Australian Government

Department of Agriculture, Fisheries and Forestry

AUSTRALIAN Pork®

Development of a predictive model for vacuum-packed pork

Final Report
APL Project 2020/0083

February 2023

University of Tasmania
Dr Laura Rood
Prof Tom Ross
Dr Chawalit ('Jay') Kocharunchitt
Private Bag 98
Hobart, TAS, 7001

Acknowledgements

This project was supported by funding from Australian Pork Limited, industry levy payers and the Commonwealth.

Executive Summary

There is little published data about the current shelf-life of Australian VP pork. This makes it particularly challenging for the pork industry to manage its cold chains to maintain/optimise product quality, while maximising productivity and reducing wastage. Therefore, the overall aim of this study was to develop a tool for the industry to predict the shelf-life of VP pork in cold chains. The following objectives are set out to achieve the aim: i) to determine the shelf-life of VP pork at different storage temperatures; and ii) to use the data generated to develop a tool for shelf-life prediction of VP pork. This tool (once validated) will enable Australian pork producers/processors to better manage their cold chains, enabling them to optimise product quality, reduce wastage due to unexpected loss of quality, reduce the need for markdowns, and reduce customer complaints. This report describes that work including a first version of the predictive tool, and can be summarised as follows:

- A series of shelf-life trials were conducted using different products (*i.e.,* rind-on, rindless, bone-in and boneless) of VP pork leg and shoulder at four different storage temperatures (ranging from -0.5°C to 10°C). Throughout storage, organoleptic (odour and colour) and microbial (total viable count and lactic acid bacteria) assessments, and pH measurement were conducted to determine the products' shelf-life.
- As expected, all VP pork products achieved a longer shelf-life (based on odour score) as the storage temperature decreased with a shelf-life of up to 82 days at approximately -0.5°C. This was in line with the observations that bacterial growth rates decreased with a decrease in temperature.
- It was found that total viable count and odour score were amongst the most suitable indicators to estimate the shelf-life of different VP pork products as a function of temperature.
- Different VP pork products exhibited a different response for total viable count growth and odour development, indicating that different mathematical models are required to provide an accurate prediction of the shelf-life of different VP pork.
- New mathematical models for shelf-life prediction of VP pork products were successfully developed and incorporated into a first version of the tool in MS 365 ®Excel.
- Preliminary analysis suggested that the developed tool may outperform the DMRI tool in predicting the shelf-lives of VP pork calculated by either odour or TVC. However, this was as expected given that the data used for comparison was used to develop the tool.
- Evaluation of the developed tool using independent data is required to specially determine its performance prior to its commercial adoption.

Table of Contents

List of Tables

List of Figures

1. Background to Research

The Australian pork industry aims to produce pork efficiently and sustainably to meet consumer demand, while assuring its premium quality. However, this is constantly challenged by the need to minimise the loss of product shelf-life along different supply chains, and to meet a wide range of shelf life-related specifications imposed by intended markets. Accordingly, being able to assure quality remaining for products is critical for the success of the Australian pork industry.

There is little published data about the current shelf-life of Australian vacuum-packed (VP) pork. The last reported shelf-life was in 1988 in which VP pork achieved a shelf-life of 4-6 weeks at ~0°C (Egan et al., 1988) – much shorter than that of other meat types such as beef and lamb in the same packaging format (Huynh et al., 2016). This is despite pork having a similar pH (pH 5.4-5.7) to beef (pH 5.5-5.7) and lower pH than lamb (pH 5.6-6.8). Previous research to specifically determine the differences between the shelf-life of VP pork and beef indicated that the shorter shelf-life of pork might be explained by a higher level of post-slaughter microbial contamination on pork compared to beef. Other factors that might contribute to the shorter shelf life include changes in pH and the concentration of lactic acid in pork during storage (Blixt and Borch, 2002). To this end, we systematically assessed the shelf-life of Australian VP pork products across storage temperature ranges (from -0.5˚C to 10˚C) to define its best-before dates or quality status in distribution. The data generated and the interpretation based on this were also used to develop a tool for predicting shelf-life of VP pork in supply chains. Achieving this, and being able to demonstrate its scientific basis, would enable the industry to further develop appropriate national standards (*i.e.,* standardising methodology) for shelf-life assessment, while having the ability to better understand and manage diverse supply chains, *i.e.,* helping to decide on product disposition as it moves through a supply chain, reducing wastage through premature quality loss and addressing market access challenges etc.

2. Objectives of the Research Project

The major objectives of this project are:

I. to determine shelf-life of Australian vacuum-packed pork at different storage temperatures; and;

II. to develop a tool for shelf-life prediction of VP pork.

3. Introductory Technical Information

Meat spoilage is a complex phenomenon involving interactions among growing microorganisms, various biochemical reactions, and storage environment. However, the growth of these microorganisms and the processes of spoilage are predictable in a well-defined ecosystem (Huynh et al., 2016). Through our previous projects with Meat and Livestock Australia, we demonstrated that and successfully developed shelf-life predictive models for vacuum-packed (VP) beef and lamb based on the growth rate of microorganisms present and processes of spoilage (based on odour) as a function of temperature (Ross et al., 2019). The models were well-validated and have now been adopted by many red meat processors and exporters as a reliable and cost-effective decision-support tool for better management of their cold chains, *i.e.,* to optimise product quality, to avoid unexpected loss of quality, to reduce wastage, reduce the need for markdowns, and, more importantly, reduce customer complaints. This tool has shown to have enormous benefits, such as cost savings of $9.1 million per annum for a domestic retail beef supply chain alone, with 99.5% of this benefit deriving from reduced waste and markdowns (Ross et al., 2019).

As mentioned in Section 1, there is not much published data on the current shelf-life of Australian VP pork, especially at different storage temperatures. This makes it particularly challenging for the pork industry to manage its cold chains to maintain/optimise product quality, while maximising productivity and reducing wastage. Therefore, the overall aim of this study was to develop a predictive tool for shelf-life of Australian VP pork. This aim was achieved through the following objectives: i) to determine the shelf-life of Australian VP pork at different storage temperatures; and ii) to use the data generated to develop a mathematical model(s) for shelf-life prediction of VP pork. This model (once validated) will enable Australian pork producers/processors to better manage their cold chains. This report, therefore, describes that work and presents a first version of the predictive tool.

4. Research Methodology

4.1 Meat samples

- Two sets of trials were conducted in January and May 2022 using fresh vacuum-packed (VP) boneless pork leg (n = 120 rind-on; n = 120 rindless) sourced from an abattoir in SA, Australia, and VP pork shoulder (n = 120 rind-on bone-in; n = 120 rindless boneless) from an abattoir in NSW, Australia, respectively. Both leg and shoulder primals were sourced from single herds of animals and were received by the University of Tasmania via cold freight seven days after slaughter (temperature during transit: leg = 3.8 ± 0.9°C; shoulder = 3.9 ±1.5°C).
- Upon arrival, samples of each cut were randomly selected and stored at four different temperatures (30 samples per temperature) ranging from commercial storage to temperature abuse conditions (from -0.5°C, 2°C, 5°, and up to 10°C) for shelf-life assessment. Product temperature was recorded at 10 min intervals using data loggers. The shelf-life of each cut at each temperature was determined by organoleptic and microbial assessments as previously described by Jolley et al. (2019) and Kaur et al. (2021) with some modifications as described below.

4.2 Organoleptic and pH assessment

- Sensory attributes of samples (three replicates/packs at each time point for each temperature) were periodically assessed by a semi-trained sensory panel (comprising of three to six people) over the course of storage. Specifically, prior to opening, each pack was scored on an appropriate scale for vacuum/packaging integrity (0 = no vacuum, leaker; 2 = poor vacuum; 4 = moderate vacuum; 6 = good vacuum; 8 = complete vacuum, tight package adhesion), seal (0 = no seal, leaker; 2 = poor seal, 3 = good seal) and purge (0 = excessive purge; 2 = significant purge; 4 = moderate purge; 6 = minimal purge; 8 = no purge). Each pack was then sterilised using 70% ethanol and aseptically opened using a scalp blade and transferred to a sterile container.
- Persistent odour and colour/bloom were measured 10 mins after opening the pack. Odour was assessed on a 9-point categorical hedonic scale (0 = off-odour, "spoiled", to 8 = no odour, normal meaty odour), while colour was determined by a visually anchored scale (0 = other colour e.g., green colouration; 2 = very poor bloom, grey colouration; 4 = poor bloom, some greyness; 6 = bloom, light red colouration; 8 = bloom, red colouration). A mean score of <4 was considered as commercially unacceptable to consumers.
- Meat surface pH was measured using a handheld pH meter (Model 206-pH2, Testo, Australia) at eight random locations (4 x locations on both fat and lean tissue) on the meat sample. The pH meter was initially calibrated according to manufacturer's instructions.

4.3 Microbial assessment

- After sensory assessment, samples were assessed for bacterial numbers. Each meat piece was transferred into a sterile stomacher bag containing an appropriate amount of 0.1% (w/v) bacteriological peptone water (LP0037, Oxoid, Australia) followed by massaging by hand for a total of 2 min. Rinsate aliquots were removed and used to prepare tenfold serial dilutions in 0.1% bacteriological peptone water and appropriate dilutions were spread plated. Total viable counts were enumerated on tryptone soya agar (TSA, CM0131, Oxoid, Australia), and lactic acid bacteria on de Man-Rogosa-Sharpe agar (MRS, CM0361, Oxoid, Australia) under anaerobic conditions using Anaerogen Compact pouches (Anaerogen Compact pouch, Oxoid, Australia). Both TSA and MRS were incubated at 25°C for 5 days before colonies present were enumerated.

