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1 Predictive models for the shelf life of Australian vacuum-packed beef

2 and lamb: development and evaluation

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21 Abstract

22 The Australian meat industry exports vacuum packed beef and lamb to more than one 23 hundred markets, some of them involving containerised sea voyages of 40-50 days. On 24 occasion, product may be subjected to temperature increase due to refrigeration problems, 25 or to extended shipping times due to strikes or to delays in clearing the destination port, as 26 happened in 2020 due to the Coronavirus pandemic. Such deviations in temperature and 27 time have a pronounced effect on the shelf life of the consignment which, traditionally, has 28 required sampling of the cargo for microbiological and sensory testing prior to making a 29 disposition - adding to the loss of shelf life. In this study, we describes a predictive tool that 30 provides rapid and accurate assessment of the product shelf life remaining for Australian 31 vacuum packed beef and lamb cuts, based on their initial total bacterial count, and 32 time:temperature parameters. The models were validated by independent data and have a 33 bias factor of 1.02 and 0.90, and an accuracy factor of 1.10 and 1.11 when used for 34 predicting the shelf life of beef and lamb cuts, respectively packed under vacuum. This 35 indicates a good agreement between the observed and predicted shelf lives of VP and VSP 36 cuts. The models will allow the Australian meat industry to manage their supply chains 37 effectively and reliably to ensure high quality meat products with excellent shelf life.

38 **1. Introduction**

39 Since its first exports in 1880, Australia has developed a reputation for producing red 40 meat with excellent shelf life, servicing markets, especially for chilled, vacuum-packed (VP) 41 beef and lamb, to more than 100 countries (Small et al., 2012). Supply to distant markets 42 means that Australian exporters are constantly challenged to minimise the loss of product 43 quality (including shelf life) along different supply chains and to meet a wide range of shelf 44 life-related specifications imposed by intended markets. For instance, China specifies a shelf 45 life of 120 and 80 days from slaughter for vacuum packed beef and lamb/mutton cuts, 46 respectively, while some Middle Eastern countries require at least 50% shelf life to remain 47 when the consignment is landed (Huynh et al., 2016). 48 It has long been known that shelf life depends upon the degree of bacterial 49 contamination at packing and the growth conditions: temperature, pH and oxygen permeability of the packaging film, of which temperature is considered the most important 50 51 factor. Gill et al., (1988a) established the optimum temperature for storage of VP meat as -52 1.5±0.5°C, and also showed that small rises in temperature reduce shelf life significantly: at 53 temperatures of 0°, 2° or 5°C, the storage life was reduced by about 30, 50 or 70%, 54 respectively, compared with storage at -1.5°C (Gill et al., 1988b). For supply to distant 55 markets it has become customary for exporters to specify that the shipping container set-56 point is -1.5°C. 57 Temperature abuse, defined by Mills et al., (2014) as warmer than 5°C during any 58 stage of the cold chain, is onerous for the supplier, usually involves the need to evaluate the 59 sensory and microbiological condition of the shipment in order to decide its disposition. 60 Further, the time taken to unload the container, select and sample representative units, 61 then await test results increases the likelihood that the contents might be deemed unfit for 62 human consumption or to comply with importer specifications. A container of VP meat can 63 have an insured value of USD200,000 and, as a means of improving the timeliness with 64 which a disposition decision can be made, we propose a predictive model that rapidly and 65 accurately predicts the remaining shelf-life of VP beef and sheep meat. 66 Previously, we assessed the microbiological and sensory qualities of VP beef and 67 lamb cuts sourced from several Australian abattoirs through storage at temperatures 68 ranging from 0°C to 8°C (Kaur et al., submitted for publication) in which it was found that 69 total bacterial count and odour of the meat were suitable indicators for determining the 70 shelf life of VP beef and lamb. Rates of quality deterioration as indicated by odour were

found to correlate strongly with TVC growth rates across the storage temperature range,

