values were plotted against corresponding Cooling Index values (*see* Figure 6). No correlation is evident.

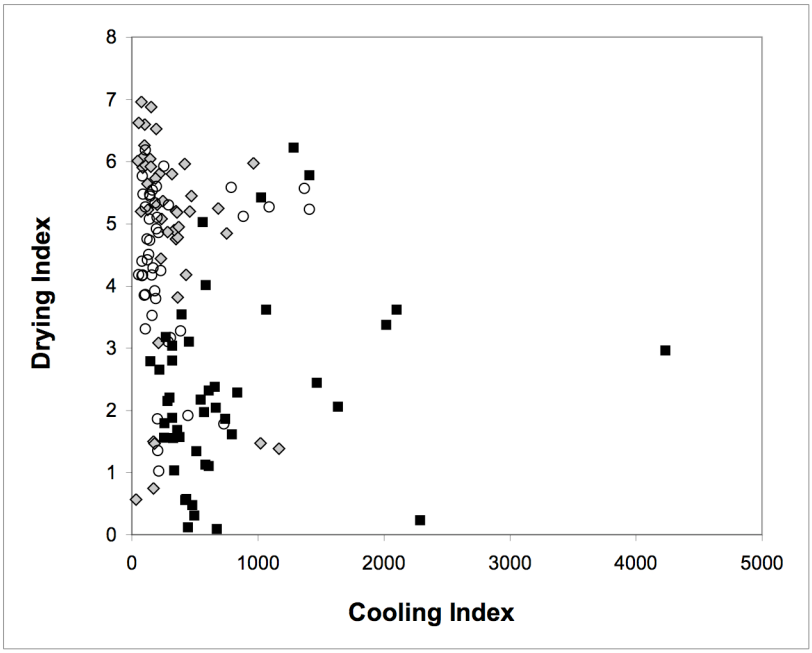

Figure 6 Evaluation of correlation between Cooling Index and Drying Index for three different sampling sites (butt, flank, brisket) on 45 sides of beef under five commercial chilling regimes. Solid squares represent butt samples; shaded diamonds are brisket samples while open circles are predictions based on data collected at the flank site.

As a final part of this preliminary investigation, the predicted generations of growth of psychrotrophic pseudomonads as a function of Cooling and Drying Indices were evaluated to explore means to minimise pseudomonas growth. Figures 7a and b illustrate the results and suggest that, in general pseudomonad growth is poorly correlated with Cooling Index. This is surprising given the dominant effect of temperature on the rates of all biological processes but is probably explained by the fact that the Cooling Index assumes a threshold for growth of 7°C, which, as noted earlier, is inappropriate for pseudomonads.

Closer examination of Figure 7a suggests that growth does, in general, increase as a function of Cooling Index, towards an asymptote around four to six generations of growth, but that in many cases growth is much reduced. This may, for example, be due to the effect of decreased water activity. As correlation between Cooling Index and Drying Index is poor (Figure 6), this possibility cannot be rigorously assessed. Drying Index, however, appears to be more closely correlated with pseudomonad growth, with growth amount increasing with Drying Index. This observation tends to support the hypothesis above and is consistent with the notion that while the Cooling Index is not a good predictor of pseudomonad growth because it employs a threshold for growth of 7°C the Drying Index, incorporating the lower water activity limit to growth of both *E. coli* and pseuomonads, is a more informative indicator of conditions that severely limit pseudomonad growth.

To explore this further, the average and standard deviation of absolute predictions of growth of pseudomonads for all butt samples, all brisket samples and all flank samples was determined. The results were 2.243 (±1.278, SD), 3.788 (±1.149, SD) and 3.009 (± 0.812, SD) respectively. All means were highly significantly different ($p \ll 0.001$). Thus, the butt site supports significantly less growth of pseudomonads, despite that it is, in general, the slowest cooling site. The effect of lower water activity at the butt may explain this apparent anomaly.

