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Microbial growth and communities of packed lamb shoulders

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Abstract

Several Australian sheep and lamb processors export vacuum packed lamb shoulders. Vacuum packed and modified atmosphere (100% CO_2) packed lamb shoulders, bone-in and bone-out, were stored for up to 12 weeks. All product types performed very well organoleptically and microbiologically, indicating that at -0.3°C, vacuum packed and modified atmosphere packed lamb shoulders can last for at least 12 weeks. No evidence could be found to support anecdotal information that bone-in product has a shorter shelf-life than the corresponding boneless primal. However, for bone-in product in modified atmosphere packaging, more holes in the pack were observed. This may be due to excessive shrinkage, which was observed in all modified atmosphere packs, though this problem may be overcome by changing the gas to meat ratio or gas mixture.

Executive Summary

Several Australian sheep and lamb processors export vacuum packed lamb shoulders. Previous research on boneless lamb shoulders indicates that consumer acceptability remains high for product that has been vacuum packed for up to 78 days (A.MFS.0185 and A.MFS.0196). However, the growth curves for Total Viable Counts (TVC) and Lactic Acid Bacteria (LAB) have not been quantified for vacuum packed lamb shoulders. In addition, anecdotal evidence from the trade suggests that bone-in product often has a shorter shelf-life than the corresponding boneless primal. There is also no information on the microbial ecology on this product.

While vacuum packing is common practice in Australia, few processors pack product in modified atmosphere, which is used more frequently in New Zealand. A storage trial of bone-in and boneless vacuum packed and modified atmosphere packed lamb shoulders was undertaken to collect local data on the shelf-life of these products and demonstrate the potential value of this packaging system to the Australian industry. The shoulders were sourced from a single lot of lambs that were slaughtered, processed and packed by a Victorian processor, using their commercial conditions. Shoulders were either vacuum packed individually (up to four per boning state per sampling time) or in packs of four using a 100% CO_2 modified atmosphere (at most one pack per sampling time).

Product was stored for up to 12 weeks at an average temperature of -0.3°C. Shoulders were tested on a weekly basis for appearance, colour and odour, pH, Total Viable Counts (TVC), and Lactic Acid Bacteria (LAB).

All four boning and packaging combinations performed well across the storage period in relation to odour and colour assessments. Vacuum packed product was consistently well packed. However, a relatively large number of damaged packs, possibly due to excessive shrinkage, were observed in bone-in modified atmosphere product.

With respect to pH, modified atmosphere product first dropped before recovering to starting levels, though considerable variability between shoulders from the same pack was observed. Vacuum packed shoulders either increased during storage (bone-in product) or decreased (bone-out product).

Vacuum packed product showed ‘traditional’ S-shaped growth curves for TVC and LAB, which were well modelled by the Gompertz and Baranyi growth models. Both models resulted in similar parameter estimates. The starting levels for TVC were 3.33 and 3.36 $\log_{10}$ cfu/g, stationary phase was reached by 7.96 and 7.66 $\log_{10}$ cfu/g, lag phase was 10.9 and 7.9 days, and maximum specific growth rate was 0.18 and 0.15 $\log_{10}$ cfu/g/day, respectively. Similarly, for LAB the two models estimated starting levels of 2.06 and 2.17 $\log_{10}$ cfu/g, stationary phase levels of 7.68 and 7.10 $\log_{10}$ cfu/g, a lag phase of 7.01 and 6.75 days, and a maximum specific growth rate of 0.24 and 0.22 respectively. No differences between bone-in and bone-out product could be determined, hence this work does not support anecdotal evidence of a shorter shelf-life for bone-in lamb shoulders. In addition, the levels were very similar to those obtained in a previous trial, but with product from a different processor.

In contrast, growth in modified atmosphere product was very limited. No growth curve could be fitted for TVC and only the Gompertz model could find estimates for LAB. The estimate for the starting level was 2.06 $\log_{10}$ cfu/g, stationary phase level was 5.83 $\log_{10}$ cfu/g (though these were not directly observed), the lag phase was 37.6 days and the maximum specific growth rate was 0.1 $\log_{10}$ cfu/g/day. Again, differences between bone-in and bone-out lamb shoulders could not be substantiated.