4.4 Data analysis

- A growth curve at each temperature was constructed by plotting the $\log_{10}$ CFU/cm^2 *versus* time (day). The 'D-model' was used to estimate growth parameters such as lag time (LT) and growth rate (GR) (Baranyi and Roberts, 1994). GR was then converted to generation time (GT). In addition, relative lag time (RLT) was calculated by dividing LT by GT, to characterise lag phase duration in the term of GT (Mellefont et al., 2003; Robinson et al., 1998; Ross, 1999).
- Wherever appropriate, a two-tailed *t*-test assuming equal variances was applied to assess statistical differences in kinetic parameters (*i.e., P-value of* ≤0.05).
- The end of shelf-life was estimated based on the time taken for samples to reach a mean sensory score (odour, colour, purge and vacuum integrity) of ≤4 at a given timepoint and subsequent timepoints. The observed shelf-life was then converted into rate of quality loss per day.
- Where relevant, parameters such as LT, RLT and shelf-life were estimated with a consideration of the time taken during transit.
- The square root model of Ratkowsky et al. (1982) was used to assess the effects of storage temperature on the rates of bacterial growth and quality loss. A one-way analysis of covariance (ANCOVA) was used to test for statistical differences in rates of bacterial growth and quality loss between product types (rind-on and rindless, and bone-in and boneless) and meat cuts (leg and shoulder).

4.5 Development of predictive models for shelf-life of VP pork

- From the data generated, mathematical models based on the square root model of Ratkowsky et al. (1982) were developed to predict the rates of TVC increase and quality loss (*i.e.,* odour development) as a function of temperature.
- In principle, the effects of temperature on the rates of TVC increase and quality loss can be described as:

$$\sqrt{\mu_{\text{TVC/odour development}}} = b(T - T_{min}) \qquad (1)$$

where $\mu_{TVC/odour\ development}$ is the specific rate of TVC growth or odour development (in days); *b* is the slope of the regression line; *T* is the temperature at which meat is stored (°C); and T_{min} is a notional minimal temperature for any given reaction and is the intercept of the regression line with the x (temperature) axis.

- With the parameters (*b* and T_{min}) obtained from Equation (1), shelf-life of a VP product based on odour development can be predicted as follows:

$$SL = [\frac{1}{b \times (T - T_{\text{min}})}]^2 \qquad (2)$$

where *SL* is the shelf life of VP pork (days); *b* is the slope of the regression line that corresponds to *b* in Equation (1); *T* is the temperature at which meat is stored (°C); and T_{min} is the minimum temperature where the rate of odour development is zero.

- Equation (2) was then modified to predict remaining shelf life by accounting for the observed initial TVC (*i.e.,* at the time of packaging). This was achieved by calculating a correction factor that considers the initial TVC numbers and the calculated TVC numbers of a given product at the time of spoilage at a given temperature. This correction factor was then incorporated to Equation (2) to predict the remaining shelf-life ($SL_{\text{remaining}}$; day).

$$SL_{\text{remaining}} = [\frac{N_0 - (N_{\text{obs}}) + N_{\text{s}}}{N_{\text{s}}}] \times [\frac{1}{b \times (T - T_{\text{min}})}]^2 \qquad (3)$$

where N_0 is the initial TVC based on the experimental data ($\log_{10}$ CFU/cm^2) for a given product stored at -0.5˚C; N_{obs} is the expected or measured TVC ($\log_{10}$ CFU/cm^2) at some time, *t*,; and N_s is the nominal TVC on VP pork at the time of spoilage ($\log_{10}$ CFU/cm^2). The N_s value was estimated by extrapolation of the regression line of the experimental data to the time at which a product was spoiled at -0.5˚C.

- Based on the developed Equations (1) and (3), a model interface was produced in MS 365 ®Excel to predict the growth of TVC and the remaining shelf-life of various VP pork cuts (leg or shoulder, bone-in or boneless and rind-on or rindless).

5. Results and Discussion

5.1 Microbial growth on VP pork meat during storage

- Figures 1 – 4 show a typical growth response of bacteria (TVC and LAB) on various VP pork products stored at a low temperature.

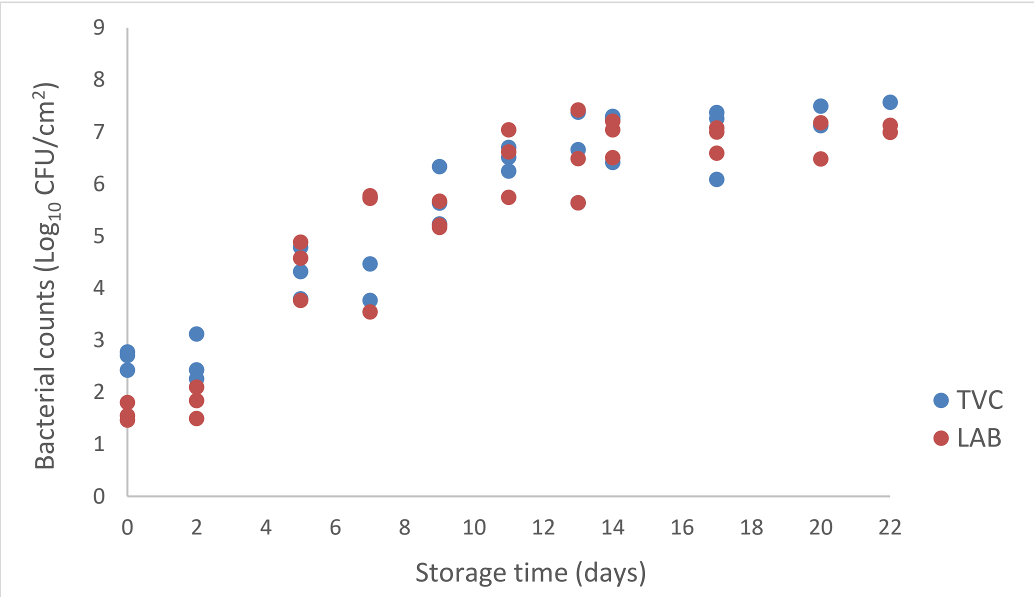

Figure 1 *Representative growth curve of total viable counts (TVC) and lactic acid bacteria (LAB) on vacuum-packed rindless pork leg over the course of storage at 5°C.*

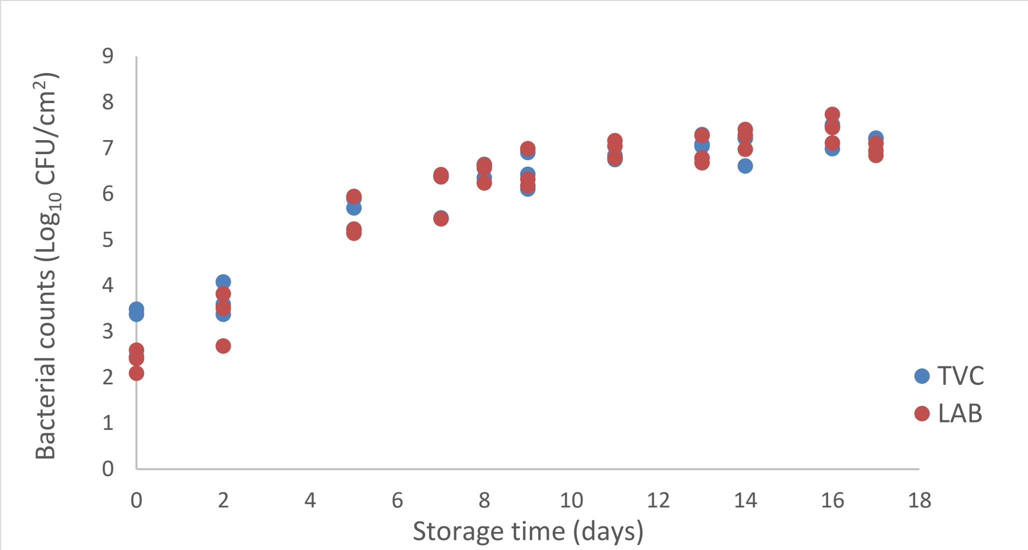

Figure 2 *Representative growth curve of total viable counts (TVC) and lactic acid bacteria (LAB) on vacuum-packed rind-on pork leg over the course of storage at 5°C.*

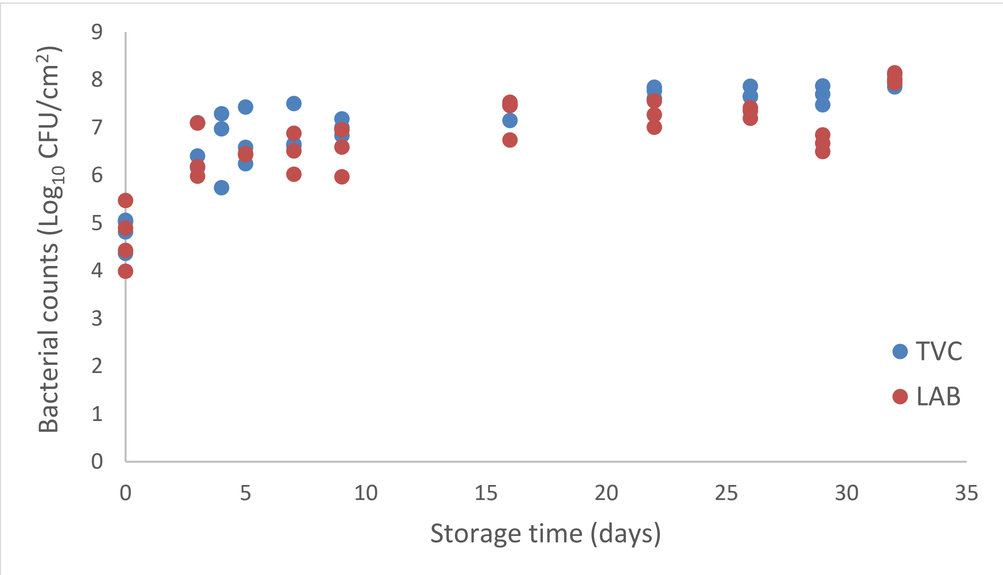

Figure 3 *Representative growth curve of total viable counts (TVC) and lactic acid bacteria (LAB) on vacuum-packed rind-on, bone-in pork shoulder over the course of storage at -0.5°C.*