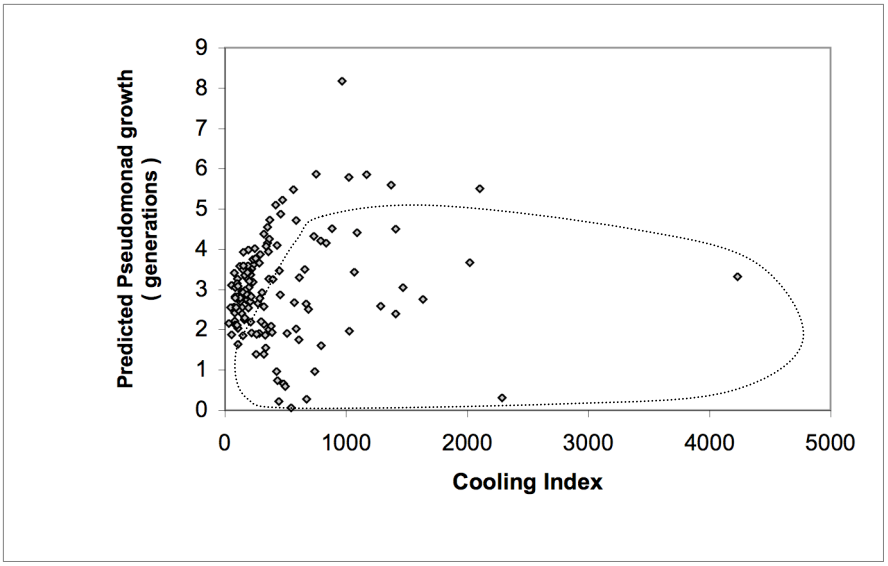

Figure 7a. Evaluation of the Cooling Index as a predictor of growth of psychrotrophic pseudomonads. The encircled data are proposed to be situations where pseudomonad growth is constrained by reduced water activity, and are highlighted to emphasise the proposed underlying relationship between predicted pseudomonad growth and Cooling Index if those data are ignored.

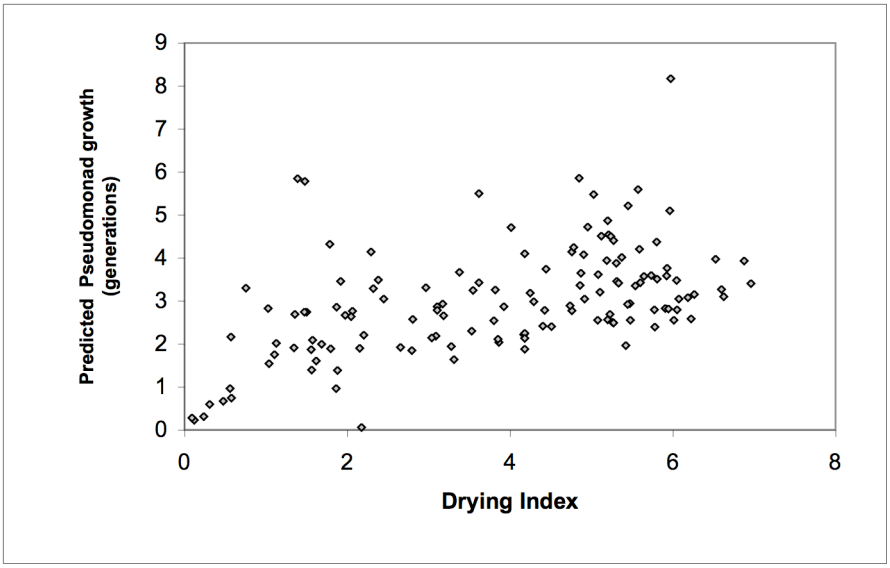

Figure 7b. Evaluation of the Drying Index as a predictor of growth of psychrotrophic pseudomonads.

4. Conclusions

To reiterate, the objectives of this project were to:

i) assess the reliability of the carcase chilling model;

ii) determine whether the RI leads to unnecessarily conservative estimates of RI; and

iii) compare predicted changes in *E. coli* and pseudomonad levels under different cooling regimes and at different sites on the carcase to determine whether there is scope for optimisation of cooling regimes.

The analyses described above have suggested that the Pham-Trujillo model, developed in PRMS.043A, in its current form does not well describe observed carcase temperature and water activity changes during air cooling. The reason for the apparently poor predictions were not pursued in this study, but it is noted that the model relies on a relatively large number of inputs. The fidelity of the data used as inputs to the carcase chilling model was not assessed in this study. Similar results were obtained by Pham and Trujillo, however, when they used the same Food Science Australia data to assess the performance of the model. As such, some of the original project aims could not be completed because the Pham-Trujillo model could not be relied upon to generate accurate predictions of cooling and drying of carcase surfaces during air chilling.

A number of approaches were used to address the second objective, relying on the Food Science Australia dataset. It was found that, as expected, the butt cools significantly more slowly than either the brisket or flank. The butt becomes drier than either of the other two sites, however, counteracting the effect on the predicted extent of *E. coli* growth due to the slower cooling. Thus, inclusion of varying water activity during carcase cooling would be expected to lead to lower RI estimates.