From the microbiological results it can be concluded that both packing methods (vacuum and modified atmosphere) result in product that microbiologically and organoleptically meets a shelf-life of at least 12 weeks at temperatures of -0.3°C. However, it was noticed that despite small variability in TVC and LAB in product prior to packing, the variability appears to increase over time during vacuum packed storage, despite product having been stored under identical conditions. Similarly, for modified atmosphere product the microbiological variability between shoulders from the same pack is large compared to the starting levels. Clearly this may affect industry's ability to produce microbiologically consistent product for long term storage, which may be an issue for some customers in overseas markets.

Despite modified atmosphere product performing very well microbiologically and organoleptically throughout the storage period, there are potential issues with respect to ensuring pack integrity. Out of the 19 packs stored, three bone-in packs had holes. The likely explanation for these holes is that they are a result of bone punctures due to excessive shrinkage, which was observed in many of the modified atmosphere packs during storage. However, this problem could possibly be overcome by using a larger gas to meat ratio than the 1:5 to 1 used here, or by introducing small amounts of other gases, such as nitrogen.

Contents

Background

Several Australian sheep and lamb processors export vacuum packed lamb shoulders. Previous research on boneless lamb shoulders indicates that consumer acceptability remains high for product that has been vacuum packed for up to 78 days (A.MFS.0185 and A.MFS.0196). However, the growth curves for Total Viable Counts (TVC) and Lactic Acid Bacteria (LAB) have not been quantified for vacuum packed lamb shoulders. In addition, there is no information on the microbial ecology on this product.

Anecdotal evidence from the trade suggests that bone-in product often has a shorter shelf-life than the corresponding boneless primal. It is not known why this should be so.

Very few Australian processors pack product in modified atmosphere, whereas it is understood to be relatively common in New Zealand. It is desirable to collect local data on the shelf-life of these products and demonstrate the potential value of this packaging system to the Australian industry.

Project Objectives

Establish growth curves for Total Viable Counts and Lactic Acid Bacteria counts for vacuum packed and modified atmosphere packed lamb shoulders stored for 84 days at -0.5°C and collect colonies of Total Viable Counts[1] for ecological analysis.

[1] The original aim was to collect colonies from Lactic Acid Bacteria for ecological analysis, but this was changed as a result of a teleconference held on 14 Jan 2011.

Methodology

Raw Materials

The experimental factors of interest were:

- Boning state: Bone-in (BI) versus Bone-out (BO), and
- Packaging Type: Vacuum Packed (V or VAC) versus Modified Atmosphere Packed (M or MAP).

Consequently, the four product types consisted of the combinations of Boning State and Packaging Type, namely:

- VBI: Vacuum Packed Bone-in
- VBO: Vacuum Packed Bone-out
- MBI: Modified Atmosphere Packed Bone-in
- MBO: Modified Atmosphere Packed Bone-out

The products were sourced from a Victorian abattoir that produces all of them commercially. Lamb shoulders were boned and packed on the morning of 2 March 2011 from a single mob of animals slaughtered on 28 February 2011. Shoulders were packed individually (VAC) or in packs of four (MAP) in Protite Ultra Shrink Bags (O_2 trans of 18.6 cc/m^2/24hr at 23 °C, 0% RH). Modified atmosphere packing was done using a ratio of 1:1.5 of meat to CO_2 (pers comm. I. Eustace). MAP product shoulders were also individually wrapped in moisture absorbing paper. To allow for weekly sampling of four shoulders over the 12 week storage trial a total of 48 shoulders were ordered per product type.

Packing was overseen by Chris Sentence, who also collected meat samples for microbiological testing from product prior to packing.

Product was sent to SARDI Food safety by a commercial carrier using refrigerated transport.

Storage

The pallet of product was delivered by truck on 4 March 2011 at approximately 14:00. A forklift delivered the pallet from the truck, which was stopped at the corner of Cross Road and Pitcairn Avenue, Urrbrae, to the SARDI, Plant Research Centre, Gate 2a Hartley Grove, Urrbrae – the distance of approximately 1 km took approximately 10 minutes.