72 with beef and lamb cuts showing different but consistently parallel rate values. These 73 findings indicated the feasibility of developing models for separately predicting the shelf-life 74 of VP beef and lamb based on the growth of microorganisms and of presence of persistent 75 odour as a function of temperature. To this end, we developed predictive models for the 76 shelf life of Australian VP beef and lamb primals that encompass and extend from the results 77 and models of Kaur et al., (submitted for publication). A number of previously published 78 work datasets were used and supply chain trials conducted to evaluate the performance of 79 the developed models in both simulated and real commercial supply chains. 80 81 2. Materials and methods 82 2.1 Model development 83 2.1.1 TVC growth models 84 Data for specific growth rates of total bacterial count (TVC) on VP beef and lamb 85 sourced from Australian abattoirs at different storage temperatures (ranging from 0°C to 86 8°C) were obtained from Kaur et al. (submitted for publication). To describe the effects of 87 storage temperature on the rates of TVC growth, linear regression analysis was performed 88 for each meat type (Ratkowsky et al., 1982) and the following model derived: $\sqrt{\mu_{\text{TVC}}} = (a \times T) + b$ 89 (1) 90 where μ_{TVC} is the specific growth rate of TVC (in hours); *a* is the slope of the regression line; *T* 91 is the temperature at which meat is stored (°C); and b is the regression coefficient. From 92 Equation (1), the theoretical minimum temperature for TVC growth on VP beef and lamb 93 was estimated by extrapolation of the regression line to $\sqrt{\mu_{\text{TVC}}} = 0$. 94 95 2.1.2 Shelf life models 96 With the parameters obtained from Equation (1), shelf life could be predicted on the 97 basis that spoilage of VP meat is mainly caused by microorganisms (Gill & Newton, 1979).

98 Equation (1) can then be expressed as:

99
$$SL = \left[\frac{1}{a \times (T - T_{\min})}\right]^2$$
 (2)

where *SL* is the shelf life of VP primals (days); *a* is the slope of the regression line that
corresponds to *a* in Equation (1); *T* is the temperature at which meat is stored (°C); and *T*_{min}
is the minimum temperature where the rate of TVC growth is zero (*i.e.*, from Equation (1)).
However, to account for the shelf life of Australian VP beef and lamb primals under ideal
storage conditions as established by industry and research data (Phillips et al., 2012; Sumner

& Jenson, 2011) Equation (2) was modified to calibrate to the established shelf life of VPmeat and is given as follows:

$$SL = \left[\frac{1}{a \times (T - T_{\min})} - c\right]^2$$
 (3)

where *c* is a factor that enables calibration to the established shelf life of VP beef and lamb.
Equation (3) was further modified to predict the remaining shelf life by accounting for the
observed initial TVC (at the time of packaging). This was achieved by calculating a correction
factor that considers the established data for the initial TVC and the calculated TVC at the
time of spoilage (Phillips et al., 2012; Sumner and Jenson, 2011). This correction factor was
then incorporated to Equation (3) to predict the remaining shelf life (*SL*_{remaining}; day).

114
$$SL_{\text{remaining}} = \left[\frac{N_0 - (N_{\text{obs}}) + N_s}{N_s}\right] \times \left[\frac{1}{a \times (T - T_{\min})} - c\right]^2$$
 (4)

115 where N_0 is the initial TVC based on the established data of Phillips et al., 2012 (log

116 CFU/cm²); N_{obs} is the observed initial TVC (log CFU/cm²); and N_s is the nominal population

117 level on VP beef and lamb at the time of spoilage (log CFU/cm²). The N_s value was estimated

118 by extrapolation of the regression line of TVC data to the time at which spoilage occurs

119 (Phillips et al., 2012; Sumner & Jenson, 2011).

120

107

121 2.1.3 Production of a model interface

Based on the developed Equations (1) and (4), a model interface was produced in
MS [®]Excel 2016 to predict the growth of TVC and the remaining shelf life of VP beef and
lamb primals.

125

126 2.2 Validation of the developed models in commercial and simulated supply chains

127 2.2.1 Previous shelf-life trials

Relevant data for initial TVC, average storage temperature and observed shelf-life were collated from studies previously reported by Sakai et al., (submitted for publication) for VP beef, and Kaur et al., (2017) for VP lamb. Altogether, these studies provide 11 time:temperature based datasets for the shelf-life of Australian VP primals stored under either constant or dynamic temperatures.