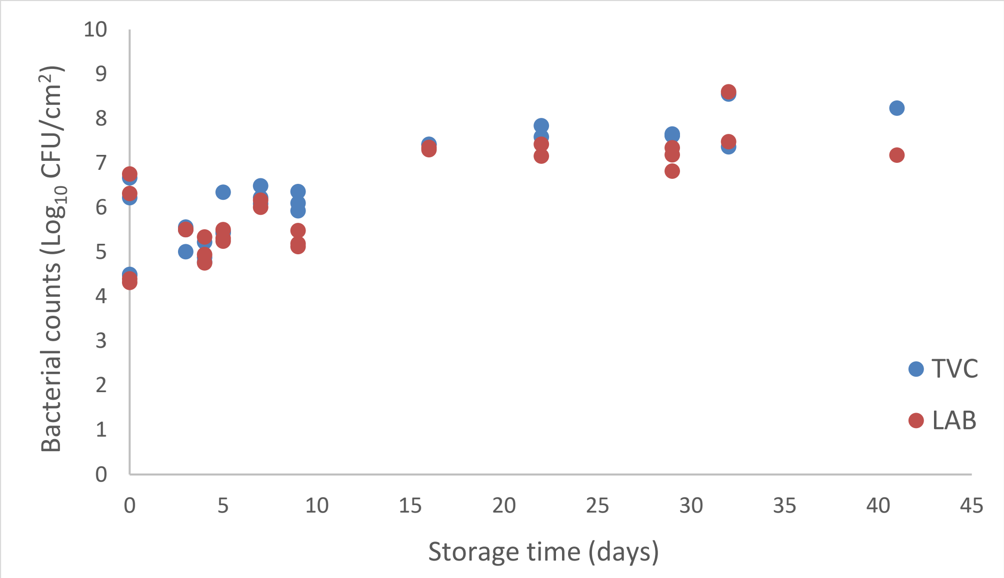

Figure 4 *Representative growth curve of total viable counts (TVC) and lactic acid bacteria (LAB) on vacuum-packed rindless, boneless pork shoulder over the course of storage at -0.5°C.*

- The initial TVC (7 days after slaughter) on VP pork leg ranged between 3.0 – 3.1 $\log_{10}$ CFU/cm^2 on rind-on products and 2.5 – 2.7 $\log_{10}$ CFU/cm^2 on rindless products. This was consistently higher compared to that of LAB counts, which ranged between 2.3 – 2.4 $\log_{10}$ CFU/cm^2 on rind-on products and 1.5 – 1.7 $\log_{10}$ CFU/cm^2 on rindless products (Figures 1 and 2).
- In contrast to VP leg, VP shoulder showed to have similar TVC and LAB numbers at the first sampling point. These ranged between 4.7 – 5.0 $\log_{10}$ CFU/cm^2 on bone-in products and 5.3 – 5.5 $\log_{10}$ CFU/cm^2 on boneless products. The apparent similarity of both TVC and LAB counts on shoulder but not on leg suggests that the microbial community on shoulder was more established as also supported by the higher counts observed at the first sampling point (Figure 3 and 4). These differences might be attributed to processing, as each cut was sourced from different processing facilities (Kaur et al., 2017; Kaur et al., 2021; Tucker and Klepper, 2005).
- TVC and LAB on all rindless products (leg and shoulder) entered a lag phase for a period of time before growth commenced (see representative growth curves in Figures 1 and 4). This is with the exception of rind-on leg stored at -0.26°C, which had no lag phase. However, there was no lag time observed for rind-on products regardless of the cut type (leg and shoulder). The basis of this variation between rind-on and rindless products might be due to the fact that rind was removed, creating a new environment for bacterial growth. It is well established that when bacteria are transferred from one environment to another, they typically display a period of delay in growth, resulting in their numbers to remain unchanged. During this period, bacterial cells modify their physiological state to adapt to their new

environment before growth can be re-established (Buchanan and Cygnarowicz, 1990; King et al., 2014; Mellefont and Ross, 2003).

- Both TVC and LAB reached similar maximum populations on each product type at each storage temperature, ranging between 6.84 – 7.57 $\log_{10}$ CFU/cm^2 on leg and 6.89 – 8.20 $\log_{10}$ CFU/cm^2 on shoulder. These results are in accordance with previous observations in which LAB population was a predominate part of the total bacterial population in meat stored at low temperatures under anaerobic condition (Jiang et al., 2010; Li et al., 2006; Pennacchia et al., 2011; Zhao et al., 2015).
- Tables 1 to 4 summarise the bacterial growth kinetics on VP pork products stored at different temperatures (ranging from -0.5 to 10°C).

Table 1 *Summary of the growth kinetics of total viable count (TVC) on vacuum-packed boneless pork leg products stored at different temperatures. RO represents rind-on products; and RL represents rindless products.*

Observed temperature (°C)*	Product type	LT(d)[a]	GR (d^{-1})[b]	RLT[c]	RMSE[d]
8.13	RO	-	0.4960	-	0.36
8.13	RL	3.62	0.5499	6.61	0.48
4.88	RO	-	0.4387	-	0.36
5.45	RL	4.15	0.4209	5.80	0.45
2.21	RO	-	0.2502	-	0.35
2.21	RL	9.74	0.2015	6.52	0.33
-0.67	RO	-	0.1564	-	0.38
-0.26	RL	-	0.0786	-	0.40
Average:				**6.31 ± 0.45**	

* Observed temperature 4 days after packaging
a LT = Lag time
b GR = Growth rate
c RLT = Relative lag time as determined by dividing lag time by generation time
d RMSE = root-mean-square error, a value between 0.2 and 0.5 shows that the model can predict the data accurately

Table 2 *Summary of the growth kinetics of lactic acid bacteria (LAB) on vacuum-packed boneless pork leg products stored at different temperatures. RO represents rind-on products; and RL represents rindless products.*

Observed temperature (°C)*	Product type	LT(d) [a]	GR (d^{-1})[b]	RLT[c]	RMSE[d]
8.13	RO	-	0.5312	-	0.41
8.13	RL	7.80	0.5186	13.45	0.51[f]
4.88	RO	-	0.5403	-	0.37
5.45	RL	7.62	0.5399	13.68	0.50[f]
2.21	RO	-	0.6433	-	0.35
2.21	RL	17.10	0.2741	15.58	0.51[f]
-0.67	RO	-	0.1720	-	0.39
-0.26	RL	-	0.0847	-	0.43
Average:				**14.23 ± 1.17**	

* Observed temperature 4 days after packaging
a LT = Lag time
b GR = Growth rate
c RLT = Relative lag time as determined by dividing lag time by generation time
d RMSE = root-mean-square error, a value between 0.2 and 0.5 shows that the model can predict the data accurately
f RMSE values indicating poor fit model

Table 3 *Summary of the growth kinetics of total viable count (TVC) on vacuum-packed pork shoulder products stored at different temperatures. RO represents rind-on products; RL represents rindless products; BI represents bone-in; and BL represents boneless.*

Observed temperature (°C)*	Product type	LT(d)[a]	GR (d^{-1})[b]	RLT[c]	RMSE[d]
10.56	RO, BI	-	0.8756	-	0.37
10.56	RL, BL	2.65	1.1467	10.09	0.21
4.43	RO, BI	-	0.6150	-	0.42
4.18	RL, BL	5.41	0.5733	10.30	0.25
2.21	RO, BI	-	0.4057	-	0.31
2.21	RL, BL	9.00	0.4035	12.07	0.30
-0.26	RO, BI	-	0.2888	-	0.35
-0.88	RL, BL	17.28	0.2419	13.89	0.34
Average:				**11.59 ± 1.77**	

* Observed temperature 5 days after packaging
a LT = Lag time
b GR = Growth rate
c RLT = Relative lag time as determined by dividing lag time by generation time
d RMSE = root-mean-square error, a value between 0.2 and 0.5 shows that the model can predict the data accurately

Table 4 *Summary of the growth kinetics of lactic acid bacteria (LAB) on vacuum-packed pork shoulder products stored at different temperatures. RO represents rind-on products; RL represents rindless products; BI represents bone-in; and BL represents boneless.*

Observed temperature* (°C)	**Product type**	**LT(d)** [a]	**GR (d^{-1})** [b]	**RLT** [c]	**RMSE** [d]
10.56	RO, BI	-	0.8235	-	0.38
10.56	RL, BL	3.91	1.2509	16.24	0.27
4.43	RO, BI	-	0.9069	-	0.46
4.18	RL, BL	6.15	0.6463	13.19	0.22
2.21	RO, BI	-	0.3566	-	0.36
2.21	RL, BL	16.11	0.2306	12.34	0.45
-0.26	RO, BI	-	0.5725	-	0.51 [f]
-0.88	RL, BL	8.93	1.3295	39.43	0.23
				20.30 ± 12.86	

* Observed temperature 5 days after packaging
a LT = Lag time
b GR = Growth rate
c RLT = Relative lag time as determined by dividing lag time by generation time
d RMSE = root-mean-square error, a value between 0.2 and 0.5 shows that the model can predict the data accurately
f RMSE values indicating poor fit model