On arrival at SARDI, the boxes were removed from the pallet and spread throughout a walk-in cool room, dedicated to this trial and set to -0.5°C, to allow for quick cooling. Boxes were opened and the contents were compared with the quantities planned for each product type.

Temperature Monitoring

Four data loggers were placed inside four separate boxes of product prior to shipping. The boxes with loggers were located at different points throughout the pallet.

For long term storage at SARDI, twelve data loggers, set to record every 20 minutes, were placed in different boxes of product to allow checking and monitoring of temperatures

throughout the trial. At each sampling time one logger was removed when product was sampled for sensory and microbiological analysis.

Sampling

One surface slice sample was collected aseptically from each of four BI and four BO shoulders prior to packing. These were placed into individual sterile plastic bags and placed on ice inside an esky. They were transported to the laboratory and tested within 10 hours of collection.

Sensory and microbiological evaluation of the various product types was conducted on a weekly basis. Because not all product requested was delivered or suitable for the storage trial a reduced sampling scheme, shown in Table 1, was developed. This table also includes modifications that were necessary when packs were found to not be intact.

Table 1: Modified sampling schedule indicating the number of samples (individual shoulders) sampled at each time point.

	MAP		VAC	
Sampling Date	**Bone-in**	**Bone-out**	**Bone-in**	**Bone-out**
10/03/2011	4		4	2
17/03/2011	4	4	4	3
24/03/2011	4	4	4	2
31/03/2011	4	4	4	2
7/04/2011	4		4	3
13/04/2011			4	3
21/04/2011	4	4	4	3
28/04/2011		4	4	2
5/05/2011	4		4	2
12/05/2011		4	4	2
19/05/2011	4		4	2
26/05/2011		4	4	2
Total	**32**	**28**	**48**	**28**

Sensory Evaluation

On each sampling occasion, each pack opened was subjected to a sensory assessment by either Chris Sentence or a SARDI Food Safety staff member, trained by Chris Sentence.

All sensory assessments utilised ordinal scales from 0 (indicating poor performance) to 8 (indicating good performance). Sensory sheets showing the description of each criterion and score are provided in Appendices 1 and 2.

Prior to opening, packs were scored for *Vacuum* (VAC packs) or *Residual gas* (MAP packs), *Seal* and overall *Appearance*.

Packs were then cut open along the seal using a pair of scissors and the *Initial odour* was scored. The bag was left open for two minutes and the *Odour after 2 minutes* and the *Colour after 2 minutes* was assessed. When the Colour score was less than 7 then the *Colour after 5 minutes* was assessed.

A summary of the scoring scheme is provided below:

- Vacuum/Residual Gas: A 0 indicates *no vacuum* (VAC) or *excess gas / tight pack* (MAP) and 8 indicates *complete vacuum* (VAC) or *loose pack with small amount of residual gas* (MAP).
- Seal: A 0 indicates *no seal / leaker* and an 8 indicates a *good seal*.
- Appearance: A 0 indicates *excess purge / unattractive* and an 8 indicates *no purge / attractive*.
- Initial Odour and Odour after 2 minutes: A 0 indicates *off odour* and an 8 indicates *no odour*.
- Colour after 2 and 5 minutes: A 0 indicates *Other colour, e.g. green*, a 2 indicates *very poor bloom, grey colour* and an 8 indicates *full bloom to red*.

Microbiological Testing and pH

pH

The pH of the uncut (freshly exposed surface) and cut surface (immediately after taking a sample) were measured using a Hanna pH electrode (HI1413B, Hanna Instruments Ltd Bedfordshire UK). The calibration of the pH probe was checked approximately twice per sampling session.

Sample collection

Excision samples were aseptically taken from each of four bone-in and bone-out lamb shoulders immediately prior to packing at the abattoir. Fat and lean surfaces were excised to a depth of approximately 2-5 mm. The total weight excised from each shoulder was 40-50 g. Excised samples were immediately placed in an esky, held at a temperature <4°C and delivered to SARDI, Waite within 10 hours of commencement and eight hours of completion of sampling. A sub-sample of 25 g was used for subsequent microbiological testing.