When both cooling and drying are taken into account for *E. coli* growth predictions, predicted *E. coli* growth is not significantly different at the butt, flank or brisket sites. The extent of the conservatism in the RI due to the assumption of water activity of 0.995, ranges from ~1.2 at the flank and brisket to 2.4 at the butt. Overall, the over-estimation is 1.6-fold.

Analysis of the potential growth of *E. coli* and potential growth of psychrotrophic pseudomonads, responsible for aerobic spoilage of red meat, indicated that under almost all commercial chilling regimes considered pseudomonads had significantly greater growth potential than *E. coli*. This is explained by the fact that, for the data used for the comparisons, temperatures were almost always in the range in which pseudomonads are expected to have faster growth rate than *E. coli* (i.e. $\leq$ 15 - 20°C). It was noted, however, that at sites in which greater drying occurred, the difference between predicted growth of *E. coli* and psychrotrophic pseudomonads was significantly less. Thus, the butt had the lowest overall ratio of growth of psychrotrophic pseudomonads:growth of *E. coli*. Moreover, the butt site was found to support significantly less growth of pseudomonads than the flank or brisket, despite that it is the slowest cooling site of the three considered.

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Appendix 1 Chiller operating parameters and carcase data used for each simulation with the Pham model

Plant A

Run 1

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**179.5**	**188.0**	**201.5**	**166.5**	**170.5**
Fat depth (mm)		**29.6**	**19.2**	**18.7**	**26.1**	**30.0**
Time on floor (min)		**~45**				
Air temp (°C)		**~25**				
RH (%)		**~70**				
Chilling time (h)		**16**	**16**	**16**	**16**	**16**
Air temp (°C)*		**1.0**	**0.5**	**0.5**	**1.5**	**1.0**
Air RH (%)		**84**	**85**	**84**	**85**	**83**
Air velocity (m/s)	Butt	**1.41**	**2.08**	**1.9**	**0.78**	**1.57**
	Flank	**1.28**	**1.5**	**1.44**	**1.15**	**1.04**
	Brisket	**0.41**	**1.43**	**0.96**	**1.53**	**1.38**
Air direction (°)	Butt	**180**	**180**	**180**	**270**	**270**
	Flank	**180**	**180**	**180**	**270**	**270**
	Brisket	**180**	**180**	**180**	**270**	**270**
Air turbulence (%)	Butt	**10**	**10**	**14**	**15**	**9**
	Flank	**12**	**14**	**18**	**17**	**13**
	Brisket	**43**	**11**	**20**	**9**	**11**

* Starting at ~10°C and reducing to this temperature after about 8 h

Run 2

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**205.5**	**207.0**	**201.5**	**239.5**	**216.0**
Fat depth (mm)		**30**	**26**	**28**	**24**	**26**
Time on floor (min)		**~45**				
Air temp (°C)		**~25**				
RH (%)		**~70**				
Chilling time (h)		**14**	**14**	**14**	**14**	**14**
Air temp (°C)*		**0.0**	**0.0**	**0.5**	**1.5**	**2.5**
Air RH (%)		**85**	**86**	**84.5**	**77**	**83**
Air velocity (m/s)	Butt	**1.35**	**1.25**	**1.62**	**0.78**	**0.82**
	Flank	**0.96**	**0.68**	**1.41**	**1.44**	**1.31**
	Brisket	**1.26**	**0.81**	**1.07**	**1.46**	**1.19**
Air direction (°)	Butt	**180**	**180**	**180**	**0**	**0**
	Flank	**180**	**180**	**180**	**0**	**0**
	Brisket	**180**	**180**	**180**	**0**	**0**
Air turbulence (%)	Butt	**28**	**13**	**22**	**23**	**11**
	Flank	**14**	**29**	**22**	**12**	**13**
	Brisket	**13**	**15**	**21**	**10**	**11**

* Starting at ~10°C and reducing to this temperature after about 10 h

Plant B

Run 1

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**131.5**	**139.0**	**311.0**	**175.5**	**175.5**
Fat depth (mm)		**16**	**12**	**27**	**27**	**17**
Time on floor (min)		**~45**				
Air temp (°C)		**~25**				
RH (%)		**~70**				
Chilling time (h)		**16**	**16**	**16**	**16**	**16**
Air temp (°C)*		**1.0**	**0.0**	**1.0**	**0.0**	**-0.5**
Air RH (%)		**82**	**82**	**80**	**88**	**89**
Air velocity (m/s)	Butt	**0.38**	**0.23**	**0.28**	**1.96**	**1.8**
	Flank	**0.52**	**0.39**	**0.09**	**0.81**	**1.8**
	Brisket	**0.51**	**0.44**	**0.57**	**2.23**	**2.07**
Air direction (°)	Butt	**0**	**0**	**0**	**0**	**0**
	Flank	**0**	**0**	**0**	**315**	**315**
	Brisket	**0**	**0**	**0**	**270**	**270**
Air turbulence (%)	Butt		**73**	**42**	**12**	**9**
	Flank			**29**	**39**	**12**
	Brisket	**51**		**35**	**9**	**13**