- In all cases, the growth kinetics of TVC as estimated by the D-Model had a root-mean-square error (RMSE) value within the range (between 0.2 and 0.5). This indicates a good fit between observed values and those predicted by the model (RMSE = 0.21 – 0.48) (Tables 1 and 3). However, the D-model fits for the LAB data were relatively poor, and in many instances, had an RMSE value that indicates poor data predictability (RMSE > 0.5) (Tables 2 and 4). This noisiness in the LAB data was likely due to inconsistent recovery of LAB onto MRS agar from meat samples, as previously reported by Kaur et al. (2021).
- The growth rates of TVC on VP pork leg and shoulder increased as the storage temperature increased (Tables 1 and 3). This was as expected from the vast amount of published data on the effects of temperature on microbial growth rates (EFSA Panel on Biological Hazards (BIOHAZ), 2016; Corkrey et al., 2012; Kaur et al., 2021). However, this trend was not clear for LAB data, which again, is likely due to its poor fit to the model (Tables 2 and 4).
- It was evident that the observed bacterial lag time typically increased as storage temperature decreased (Tables 1 and 2). The relative lag time (RLT) response of TVC and LAB also appeared to be significantly different ($P \leq 0.05$) between pork leg (TVC = 6.31 ± 0.45; LAB = 14.23 ± 1.17) and shoulder (TVC = 11.59 ± 1.77; LAB = 20.30 ± 12.86). The high variance evident for the RLT of LAB data is likely due to the poor fit to the model (*see* above).
- Analysis of the growth rate data using the square root model (Ratkowsky et al., 1982) established that the rates of TVC growth were strongly temperature dependent (R^2 = 0.94 – 0.98). This was not the case for rates of LAB growth (R^2 = 0.41 – 0.86), which was expected

due to the noisiness in the LAB data (as already described above). The growth rate of TVC, but not LAB, on VP pork could therefore be predicted as a function of temperature. Accordingly, the TVC data was used for subsequent analyses only.

- To determine the effects of different variables (*i.e.,* rind-on *vs* rindless, bone-in *vs* boneless and leg *vs* shoulder) on bacterial growth, the rates of TVC increase across storage temperatures were grouped according to their variables (to increase the number of datapoints being considered) and compared (Figures 5 – 7).

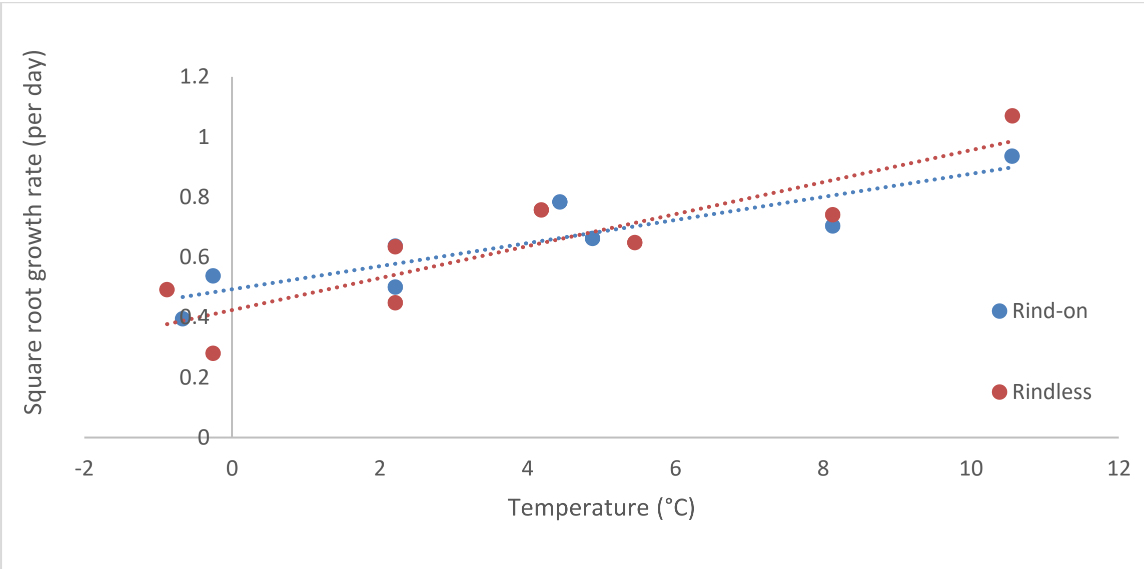

Figure 5 *Square root plot of total viable counts (TVC) on vacuum-packed pork rind-on and rindless products (including shoulder and leg) across storage temperature. ANCOVA revealed no significant difference between rind-on and rindless products (P > 0.05).*

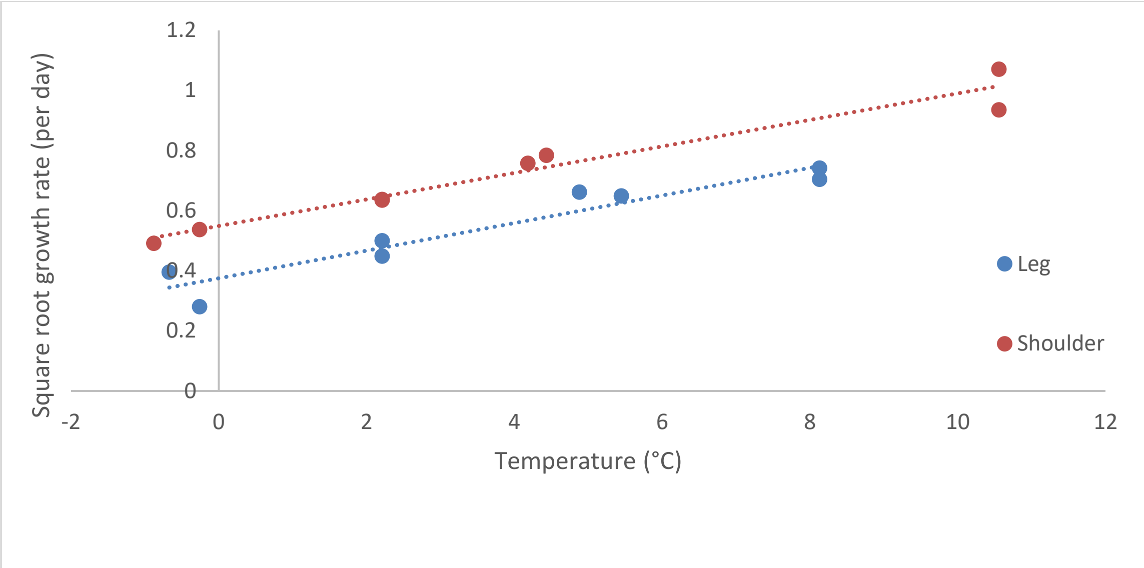

Figure 6 *Square root plot of total viable counts (TVC) on vacuum-packed pork leg and shoulder across storage temperature. ANCOVA revealed a significant difference between leg and shoulder (P < 0.05).*

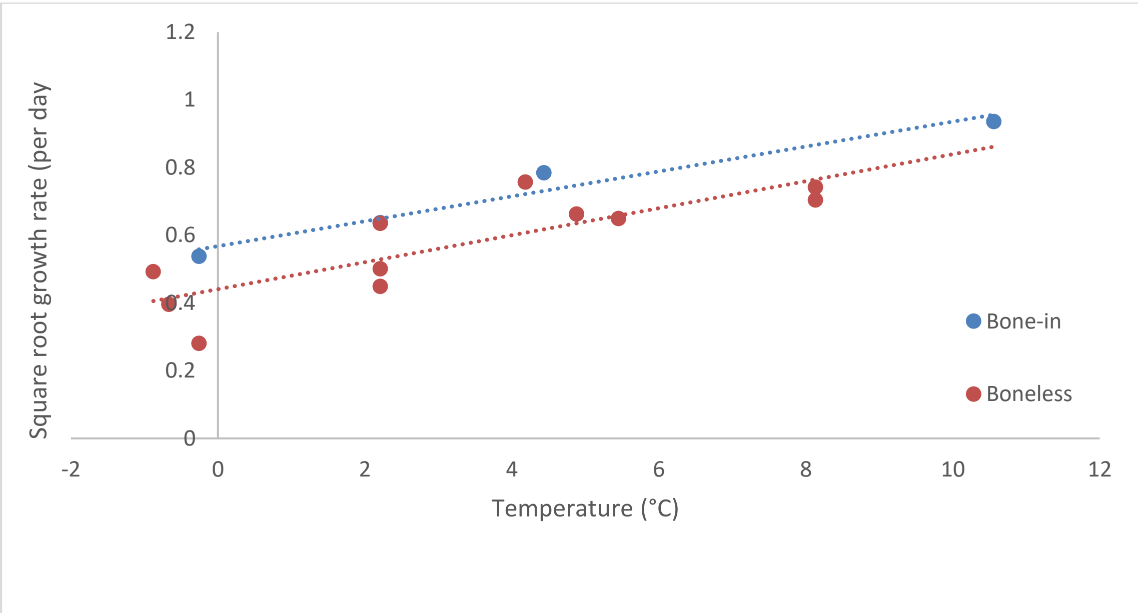

Figure 7 *Square root plot of total viable counts (TVC) on vacuum-packed bone-in and boneless pork (including shoulder and leg) across storage temperature. ANCOVA revealed a significant difference between bone-in and boneless products (P < 0.05).*

- It was found that the rates of TVC increase were not significantly different ($P > 0.05$) between rind-on and rindless products regardless of their cut (leg and shoulder) (Figure 5). However, shoulder had consistently ($P < 0.05$) faster rates of TVC increase compared to leg across all temperatures tested (Figure 6). A similar observation was also observed in which the rates of TVC were consistently faster ($P < 0.05$) on bone-in products than on boneless products (Figure 7). The faster growth rate observed for pork shoulder and bone-in products (compared to their relative cut/product) could be due to differences in their biochemistry, such as differences in meat pH (*see* the results below for more details) (Holmer et al., 2009), and/or lactic acid and glucose content as reflected by different muscle types (Knox et al., 2008). The bacterial growth rate might also be faster on bone-in products due to the higher pH of exposed bone marrow and bone dust (Field, 1999). However, it should be noted that the specific effects of cut type cannot be differentiated from potential variation that may be attributed to different processing facilities (e.g., differences in processing and abattoir specific resident microbial communities) (Hultman et al., 2015; Kaur et al., 2017), as different cuts were sourced from different facilities. Similarly, the effects of bone on the rates of TVC increase cannot be fully determined due to limited data (n=4 for bone-in products and from one source, Figure A7). Further research using pork leg and shoulder sourced from the same processing facility, and to generate more data using the same product type with and without bone from other sources, is therefore, required to confirm the observations of this study.