During the storage trial, a 25 g sample, comprising several surface pieces of 3-5 g each, was collected aseptically (as above) from each lamb shoulder after the vacuum or modified atmosphere packs were opened and after the sensory evaluation had been completed.

TVC and LAB

All meat samples were homogenised for 60 sec in 225 ml Peptone Saline Solution using a stomacher and serial dilutions prepared using 9 mL volumes of Peptone Saline Solution.

For Total Viable Counts serial decimal dilutions were inoculated (1 mL) onto Petrifilm Aerobic Count Plates (3M Corp) and incubated at 25°C ± 1°C for 96 h ± 3 h. After incubation, plates were examined as per the manufacturer's instructions and the aerobic plate count calculated. The limit of detection was 10 cfu/g.

For Lactic Acid Bacteria counts volumes of each decimal dilution (1 mL) were added to an equal volume of double-strength MRS broth (Oxoid Pty Ltd, Adelaide, Australia) and mixed thoroughly. An aliquot (1 mL) of the MRS suspension was inoculated onto Petrifilm Aerobic Count Plates (3M Corp) and incubated at 25°C ± 1°C for 96 h ± 3 h. Films were incubated in sealed pouches containing an anaerobic atmosphere generated by an Anaerogen Compact

pouch (Oxoid, Basingstoke, UK). After incubation the plates were examined as per the manufacturer's instructions and the count calculated. The limit of detection was 20 cfu/g.

Storage and shipping of diluent and isolates

Colony picks were randomly selected from each selected TVC Petrifilm, streaked onto Nutrient Agar (Oxoid) and incubated at 25°C until adequate colony size was observed. Up to 10 colony picks were obtained from individually packed vacuum packed samples and up to five colony picks were obtained from each shoulder in bulk-packed MAP samples (total of up to 20 colonies per pack). Colonies were then scraped separately into Snap Freeze Medium (Oxoid) and frozen at -80°C for later transport on dry ice to the University of Tasmania.

In addition, one 20 ml aliquot of the initial homogenate prepared above was immediately frozen and stored at -80°C. A separate 10 ml aliquot was supplemented with sterile glycerol to a final concentration of 15% glycerol, thoroughly vortexed and immediately frozen and stored at -80°C. Both aliquots were frozen and shipped with dry ice to the University of Tasmania for ecological analysis.

Statistical Analysis

The sensory, pH and microbiological results were combined into a single data set, together with additional identifying information, such as boning state (bone-in, bone-out), packaging (VAC, MAP), product age at time of testing, the pack the sample came from, replicate identifiers and any additional comments. The dataset is provided in Appendix 3.

A total of four MAP packs were found to either contain holes (3 bone-in) or exhibit a faulty seal (1 bone-out) and these were excluded from all analysis.

Sensory

The sensory information was summarised by calculating the median score for each product type at each time point. This was done because of the small number of replicates per occasion and the robust nature of the median, compared to the mean.

pH

Differences in pH between the packaging type and boning state, and their interaction, were assessed using a two-way analysis of variance.

Microbiological

For TVC and LAB two growth curves were fitted – the modified Gompertz model (Zwietering *et al.*, 1990) and the Baranyi model (Baranyi and Roberts, 1994). For model fitting, the following analytical forms (Toldrá, 2009) were used for the modified Gompertz model

$$\log_{10} N = \log_{10}(N_0) + \log_{10}\left(\frac{N_m}{N_0}\right) \exp\left\{-\exp\left[\frac{\mu e^1}{\ln N_m - \ln N_0}(\lambda - t) + 1\right]\right\}$$

and for Baranyi model

$$\log_{10} N = \log_{10}(N_m) + \log_{10}\left\{\frac{-1 + e^{A\mu} + e^{\mu t}}{e^{\mu t} - 1 + e^{A\mu} \ln N_m - \ln N_0}\right\}$$

where

- N denotes the number of organisms;
- N_0 and N_m denote the initial and the maximum number of organisms;
- μ denotes the maximum specific growth rate; and
- λ denotes the lag time.