133

134 2.2.2 Lamb primals, Australia- Middle East

The shelf-life of various lamb products was evaluated in a commercial supply chain. Lamb racks, boneless legs and bone-in legs were vacuum-packed, cartoned and shipped by sea from Australia to Bahrain where they were stored at approximately 0°C throughout the trials. All trials were conducted with triplicate samples. The time:temperature profile of
 the samples was recorded by data loggers (TG-4080 Hastings Data Loggers, Australia) from
 an abattoir to storage in Bahrain. -

142

143 2.2.3 Beef primals and cuts, simulated trial Australia

144A series of shelf-life trials were conducted at a beef processing plant in Brisbane,145Australia to determine the shelf-life of various types of commercial products in a simulated146domestic supply chain. These included VP rump roast with three ageing regimes (5, 20 and14769 days), and vacuum-skin-packed (VSP) rump streaks produced from VP primals after148ageing for different durations product (30 and 57 days).

149 In a simulated supply chain, rump primals or roasts were aged in VP (at

approximately 0°C) for different durations. Both aged and non-aged products, except for VP

151 rump roast were cut into steaks and packed in different packaging systems as appropriate.

152 All products were then subjected to different steps in a simulated supply chain: transferring

153 to and storing in a distribution centre storage, and transferring to a retail display for

154 different durations and at different temperatures as experienced in a domestic supply chain.

155 In addition, conditions that simulate consumer's practices e.g. consumers home journey and

156 storage in their fridge were included.

Each trial was conducted with at least triplicate samples. The time:temperature
profile of the samples was monitored through the trials using five data loggers (TG-4080
Hastings Data Loggers, Australia).

160

161 *2.2.4 Quality assessment*

162 An appropriate number of packs of each product types (beef and lamb) were 163 assessed on the day of packing and throughout the supply chain for sensory evaluation 164 and/or microbiological analysis (total viable counts, TVC).

165

166 2.2.4.1 Sensory analysis

167 At each sampling point, each pack of samples was evaluated for its odour attribute

168 by a trained sensory panel comprising of at least five members. Specifically, packs were

169 opened and left for 10 min before panellists assessed their odour sensory quality.

170 Assessment of odour was based on a 3-point categorical hedonic scale as follows: 0 = strong

sour odour/off odour; 1 = moderate sour odour/moderate off odour; and 2 = fresh meat

172 odour/very slight sour odour.

173 2.2.4.2 Microbiological analysis

174 Enumeration of total bacteria was performed on cuts at the time of vacuum packing. 175 For beef samples a surface section from the longest length of the cut was aseptically excised 176 $(25 \pm 2.5 \text{ g})$. Meat pieces were combined with 225 ml of buffered peptone water (BPW, 177 CM1049, Oxoid Ltd., Australia) in a sterile stomacher bag and stomached for 1 min. Serial 178 dilutions were prepared as necessary in BPW as required and aliquots (0.1 ml) of plated onto 179 the surface of Tryptone Soy agar (TSA, CM0129, Oxoid Ltd., Australia). 180 For lamb samples, a surface area measuring 200 cm² was swabbed with a sterile 181 sponge (Whirl-Pak Speci-Sponge, Nasco, USA), prewetted with 25 ml of Phosphate Buffer 182 Saline (BR0014G, Oxoid Ltd., Australia). Swabs were hand massaged in the sample bags for 183 30 seconds to release the bacteria into suspension. Suspension (1 ml) was serially diluted in 184 0.1% bacteriological peptone water (LP0037, Oxoid Ltd., Australia) as required and aliquots 185 plated onto Petrifilm aerobic count plates (3M Microbiology Products, St. Paul, MN) 186 according to the manufacturer's instruction.

187 TSA and Petrifilm plates were incubated at $20 \pm 1^{\circ}$ C for 5 days aerobically, colonies 188 were counted and reported as the mean log CFU/g or log CFU/cm² of the replicates (± 189 standard deviation).