5.2 Meat pH during storage

- The pH of each product type (rind-on *vs* rindless, bone-in *vs* boneless) was consistent for each type of cut (leg and shoulder), and typically showed a slight decline over storage period. This was expected as LAB (a major organism in VP) produce organic acid metabolic by-products, which can reduce the pH of VP meat over storage (Rood et al., 2022a) (*see* representative trend in Fig 8).

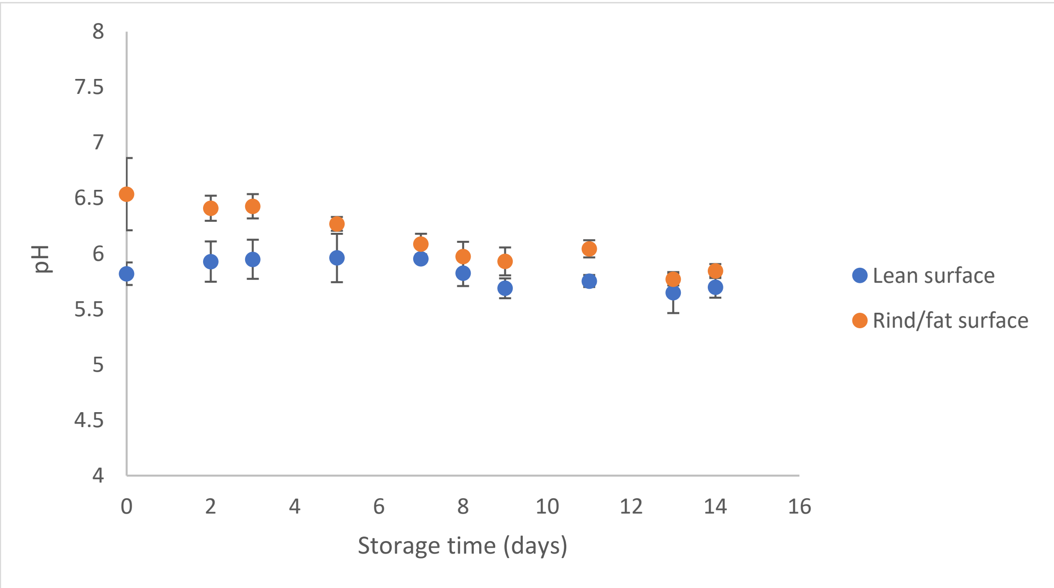

Figure 8 *Representative pH trend of vacuum-packed rind-on boneless pork leg over the course of storage at 8.13°C.*

- At the beginning of storage, the average pH of leg rind tissue was 6.54 ± 0.33, which was significantly (*P* < 0.05) higher compared to its lean tissue, which had a pH of 5.82 ± 0.10.

However, there was no significant difference ($P > 0.05$) between rind and lean tissues observed for shoulder, which had a pH of 6.53 ± 0.23 and 6.74 ± 0.34, respectively. The higher pH of the rind tissue compared to lean tissue observed for leg but not for shoulder remains to be elucidated. Furthermore, it is important to note that the rind tissue did not have an influence on the bacterial growth rate between rind-on and rindless products for both cut type as described above (Fig. 5).

- The pH of pork shoulder was significantly higher ($P < 0.05$) compared to leg regardless of the product type. The differences were more pronounced at the initial timepoint, which can be summarised in Table 5. Meat with a higher pH can increase bacterial growth rates, which likely explains the significantly faster growth rates observed on shoulder compared to leg (Figure 6) (EFSA Panel on Biological Hazards (BIOHAZ), 2016; Holmer et al., 2009; Knox et al., 2008). However, as mentioned above, the effects of cut type cannot be differentiated from potential variation that may be attributed to different processing facilities.

Table 5 *pH average and standard deviation of vacuum-packed pork products seven days after slaughter.*

Product	**Tissue**	**Leg**	**Shoulder**
Rind-on	lean	5.82 + 0.10	6.53 + 0.23
	rind/fat	6.54 + 0.33	6.74 + 0.34
Rindless	lean	5.67 + 0.03	6.28 + 0.28
	fat	5.73 + 0.11	6.41 + 0.11

5.3 Shelf-life of VP pork products

- In all cases, meat odour score was the key indicator for meat spoilage as it was the first parameter to reach an unacceptable limit of ≤4 at a given timepoint and all subsequent timepoints (Figure 9). Therefore, odour score was the sensory parameter used in subsequent analyses. This agrees well with previous studies that reported odour score was the main parameter indicative of spoilage of VP red meats (Kaur et al., 2021; Meinert and Christensen, 2014; Rood et al., 2022a; Rood et al., 2022b).

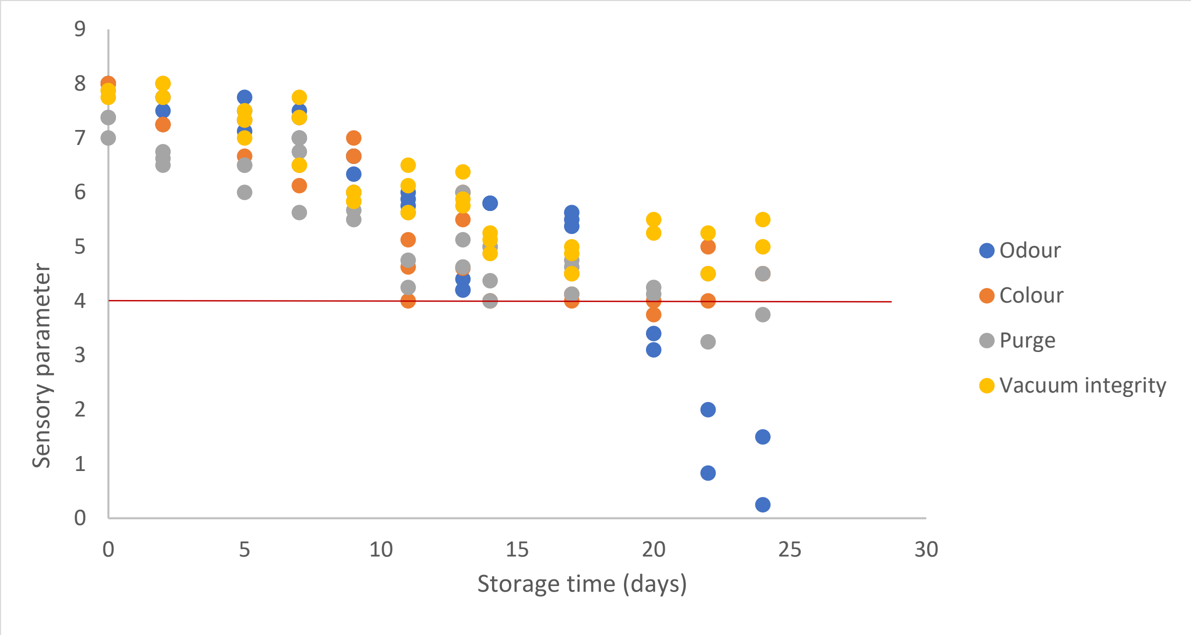

Figure 9 *Representative plot of sensory parameter scores of vacuum-packed pork over storage at 8.13°C. A mean score of ≤4, highlighted by the horizontal line, at a given timepoint and all subsequent timepoints, is regarded as commercially unacceptable.*

- Tables 6 and 7 summaries the shelf-lives of VP pork products stored at different temperatures (ranging from -0.5°C to 10°C). As expected, the shelf-life of VP pork products increased as the storage temperature decreased.

Table 6 *Summary of the shelf-lives (based on odour score) of vacuum-packed boneless pork leg products stored at different temperatures. RO represents rind-on products; and RL represents rindless products.*

Temp (°C)	Product	Observed shelf-life (days)*
8.13	RO	11
8.13	RL	13
4.88	RO	16
5.45	RL	20
2.21	RO	44
2.21	RL	40
-0.67	RO	75
-0.26	RL	82

* Time in transit taken into account

Table 7 *Summary of the shelf-lives (based on odour score) of vacuum-packed pork shoulder products stored at different temperatures. RO represents rind-on products; RL represents rindless products; BI represents bone-in; and BL represents boneless.*

Temp (°C)	Product	Observed shelf-life (days)*
10.56	RO, BI	6
10.56	RL, BL	7
4.43	RO, BI	12
4.18	RL, BL	16
2.21	RO, BI	21
2.21	RL, BL	27
-0.26	RO, BI	39
-0.88	RL, BL	72

* Time in transit taken into account

- Analysis of the shelf-life data using the square root model (Ratkowsky et al., 1982) established that the rates of quality loss were strongly temperature dependent (R^2 = 0.95 – 1.0). The rate of quality loss (based on odour score) can, therefore, be predicted as a function of temperature.
- To determine the effects of different variables (*i.e.,* rind-on *vs* rindless, bone-in *vs* boneless and leg *vs* shoulder) on the shelf-life, the rates of quality loss across storage temperatures were grouped according to their variables and compared (Figures 10-12).