* Starting at ~10°C and reducing to this temperature after about 8 h

Run 2

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**194.5**	**225.5**	**229.5**	**243.0**	**233.0**
Fat depth (mm)		**24**	**13**	**12**	**20**	**20**
Time on floor (min)		**~45**				
Air temp (°C)		**~25**				
RH (%)		**~70**				
Chilling time (h)		**14**	**14**	**14**	**14**	**14**
Air temp (°C)*		**1.0**	**1.5**	**1.8**	**0.0**	**0.0**
Air RH (%)		**79**	**76**	**80**	**89**	**88**
Air velocity (m/s)	Butt	**0.16**	**0.35**	**0.33**	**1.28**	**1.71**
	Flank	**0.51**	**0.24**	**0.13**	**1.78**	**0.78**
	Brisket	**0.51**	**0.18**	**0.13**	**1.44**	**0.63**
Air direction (°)	Butt	**0**	**0**	**0**	**0**	**0**
	Flank	**0**	**0**	**0**	**315**	**315**
	Brisket	**0**	**0**	**0**	**270**	**270**
Air turbulence (%)	Butt			**84**	**15**	**16**
	Flank				**14**	**11**
	Brisket				**17**	**25**

* Starting at ~10°C and reducing to this temperature after about 8 h

Plant C

Run 1

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**120.0**	**125.0**	**130.0**	**120.0**	**120.5**
Fat depth (mm)		**10**	**8**	**9**	**12**	**9**
Time on floor (min)		**~45**				
Air temp (°C)		**~25**				
RH (%)		**~70**				
Chilling time (h)		**15**	**15**	**15**	**15**	**15**
Air temp (°C)*		**5.0**	**4.0**	**2.5**	**3.5**	**2.5**
Air RH (%)		**86**	**85**	**87**	**88**	**83**
Air velocity (m/s)	Butt	**0.44**	**0.24**	**0.23**	**0.58**	**0.21**
	Flank	**0.53**	**0.23**	**0.21**	**0.40**	**0.50**
	Brisket	**0.76**	**0.26**	**0.18**	**0.15**	**0.91**
Air direction (°)	Butt	**180**	**180**	**180**	**0**	**180**
	Flank	**180**	**90**	**180**	**0**	**180**
	Brisket	**180**	**180**	**180**	**90**	**180**
Air turbulence (%)**	Butt					
	Flank					
	Brisket					

* Starting at ~13°C and reducing to this temperature after about 14 h

** No turbulence readings taken

Run 2

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**123.5**	**118.0**	**129.5**	**127.0**	**119.5**
Fat depth (mm)		**10**	**10**	**12**	**7**	**12**
Time on floor (min)		**~45**				
Air temp (°C)		**~25**				
RH (%)		**~70**				
Chilling time (h)		**14**	**14**	**14**	**14**	**14**
Air temp (°C)*		**5.5**	**5.5**	**4.0**	**5.0**	**4.0**
Air RH (%)		**87**	**91**	**89**	**91**	**90**
Air velocity (m/s)	Butt	**0.69**	**0.58**	**0.46**	**0.43**	**0.14**
	Flank	**0.45**	**0.62**	**0.43**	**0.18**	**0.07**
	Brisket	**0.56**	**0.66**	**0.26**	**0.26**	**0.11**
Air direction (°)	Butt	**180**	**180**	**180**	**0**	**180**
	Flank	**180**	**90**	**180**	**0**	**180**
	Brisket	**180**	**180**	**180**	**90**	**180**
Air turbulence (%)	Butt	**30**	**14**	**25**	**42**	**71**
	Flank	**34**	**18**	**35**	**33**	**60**
	Brisket	**22**	**26**	**28**	**40**	**61**