190

191 2.2.5 Determination of the shelf life

192The shelf-life of each product type was determined from odour assessments193described above. Specifically, products that were rated as 'marginal – smell off' were194considered as commercially unacceptable and the time taken to reach that endpoint was195recorded as the shelf life of the product. Due to the variability of product characteristics196even within the same trial, the shelf life was determined when at least one of the replicates197were rated as unacceptable at any given time point and subsequent time points.

198

199 2.2.6 Comparison between observed and predicted growth

The shelf-life of each product type were estimated using the developed predictive
 models based on initial TVC, and time:temperature history in the supply chain.

202 The performance of the developed models to predict the shelf-lives of VP red meat was

203 evaluated using the methods described by Ross (1996). Bias and accuracy factors for the

204 models were calculated from observed and predicted shelf lives (days) of each meat type.

205 **3.** Results and Discussion

There have been several models developed to predict the shelf life of particular products, most of which have remained a research tool rather than an effective industrial application. This is mainly because: i) the models were based on observations in wellcontrolled laboratory environments with microbiological media rather than complex food environments such as on meat; and ii) mode models validated under static temperature conditions rather than temperature fluctuations as occur during storage and distribution of foods (McDonald & Sun, 1999).

213 To develop effective spoilage models for Australian VP primals, a comprehensive 214 study was conducted to determine the microbiological and sensory qualities of meat as they 215 relate to spoilage (Kaur et al., submitted for publication). That study, congruent with 216 previous studies, indicated a robust temperature dependency of spoilage rates of VP beef 217 and lamb primals as reflected by the different rates of microbial growth (Gill et al., 1988b; 218 Kaur et al., 2017; Sumner & Jenson, 2011). However, due to differences in meat 219 biochemistry (especially glycogen and lactic acid contents), beef (pH 5.5-5.8) tends to have a 220 lower pH than lamb (pH 5.6-6.8) (Carse & Locker, 1974). Such differences affect the growth 221 of bacteria, with growth rates being faster on VP lamb than VP beef, with consequential 222 effects on shelf life, necessitating the development of two independent models to predict 223 the shelf life of each through the supply chain.

224

225 3.1 Development of predictive models for the shelf-life of Australia VP primals

Using the relevant data of Kaur et al, (submitted for publication), we applied the square root model of Ratkowsky et al., (1982) to describe the effects of storage temperature on the rates of TVC growth on VP beef and lamb in accordance with Equation (1). Table 1 shows the model parameters for different meat types. As expected, these parameters (*a* and T_{min}) differ between meat types, reflecting the differences in their biochemistry (*i.e.*, meat pH as described above).

With the parameters (*a* and T_{min}) obtained above, predictive models for the shelf life of VP beef and lamb were developed in accordance with Equation (2). However, such models could not be used to specifically predict the remaining shelf life of Australian VP meat (*i.e.*, as defined in Equation (4)). This requires a number of factors (*i.e.*, N_0 , N_s , and *c*) to be determined based on previous data for the shelf life of VP meat produced in Australia. VP beef and lamb with the initial TVC of approximately 3.0 log CFU/cm² (*i.e.* N_0 , the initial TVC) typically have an acceptable shelf life of 160 and 90 days when stored at -0.5°C, respectively, as determined by odour assessment (Phillips et al., 2012; Sumner & Jenson, 2011). Using
these values, the extrapolated N_s value was estimated to be 11.4 log CFU/cm² for beef and
11.0 log CFUcm² or lamb, whereas the *c* value (the factor required for calibration to
established shelf lives) was 0.21448 and 0.49728 for VP beef and lamb, respectively. These,
taken together, allow Equation (4) to be 'calibrated' (after inclusion of correction factors) to
predict the remaining shelf life of Australian VP beef (Equation (5)) and lamb (Equation (6))
as follows:

246
$$SL_{\text{remaining}} = \left[\frac{3.0 - (N_{\text{expt}}) + 11.4}{11.4}\right] \times \left[\frac{1}{0.01964 \times (T - 4.45861)} - 0.21448\right]^2$$
(5)