Because of the clear differences between vacuum packed and MAP product, the two packing types were modelled separately. For vacuum packed product the four replicates represented separately packed samples and hence they were treated as true replicates. For MAP product the four replicates originated form a single pack and hence cannot be considered independent. To fit the growth model, the average of the four results was therefore used.

For each packing type, the parameters were allowed to differ between bone-in and bone-out products. The likelihood ratio test was used to assess whether this resulted in a significant improvement of the model fit, compared to using pooled parameter estimates (over the two boning states).

All statistical analyses were undertaken in R 2.13 (R Development Core Team, 2011). Nonlinear models were fitted using the R package ‘nlme’ (Pinheiro *et al.*, 2011) and the R package ‘nlstools’ (Baty and Delignette-Muller, 2011) was used to find starting values for the nonlinear regression. Unless specified otherwise, a significance level of 0.05 was used.

Results

Temperature

Transport temperatures

Temperature loggers were placed in boxes at different points throughout the pallet (bottom, top and middle rows). A graph of the four temperature profiles is presented in Figure 1. The spike near the end of this graph is a result of the truck unloading at a depot in Pooraka, just prior to delivery to SARDI.

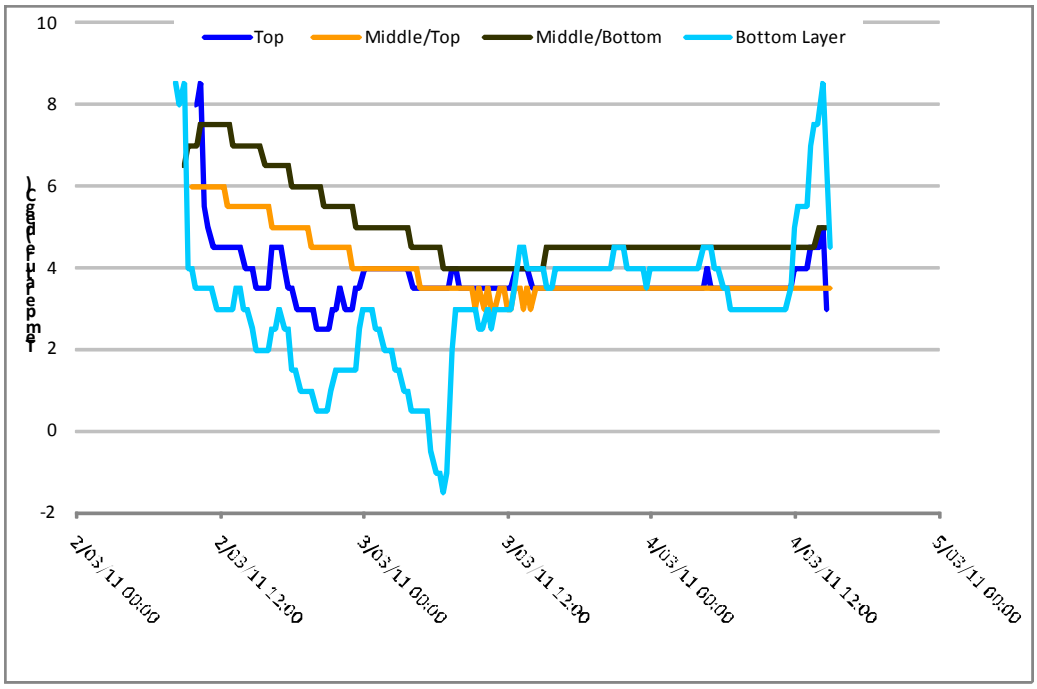

Figure 1: Temperature profiles of product post packing and during transport to SARDI. Different lines indicate temperatures in boxes at different locations in the pallet.

Storage temperatures

During storage, boxes were spread out in a single layer through the cool room, which was set to -0.5°C. A summary of the storage / product temperatures is provided in Table 2 and Figure 2, from which the following observations can be made.

- Initial cooling of product to 0°C took 24 hours and to -0.5°C took 48 hours.
- The average temperature achieved throughout the trial was -0.3°C.
- A compressor breakdown affecting all SARDI cool rooms at 5 am on Monday 11 April 2011 resulted in a spike in temperature, which reached its maximum of 4.8°C after 8 hours. By this time the system had been repaired and product cooled to 0°C by 3 am on 12 April 2011 and to -0.5°C by 21:40 on 12 April 2011.