* Starting at ~13°C and reducing to this temperature after about 14 h

Plant D

Run 1

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**184.6**	**214.0**	**210.2**	**195.8**	**180.8**
Fat depth (mm)		**24**	**14**	**14**	**11**	**19**
Time on floor (min)		**~50**				
Air temp (°C)		**~28**				
RH (%)		**~70**				
Chilling time (h)		**14**	**14**	**14**	**14**	**14**
Air temp (°C)*		**0.0**	**0.0**	**0.0**	**-0.5**	**-0.5**
Air RH (%)		**75**	**84**	**87**	**82**	**84**
Air velocity (m/s)	Butt	**0.49**	**0.66**	**0.48**	**0.26**	**0.38**
	Flank	**0.43**	**0.81**	**0.55**	**0.14**	**0.91**
	Brisket	**0.21**	**1.01**	**1.03**	**0.49**	**1.82**
Air direction (°)	Butt	**0**	**0**	**0**	**0**	**270**
	Flank	**0**	**0**	**0**	**270**	**315**
	Brisket	**0**	**0**	**0**	**270**	**315**
Air turbulence (%)**	Butt					
	Flank					
	Brisket					

* Starting at ~10°C and reducing to this temperature after about 3 h

** No turbulence readings taken

Run 2

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**158.8**	**142.8**	**159.8**	**168.2**	**150.2**
Fat depth (mm)		**9**	**15**	**18**	**12**	**6**
Time on floor (min)		**~50**				
Air temp (°C)		**~25**				
RH (%)		**~70**				
Chilling time (h)		**14**	**14**	**14**	**14**	**14**
Air temp (°C)*		**0.0**	**0.0**	**0.0**	**-0.5**	**0.0**
Air RH (%)		**90**	**91**	**91**	**91**	**88**
Air velocity (m/s)	Butt	**0.91**	**0.64**	**0.58**	**0.51**	**0.12**
	Flank	**0.34**	**0.62**	**0.43**	**0.50**	**0.45**
	Brisket	**0.48**	**0.68**	**0.98**	**0.29**	**0.23**
Air direction (°)	Butt	**0**	**0**	**0**	**0**	**270**
	Flank	**0**	**0**	**0**	**270**	**315**
	Brisket	**0**	**0**	**0**	**270**	**315**
Air turbulence (%)	Butt	**26**	**37**	**34**	**28**	**47**
	Flank	**37**	**22**	**38**	**36**	**33**
	Brisket	**38**	**31**	**14**	**54**	

* Starting at ~10°C and reducing to this temperature after about 3 h

Plant E

Run 1

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**185.0**	**163.0**	**121.5**	**117.0**	**139.5**
Fat depth (mm)		**15**	**27**	**15**	**6**	**26**
Time on floor (min)		**~50**				
Air temp (°C)		**~26**				
RH (%)		**~75**				
Chilling time (h)		**15**	**15**	**15**	**15**	**15**
Air temp (°C)*		**0.0**	**1.5**	**1.5**	**3.0**	**4.0**
Air RH (%)		**94**	**95**	**94**	**85**	**87**
Air velocity (m/s)	Butt	**1.56**	**1.63**	**1.36**	**0.24**	**0.20**
	Flank	**0.44**	**0.38**	**0.58**	**0.11**	**0.08**
	Brisket	**0.77**	**0.62**	**0.87**	**0.07**	**0.07**
Air direction (°)	Butt	**0**	**0**	**0**	**0**	**0**
	Flank	**0**	**0**	**0**	**0**	**0**
	Brisket	**0**	**0**	**0**	**0**	**0**
Air turbulence (%)**	Butt					
	Flank					
	Brisket					

* Starting at ~8-10°C and reducing to this temperature after about 4 h

** No turbulence readings taken

Run 2

		Side 1	Side 2	Side 3	Side 4	Side 5
Weight (kg)		**164.0**	**145.0**	**158.5**	**158.0**	**156.**
Fat depth (mm)		**8**	**12**	**15**	**11**	**16**
Time on floor (min)		**~50**				
Air temp (°C)		**~26**				
RH (%)		**~70**				
Chilling time (h)		**14**	**14**	**14**	**14**	**14**
Air temp (°C)*		**1.5**	**2.5**	**2.0**	**6.0**	**5.0**
Air RH (%)		**95**	**95**	**94**	**95**	**85**
Air velocity (m/s)	Butt	**1.81**	**0.99**	**1.21**	**0.06**	**0.13**
	Flank	**1.42**	**1.20**	**0.94**	**0.07**	**0.23**
	Brisket	**1.36**	**1.23**	**0.76**	**0.05**	**0.13**
Air direction (°)	Butt	**0**	**0**	**0**	**0**	**0**
	Flank	**0**	**0**	**0**	**0**	**0**
	Brisket	**0**	**0**	**0**	**0**	**0**
Air turbulence (%)	Butt	**11**	**18**	**12**	**62**	**52**
	Flank	**10**	**11**	**22**	**48**	**29**
	Brisket	**10**	**11**	**18**		**36**

* Starting at ~8-10°C and reducing to this temperature after about 4 h

This plant was the only one with complete washing of the sides – through HW decontamination cabinet.