247
$$SL_{\text{remaining}} = \left[\frac{3.0 - (N_{\text{expt}}) + 11.0}{11.0}\right] \times \left[\frac{1}{0.01986 \times (T - 5.54856)} - 0.49728\right]^2$$
(6)

248

The developed models were incorporated into a software tool (implemented in MS ®Excel) that allows prediction of the growth of TVC and calculates the remaining shelf life of VP beef and lamb primals in cold chains, (*see* Supplementary File 1). To use this tool, the user selects the product type (beef or lamb), enters the starting TVC, and a time:temperature profile, typically collected by a temperature datalogger. The tool then predicts the TVC growth profile and remaining shelf life of the product based on assessment

- 255 of predicted growth and odour kinetic responses.
- 256

257 3.2 Shelf-life data for model validation

In Table 2 are summarised the shelf-life data (n=11) obtained for various beef and lamb products in both simulated and actual commercial supply chain (*i.e.* at fluctuating temperatures) (Kaur et al., 2017; Sakai et al., submitted for publication). These include TVC at the time of packaging, average storage temperatures and the observed shelf lives. The data were then used to evaluate the performance of the developed models.

A series of trials was also conducted to provide additional data for the shelf life of Australian beef and lamb products in a commercial supply chain. In Table 3 are summarised the data for all product types (n=15), including TVC at the time of packaging, average storage temperature and the observed shelf lives (ranging from 37 to 85 days).

267

268 *3.4 Performance of predictive models*

269The predictive models for VP beef and lamb shelf life were evaluated for their270performance by comparison with independent data not used to generate the models. The

271 MS [®]Excel-based tool as described above was then used to predict the shelf life of different

272 meat products based on their time:temperature history and initial microbial counts. The

273 observed vs. predicted shelf lives (days) of each product are shown in Tables 2 and 3.

274 The bias and accuracy factor analyses of Ross (1996) were used to assess the 275 performance of model predictions of shelf life compared with the observed data. Ross 276 (1996) reported that the bias factor serves as a measurement index for the average variation 277 between the predicted and observed values, whereas the accuracy factor is used to estimate 278 the accuracy of an established model. Bias and accuracy factor values of 1 indicate a perfect 279 agreement between observed and predicted values. In this study, the models were found to 280 have a bias factor of 1.02 and 0.90, and an accuracy factor of 1.10 and 1.11 when used for 281 predicting the shelf life of beef and lamb in VP and/or VSP, respectively. These observations 282 indicate a good agreement between the observed and predicted shelf lives of VP and VSP 283 cuts. The models systematically underpredict the shelf life of VP meats with approximately 284 10% deviation, providing 'fail-safe' predictions, and it is noted that an over-prediction of 285 time to spoilage was also noted by Albrecht et al., (2019), Bruckner et al., (2013) and Tang et 286 al., (2013) for their shelf life predictive models for poultry and pork meat.

287 Development of the models here use the square root model of Ratkowsky et al., 288 (1982) to predict the remaining shelf life of VP meats, consistent with other studies 289 indicating microbial spoilage of foods can be described by the square root model 290 (Kreyenschmidt et al., 2010; Mataragas et al., 2006). However, it should be noted that, in 291 contrast to those studies, the developed models provide shelf life predictions based on the 292 growth of TVC rather than that of a specific group of organisms (collectively known as 293 'specific spoilage organisms' or SSOs). This suggests that spoilage in VP meats might be 294 facilitated by a complex phenomenon involving interactions among growing bacteria (*i.e.*, by 295 community effects). Further investigation involving inoculation of specific bacteria into 296 sterile meat to test for their spoilage capability is required to elucidate this. 297 From the above, the developed models were successfully validated to provide an accurate 298 and reliable prediction of the shelf life of beef and lamb stored under vacuum packaging 299 conditions. Such models can be readily adopted as a reliable decision-making tool in 300 commercial supply chains for VP beef and lamb. This tool offers a cost-effective approach for 301 the meat exporters to optimise and better understand their supply chains. Disposition of 302 product affected by adverse events, such as temporary loss of refrigeration on the vessel or 303 extended delivery times can be resolved speedily by using this tool. 304