- Because of the short period (36 hours) of increased temperature, it is not expected that significant growth would have occurred to jeopardise the remainder of the trial.

Table 2: Summary of temperature throughout storage trial

Minimum Temperature	-0.60°C
Average Temperature	-0.30°C
Average Temperature (excluding initial pull down & break down)	-0.34°C
Maximum Temperature (excluding initial pull down & break down)	0.30°C

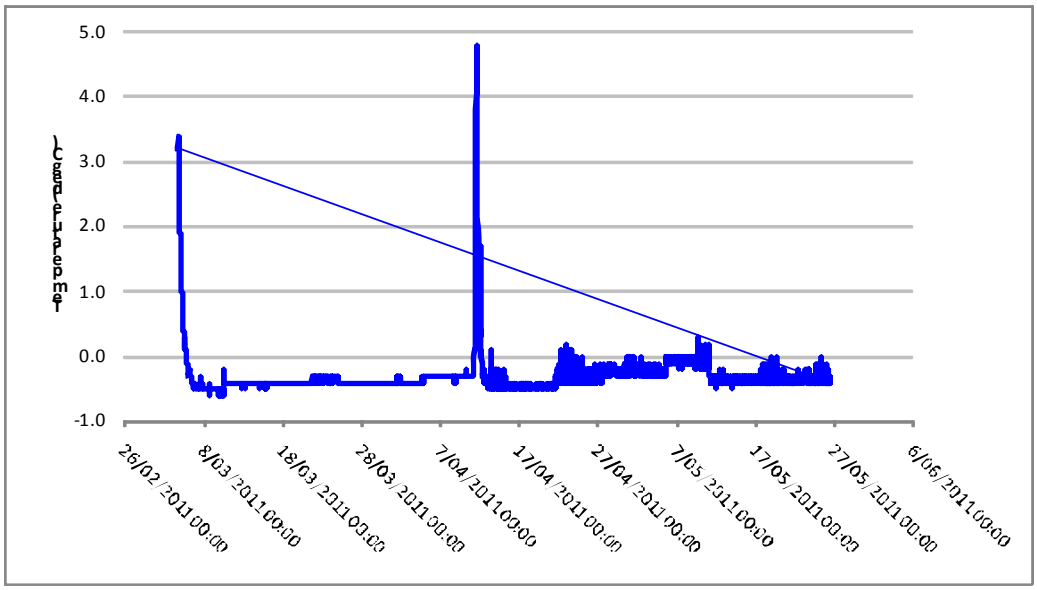

Figure 2: Temperature profiles of product throughout the storage trial.

Sensory

Sensory assessments of the various product types were undertaken prior to microbiological sampling. For MAP product, this assessment was based on the single pack available, while for VAC product between two and four packs were used (Table 1).

Vacuum / Residual Gas

A summary of the vacuum / residual gas scores is presented in Table 3. From this table it can be seen that vacuum packed product generally had a complete vacuum with tight package adhesion. However, MAP product appears to be much more variable in terms of consistency of packages, with many packages resulting in a very strong vacuum that resulted in product distortion.

Table 3: Median scores for vacuum/residual gas for Vacuum and MAP packed lamb shoulders by storage time, respectively. For Vacuum packed product the median is calculated from multiple packs, for MAP the median represents a single pack.

		MAP		VAC	
Sampling Date	**Storage Time**	**Bone-in**	**Bone-out**	**Bone-in**	**Bone-out**
10/03/2011	8	8		8	8
17/03/2011	15	2	6	8	8
24/03/2011	22	2	6	8	8
31/03/2011	29	4	4	8	8
7/04/2011	36	3		8	8
13/04/2011	42			8	8
21/04/2011	50	2	4	8	8
28/04/2011	57		4	8	8
5/05/2011	64	2		8	8
12/05/2011	71		6	8	8
19/05/2011	78	2		8	8
26/05/2011	85		8	8	8

Seal / Shrink

A summary of the seal / shrink scores is presented in Table 4, which shows that the packaging for all product types had a good seal. The exception was one bone-out MAP pack which was found to have an incomplete seal (a 0.5 cm gap at the end). In addition, three bone-in MAP packs were found to have holes in them. These faulty packs are not included in the summaries.