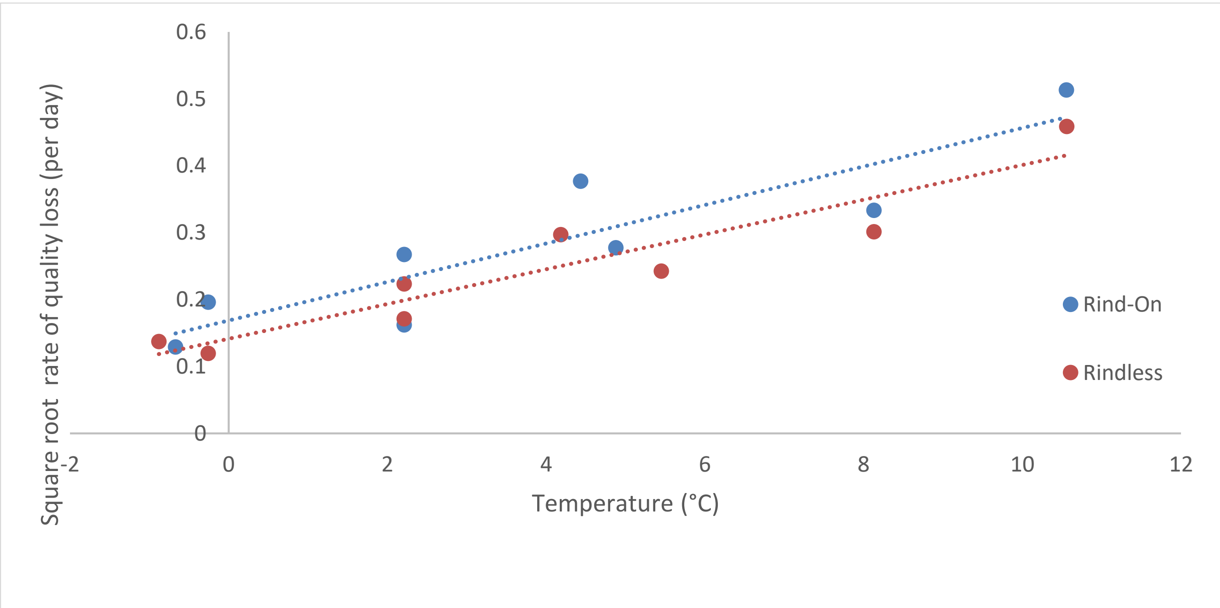

Figure 10 *Square root plot of odour score of vacuum-packed rind-on and rindless pork products (including shoulder and leg) across storage temperature. ANCOVA revealed no significant difference between rind-on and rindless products (P > 0.05).*

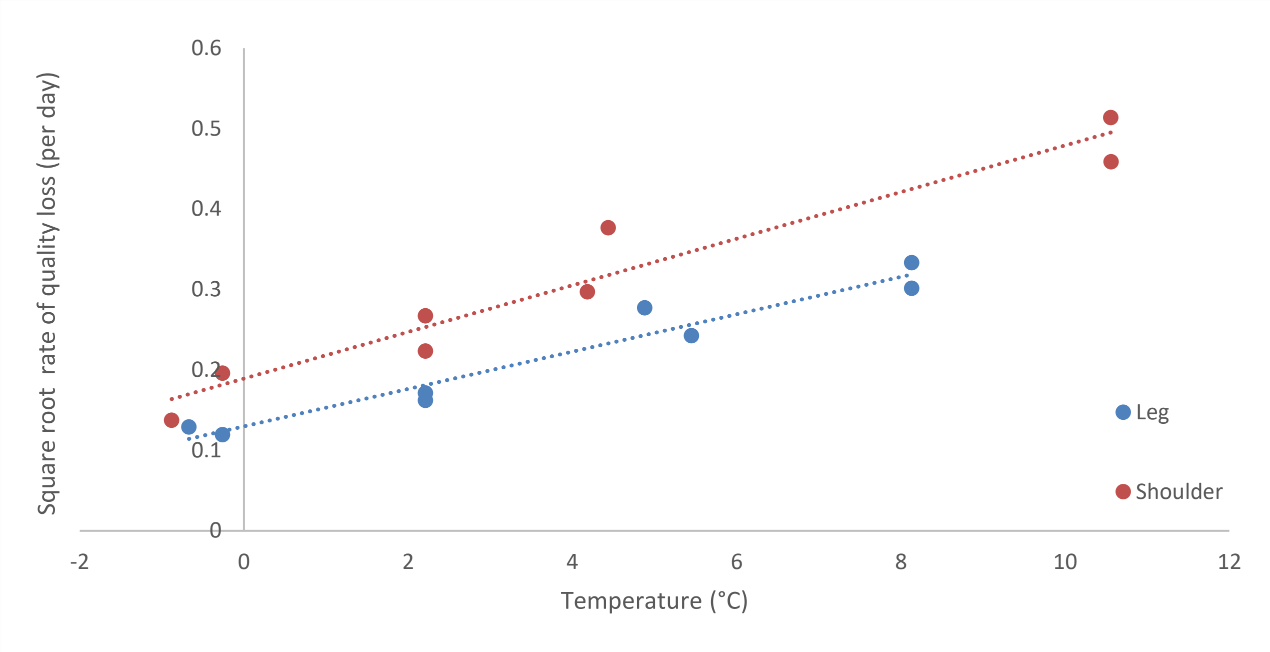

***Figure 11** Square root plot of odour score of vacuum-packed pork leg and shoulder across storage temperature. ANCOVA revealed a significant difference between leg and shoulder (P < 0.05).*

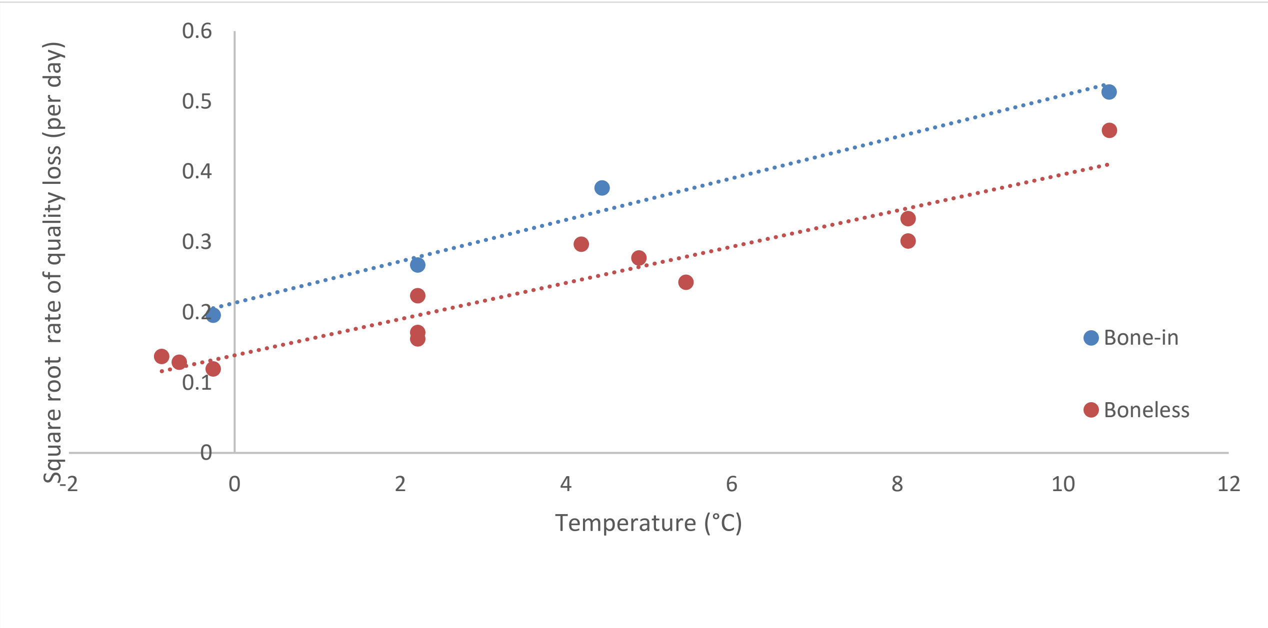

***Figure 12** Square root plot of odour score of vacuum-packed bone-in and boneless pork products (including shoulder and leg) across storage temperature. ANCOVA revealed a significant difference between leg and shoulder (P < 0.05).*

- It was found that the rates of quality loss were not significantly different ($P > 0.05$) between rind-on and rindless products (Figure 10). In contrast, shoulder and bone-in products were found to have consistently ($P < 0.05$) faster rates of quality loss compared to leg and boneless products across all temperatures tested, respectively (Figures 11 and 12). The faster rates of quality loss of shoulder and bone-in products are likely due to the faster rates of TVC increase compared to their relative product, as discussed above.

5.4 *Development of* shelf-life predictive tool for VP pork

- Based on earlier analysis of data, it was determined that different mathematical models were required to accurately predict TVC growth and shelf-life of different VP pork products. This is due to the observations that different products exhibited a different response for TVC growth and odour development. Specifically, TVC entered a lag phase before growth occurred on rindless but not on rind-on (Tables 1-4). This was despite no significant difference in the rates of TVC increase between these two product types (Figure 5). The rates of quality loss (based on odour development) were also different between leg and shoulder, and bone-in and boneless products (Figures 11 and 12, respectively).
- Tables 8 and 9 describe the model parameters used in Equations (1) and (3) for TVC and odour development for different pork products, respectively.

Table 8 *Estimated values of the parameters of Equation (1) for the specific rate of TVC increase, and relative lag time observed on different VP pork products.*

Product type	b^a	T_{min} (°C)^b	***Relative lag time***^{c}
Leg			
Rind-on boneless	0.0459	-8.1721	N/A
Rindless boneless	0.0459	-8.1721	6.3088
Shoulder			
Rind-on bone-in and boneless	0.0441	-12.4535	N/A
Rindless bone-in and boneless	0.0441	-12.4535	11.5881

a b = the slope of the regression line
b T_{min} = a notional minimal temperature for any given reaction and is the intercept of the regression line with the x (temperature) axis.
c Relative lag time as determined by dividing lag time by generation time.