305

4. Conclusion

307	This study provides "ready-to-use" models for predicting the shelf life of VP primals. The
308	models were well-validated by independent data from commercially available products in
309	both simulated and commercial supply chains. The use of the models by the Australian meat
310	industry has already led to effective management systems for optimising and monitoring
311	meat quality. At the time of writing the Coronavirus emergency has resulted significant
312	volumes of product landed in many countries requiring extended storage at the port. and
313	the tool has provided both the exporter and importer with a confident estimate of the shelf
314	life remaining.
315	
316	5. Acknowledgments
317	This work was funded by Meat & Livestock Australia (MLA) and Australian Meat Processor
318	Corporation (AMPC) (project no. G.MFS.0289). The support and guidance of Dr. Ian Jenson
319	(MLA) is gratefully acknowledged.
320	
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385

386 **Table 1.**

387 Estimated values of the parameters of Equation (1) for the specific growth rate of TVC.

Product type	а	b	7 _{min} (°C) ¹
VP beef	0.01964	0.08757	-4.45861
VP lamb	0.01986	0.11021	-5.54856

 $\overline{T_{\min}}$ is the theoretical minimum temperature and was estimated by extrapolation of the

389 regression line to $\sqrt{\mu_{\rm TVC}} = 0$.

390 Table 2.

391 Summary of previously published data used for evaluation of the models for shelf life

392 predictions.

Type of meat	Average storage	Initial TVC (log	Observed	Predicted	Study
cut	temperature ^a	CFU/cm ²) ^b	shelf-life	shelf-life	
			(days) ^c	(days)	
Vacuum-packed	beef products				
Striploin	-0.47	1.91	140	159	
Striploin	-0.44	1.91	140	158	
Chuck tender	1.45	2.47	90	81	Sakai at al
Chuck tender	2.27	2.47	90	63	Sakai et al.,
Chuck tender	1.54	2.47	90	83	for
Striploin	2.22	1.54	70	75	nublication)
Striploin	1.98	1.54	70	83	publication
Striploin	2.21	1.54	70	75	
Striploin	2.34	1.54	70	72	
Vacuum-packed	lamb products				
Bone-in hind	8.00	3.15	13	10	
shank					Kaur et al.,
Bone-in hind	-1.20	3.15	124	122	(2017)
shank					

393 a. Average temperature from packaging to the end of the trials

b. Average TVC at the time of packaging

395 c. The time taken for each product to reach its end of shelf-life based on odour attribute.

Type of meat cut	Packaging	Average storage	Initial TVC (log	Observed shelf-	Predicted shelf-
	types ^a	temperature ^b	CFU/cm ²) ^c	life (days) ^d	life (days)
Beef products					
Rump roast with 5-day aging	VP	3.97	1.03	60	56
Rump roast with 20-day aging	VP	3.03	1.03	70	75
Rump roast with 69-day aging	VP	1.04	1.03	85	06
Rump steaks produced from VP primals	VSP	3.25	2.30	41	43
without aging					
Rump steaks produced from VP primals	VSP	3.21	3.23	38	40
after aging for 30 days					
Rump steaks produced from VP primals	VSP	3.16	4.00	37	38
after aging for 57 days					
Lamb products					
Boneless legs	VP	1.36	0.12	103	88
Bone-in legs	VP	1.36	0.16	97	92
Racks	VP	1.36	0.07	94	92

Summary of the data generated for different meat products in their supply chains and its comparison with the shelf life predictive models. 397 VP = vacuum packaging; MAP = modified atmosphere packaging (75-82% O₂ and 18-25% CO₂); OW trays = over-wrapped trays; and VSP = vacuum skin packaging Average temperature from packaging to the end of the trials. þ. a. 398 399

Average TVC at the time of packaging. ن 400 The time taken for each product to reach its end of shelf-life based on odour attribute q. 401

Table 3 396