Table 9 *Estimated values of the parameters of Equations (3) for the specific rate of odour development, on different VP pork products.*

Product type	b^a	T_{min} (°C)^b	N_0 (Log$_{10}$ CFU/cm^2)^c	N_s (Log$_{10}$ CFU/cm^2)^d
Leg				
Rind-on and rindless boneless	0.0213	-5.5681	2.87	9.79
Shoulder				
Rind-on and rindless bone-in	0.0238	-7.2143	4.82	11.80
Rind-on and rindless boneless	0.0240	-5.8875	5.47	13.53

a b = the slope of the regression line
b T_{min} = a notional minimal temperature for any given reaction and is the intercept of the regression line with the x (temperature) axis.
c N_0 is the initial TVC based on the experimental data (log CFU/cm2) for a give product stored at -0.5˚C
d N_s is the nominal TVC on VP pork at the time of spoilage (log CFU/cm^2), as estimated by extrapolation of the regression line of the experimental data to the time at which a product was spoiled at -0.5˚C

- With the parameters (b and T_{min}) obtained above, predictive models for the TVC growth and shelf-life (based on odour development) of different VP pork were developed in accordance with Equations (1) and (2). However, such models could not be used to specifically predict the remaining shelf life of a given VP product (*i.e.*, as defined in Equation (3)). This requires a correction factor to be determined, which is based on the following parameters (*i.e.*, N_0, and N_s). These parameters were determined based on the current data for the shelf-life of different VP pork products stored at -0.5˚C (Table 9).
- The developed models were incorporated into a standalone tool (implemented in MS 365 ®Excel) that allows prediction of the growth of TVC and calculates the remaining shelf life of various pork products in cold chains (Figure 1 and also *see* Supplementary File 1).
- To use this tool, the user selects the product type (leg or shoulder, bone-in or boneless, and rind-on or rindless), enters the starting TVC (*e.g.*, at packing or at the beginning of cold storage), and a time:temperature profile, typically collected by a temperature datalogger. The tool then predicts the TVC growth response and remaining shelf-life of the product based on the time taken to develop unacceptable odour. To account for different market shelf-life specifications, the tool was also designed to predict a second shelf-life based on the time taken for TVC on samples to reach 10^7 CFU/cm^2 (based on the "D-model').

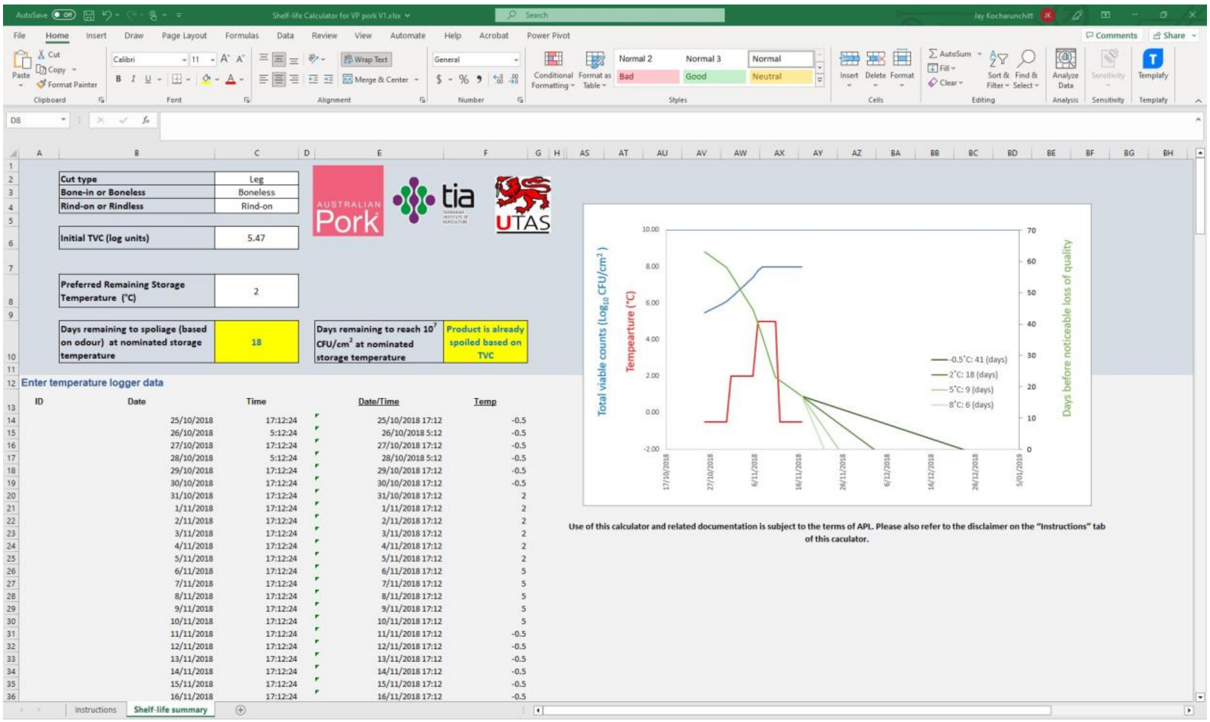

Figure 13 *A screenshot of a tool for shelf-life prediction of Australian VP pork.*

5.5 Comparison of observed data with predictive models

- To test the developed predictive tool, comparisons were made between the observed and predicted shelf-life data for each VP pork product at different storage temperatures. A publicly available tool for shelf-life prediction of VP pork developed by The Danish Meat Research Institute (DMRI) (Meinert and Christensen, 2014; access via http://dmripredict.dk) was also included for comparison. This tool can be used to predict the shelf-life of all pork cuts (regardless of their type, *i.e.,* with or without rind, fat and bone) either based on psychotropic growth or odour decline as a function of storage temperature. The input for the DMRI tool also includes initial count and its standard deviation, storage temperature. Table 10 -13 summarise these comparisons.

Table 10 *Summary of the observed and predicted shelf-lives of vacuum-packed rind-on, boneless pork leg stored at different temperatures.*

Temp (°C)	Initial TVC (log_{10} CFU/cm²)	Shelf-life based on odour			Shelf-life based TVC to Log 7		
		Observed*	Predicted (current tool)	Predicted (DMRI tool)	Observed*	Predicted (current tool)	Predicted (DMRI tool)
8.13	3.11	11	11	-	8	6	-
4.88	3.11	16	19	20	11	10	16
2.21	3.11	44	35	35	20	17	28
-0.67	3.11	75	89	63	30	32	52

* Time in transit taken into account
- Temperature outside DMRI limits

Table 11 *Summary of the observed and predicted shelf-lives of vacuum-packed rindless, boneless pork leg stored at different temperatures.*

Temp (°C)	Initial counts (log_{10} CFU/cm²)	Shelf-life based on odour			Shelf-life based TVC to Log 7		
		Observed*	Predicted (current tool)	Predicted (DMRI tool)	Observed*	Predicted (current tool)	Predicted (DMRI tool)
8.13	2.64	13	12	-	13	11	-
5.45	2.64	20	18	18	18	16	15
2.21	2.64	40	37	37	35	27	31
-0.26	2.64	82	80	62	52	47	54

* Time in transit taken into account
- Temperature outside DMRI limits

Table 12 *Summary of the observed and predicted shelf-lives of vacuum-packed rind-on, bone-in pork shoulder stored at different temperatures.*

Temp (°C)	Initial counts (log_{10} CFU/cm²)	Shelf-life based on odour			Shelf-life based TVC to Log 7		
		Observed*	Predicted (current tool)	Predicted (DMRI tool)	Observed*	Predicted (current tool)	Predicted (DMRI tool)
10.56	4.82	6	5	-	2	2	-
4.43	4.82	12	13	16	3	3	10
2.21	4.82	21	19	26	6	5	16
-0.26	4.82	39	36	44	7	7	28

* Time in transit taken into account
- Temperature outside DMRI limits

Table 13 *Summary of the observed and predicted shelf-lives of vacuum-packed rindless, boneless pork shoulder stored at different temperatures.*

Temp (°C)	Initial counts (log_{10} CFU/cm²)	Shelf-life based on odour			Shelf-life based TVC to Log 7		
		Observed*	Predicted (current tool)	Predicted (DMRI tool)	Observed*	Predicted (current tool)	Predicted (DMRI tool)
10.56	5.47	7	6	-	5	4	-
4.18	5.47	16	17	16	9	9	8
2.21	5.47	27	26	23	14	11	12
-0.88	5.47	72	69	43	26	19	22

* Time in transit taken into account
- Temperature outside DMRI limits

- To compare between the observed shelf-life data and that predicted by the developed tool and the DMRI tool (for shelf-lives based on either odour or TVC), the bias and accuracy factor indices of Ross (1996) were used to assess the performance of model predictions of shelf-life compared with the observed data. Ross (1996) noted that the bias factor serves as a measurement index for the average deviation between the observed and predicted values, whereas the accuracy factor is used to estimate the accuracy of an established model. Bias and accuracy factor values of 1 indicate a perfect agreement between observed and predicted values. Table 14 summarises the bias and accuracy factors for different comparisons.

Table 14 *Summary of the bias and accuracy factors between the observed shelf-life data and that predicted by different predictive models.*

Performance indices[a]	Shelf-life based on odour		Shelf-life based on TVC	
	Current tool	DMRI tool	Current tool	DMRI tool
Bias factor	0.97	0.95	0.88	1.41
Accuracy factor	1.10	1.22	1.14	1.60

a Both bias and accuracy factors were determined as described by Ross (1996).

- The data indicates that the developed tool overall provides a more accurate prediction (*i.e.,* having both bias and accuracy factors closer to 1) when compared to the DMRI tool (Table 14). However, given that the comparison was made based on the data used to develop the tool, these results were as expected, and can only be interpreted as the tool effectively generating a predicted shelf-life, rather than the accuracy of its prediction. Evaluation of the developed tool using independent shelf-life data is still required to evaluate its true performance.
- It was also found that the developed tool systematically underpredicts the shelf-life of VP pork (based on either odour or TVC) providing 'fail-safe' predictions (Table 14). The tool also showed to predict the shelf-life within 10% deviation of the observed data. This is in contrast to the DMRI tool which provides an over-prediction ('fail dangerous' predictions) of time to spoilage based on TVC but not odour (with approximately 20% deviation). However, Meinert and Christensen (2014) found that the DMRI tool over-estimate shelf-life based on odour. The apparent differences in their performance might be because the developed tool uses different models (as opposed to one model in the DMRI tool) to account the differences in the shelf-lives of different VP pork products (*i.e.,* cut type, rind-on *vs* rindless, and bone-in *vs* boneless). However, it is worthwhile noting that the DMRI tool

accounts for differences in product shelf-life by providing a shelf-life range based on standard deviation for TVC data and 80% confidence intervals for odour score. For instance, for VP pork with an initial count of 2.64 log CFU/cm^2 stored at -0.26, the DMRI tool predicts an average shelf-life of 62 days but provides a shelf-life range of 45 – 79 days.

6. Conclusions

- This study found that TVC and odour score were the most appropriate parameters to estimate the shelf-life of different VP pork products as a function of temperature.
- As expected, all VP pork products achieved a longer shelf-life as the storage temperature decreased with a shelf-life of up to 82 days at approximately -0.5°C. This was in line with the observations that bacterial growth rates decreased with a decrease in temperature.
- Different VP pork products (*i.e.,* leg *vs* shoulder, bone-in *vs* boneless, and rind-on *vs* rindless) exhibited a different response for TVC growth and odour development, indicating that different mathematical models are required to provide an accurate prediction of the shelf-life of different VP pork.
- New mathematical models for shelf-life prediction of VP pork products were successfully developed and incorporated into a first version of the tool in MS 365 ®Excel.
- Preliminary analysis suggested that the developed models may outperform the DMRI tool in predicting the shelf-lives of VP pork calculated by either odour or TVC. This was as expected given that the data used for comparison was used to develop the models.
- Evaluation of the developed models using independent data is required to specially determine their performance.

7. Implications & Recommendations

The shelf-life data generated in this research has been used to develop a shelf-life predictive tool. This will provide the Australian pork industry with a reliable and cost-effective decision-support tool for better management of its cold chains, *i.e.*, to optimise product quality, reduce wastage due to unexpected loss of quality, reduce the need for markdowns, and, more importantly, reduce customer complaints. However, before the tool can be adopted by the industry, future research to specifically evaluate the performance of the tool is required for a range of different processing facilities and product types.

8. Intellectual Property

A shelf-life predictive tool for Australian VP pork was developed from this research. It is anticipated that the tool will not be patented or require licensing.

9. Technical Summary

This project has provided/developed:

- the bases of Australian pork shelf-life at different storage temperatures that can be used to support negotiations with retailers and/or international markets to develop science-based shelf-life criteria to replace currently empirical and outdated or irrelevant regulations; and
- mathematical models, which are developed as a standard tool implemented in MS 365 ®Excel for shelf-life prediction of Australian VP pork , or can be incorporated into an existing tool that allows prediction of the remaining shelf-life of VP pork cuts, and also beef and lamb primals in cold chains (*see* Huynh *et al.*, 2016, for a screenshot of the existing tool – 'Shelf-life Calculator'). More specifically, the user will be able to select the product type (beef, lamb or pork), and enter the starting TVC, and a time:temperature profile, typically collected by a temperature datalogger. The tool will then predict the TVC growth profile and remaining shelf life of the product based on assessment of predicted growth and odour kinetic responses.

10. Literature cited

EFSA Panel on Biological Hazards (BIOHAZ), 2016. Growth of spoilage bacteria during storage and transport of meat. European Food Safety Authority Journal 14, 4523.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology 23, 277 - 294.

Blixt, Y., Borch, E., 2002. Comparison of shelf life of vacuum-packed pork and beef. Meat Science 60, 371-378.

Buchanan, R.L., Cygnarowicz, M.L., 1990. A mathematical approach toward defining and calculating the duration of the lag phase. Food Microbiology 7, 237-240.

Corkrey, R., Olley, J., Ratkowsky, D., McMeekin, T., Ross, T., 2012. Universality of thermodynamic constants governing biological growth rates. PLoS One 7, e32003.

Egan, A.F., Eustace, I.J., Shay, B.J., 1988. Meat packaging - maintaining the quality and prolonging the storage life of chilled beef, pork and lamb. Proceedings of the 34th International Congress of Meat Science and Technology, 68 - 75.

Field, R.A., 1999. Bone marrow measurements for mechanically recovered products from machines that press bones. Meat Science 51, 205-214.

Holmer, S.F., McKeith, R.O., Boler, D.D., Dilger, A.C., Eggert, J.M., Petry, D.B., McKeith, F.K., Jones, K.L., Killefer, J., 2009. The effect of pH on shelf-life of pork during aging and simulated retail display. Meat Science 82, 86-93.

Hultman, J., Rahkila, R., Ali, J., Rousu, J., Bjorkroth, K.J., 2015. Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages. Applied and Environmental Microbiology 81, 7088-7097.

Huynh, L., Jenson, I., Kaur, M., Kiermeier, A., Kocharunchitt, C., Miles, D., Ross, T., Sumner, J., Vanderline, P., 2016. Shelf life of Australian Red Meat, Second ed, Sydney, NSW: Meat and Livestock Australia.

Jiang, Y., Gao, F., Xu, X.L., Su, Y., Ye, K.P., Zhou, G.H., 2010. Changes in the bacterial communities of vacuum-packaged pork during chilled storage analyzed by PCR-DGGE. Meat Science 86, 889-895.

Jolley, J., Hamilton, D., Holds, G., Pointon, A., Kiermeier, A., 2019. Standardised shelf-life testing of Australian pork. Final Report (APL Project 2016/2203). South Australian Research and Development Institute, Adelaide, South Australia.

Kaur, M., Bowman, J.P., Porteus, B., Dann, A.L., Tamplin, M., 2017. Effect of abattoir and cut on variations in microbial communities of vacuum-packaged beef. Meat Science 131, 34-39.

Kaur, M., Williams, M., Bissett, A., Ross, T., Bowman, J.P., 2021. Effect of abattoir, livestock species and storage temperature on bacterial community dynamics and sensory properties of vacuum packaged red meat. Food Microbiology 94, 103648.

King, T., Kocharunchitt, C., Gobius, K., Bowman, J.P., Ross, T., 2014. Global genome response of Escherichia coli O157ratioH7 Sakai during dynamic changes in growth kinetics induced by an abrupt temperature downshift. PLoS One 9, e99627.

Knox, B.L., van Laack, R.L., Davidson, P.M., 2008. Relationships between ultimate pH and microbial, chemical, and physical characteristics of vacuum-packaged pork loins. Journal of Food Science 73, M104-110.

Li, M.Y., Zhou, G.H., Xu, X.L., Li, C.B., Zhu, W.Y., 2006. Changes of bacterial diversity and main flora in chilled pork during storage using PCR-DGGE. Food Microbiology 23, 607-611.

Meinert, L., Christensen, H., 2014. DMRI Predict, a tool to determine shelf-life of fresh meat, 60th International Congress of Meat Science and Technology, Punta Del Este, Uruguay.

Mellefont, L.A., McMeekin, T.A., Ross, T., 2003. The effect of abrupt shifts in temperature on the lag phase duration of foodborne bacteria. International Journal of Food Microbiology 83, 281-293.

Mellefont, L.A., Ross, T., 2003. The effect of abrupt shifts in temperature on the lag phase duration of Escherichia coli and Klebsiella oxytoca. International Journal of Food Microbiology 83, 295-305.

Pennacchia, C., Ercolini, D., Villani, F., 2011. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. Food Microbiology 28, 84-93.

Ratkowsky, D., Olley, J., T., M., Ball, A., 1982. Relationship between temperature and growth rate of bacterial cultures. Journal of Bacteriology 149, 1-5

Robinson, T.P., Ocio, M.J., Kaloti, A., Mackey, B.M., 1998. The effect of growth environment on the lag phase of Listeria monocytogenes. International Journal of Food Microbiology 44, 83-92.

Rood, L., Bowman, J.P., Ross, T., Corkrey, R., Pagnon, J., Kaur, M., Kocharunchitt, C., 2022a. Spoilage potential of bacterial species from chilled vacuum-packed lamb. Food Microbiology 107.

Rood, L., Bowman, J.P., Ross, T., Corkrey, R., Pagnon, J., Yang, S.W.T., Kocharunchitt, C., 2022b. The effects of glucose on microbial spoilage of vacuum-packed lamb. Meat Science 188.

Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. Journal of Applied Bacteriology 81, 501-508.

Ross, T., 1999. Predictive food microbiology models in the meat industry (MSRC.003). Meat and Livestock Australia, North Sydney, Australia.

Ross, T., Bowman, J.P., Kocharunchitt, C., Mellefont, L., 2019. Microbial ecology and physiology (V.MFS.0402). Meat and Livestock Australia, North Sydney, Australia.

Tucker, R., Klepper, K., 2005. Review of on-farm food safety best practice. Final report. Project code PRMS.075, Sydney, NSW: Meat and Livestock Australia.

Zhao, F., Zhou, G., Ye, K., Wang, S., Xu, X., Li, C., 2015. Microbial changes in vacuum-packed chilled pork during storage. Meat Science 100, 145-149.

11. Publications Arising

- Rood, R., Ross, T., Pagnon, J., Yang, S. W. T., and Kocharunchitt, C. The shelf-life of Australian vacuum-packed pork at different storage temperatures. *In preparation*.