Table 4: Median seal/shrink scores for Vacuum and MAP packed lamb shoulders by storage time, respectively. For Vacuum packed product the median is calculated from multiple packs, for MAP the median represents a single pack.

		MAP		VAC	
Sampling Date	**Storage Time**	**Bone-in**	**Bone-out**	**Bone-in**	**Bone-out**
10/03/2011	8	8		6	6
17/03/2011	15	8	8	6	8
24/03/2011	22	8	8	6	8
31/03/2011	29	8	8	8	8
7/04/2011	36	8		6	8
13/04/2011	42			6	8
21/04/2011	50	8	8	6	8
28/04/2011	57		8	8	8
5/05/2011	64	8		6	8
12/05/2011	71		8	6	8
19/05/2011	78	8		6	8

26/05/2011	85		8	8	8

Appearance

The appearance score, which also indicates the amount of purge in the back, is summarised in Table 5 for each product type over time. From this table it can be seen that appearance scored dropped (amount of purge increased) over the storage trial. This is despite MAP product being wrapped in absorbent paper.

Table 5: Median appearance scores for Vacuum and MAP packed lamb shoulders by storage time. For Vacuum packed product the median is calculated from multiple packs, for MAP the median represents a single pack.

		MAP		VAC	
Sampling Date	**Storage Time**	**Bone-in**	**Bone-out**	**Bone-in**	**Bone-out**
10/03/2011	8	8		8	8
17/03/2011	15	6	6	6	6
24/03/2011	22	6	6	6	6
31/03/2011	29	4		4	4
7/04/2011	36	5		5	6
13/04/2011	42			5	4
21/04/2011	50	2	4	5	4
28/04/2011	57		4	2	3
5/05/2011	64	4		4	6
12/05/2011	71		2	4	3
19/05/2011	78	4		4	3.5
26/05/2011	85		3	3	4

Odour

The initial odour score is summarised in Table 6, which shows that vacuum packed product generally exhibited little variability in the median score over time – the score of 6 indicates a very slight sour odour. In contrast, MAP showed more variability, but this is probably due to only single packs being available for assessment. While bone-in MAP product appears to have scored marginally better than bone-out MAP product no firm conclusions can be drawn.

Table 6: Median scores for initial odour for Vacuum and MAP packed lamb shoulders by storage time.

		MAP		VAC	
Sampling Date	Storage Time	Bone-in	Bone-out	Bone-in	Bone-out
10/03/2011	8	8		6	6
17/03/2011	15	8	6	6	6
24/03/2011	22	8	8	6	7
31/03/2011	29	6	6	6	6
7/04/2011	36	7		6	6
13/04/2011	42			6	6
21/04/2011	50	6	6	6	5
28/04/2011	57		4	6	6
5/05/2011	64	8		7	7
12/05/2011	71		6	6	7
19/05/2011	78	8		6	7
26/05/2011	85		4	5	6

The median odour score after two minutes is provided in Table 7 for each product type over time. From this table it can be seen that both products consistently yielded a very high score (no odour). In total, only five packs assessed did not score an 8. One MAP bone-in pack scored a 4, two VAC bone-in packs scored a 6, one VAC bone-in and one VAC bone-out product scored 7.

Table 7: Median scores for odour after two minutes for Vacuum and MAP packed lamb shoulders by storage time.

		MAP		VAC	
Sampling Date	Storage Time	Bone-in	Bone-out	Bone-in	Bone-out
10/03/2011	8	8		8	8
17/03/2011	15	8	8	8	8
24/03/2011	22	8	8	8	8
31/03/2011	29	8	8	8	8
7/04/2011	36	8		8	8
13/04/2011	42			8	8
21/04/2011	50	8	8	8	8
28/04/2011	57		8	8	8
5/05/2011	64	8		8	8
12/05/2011	71		8	8	7.5
19/05/2011	78	8		8	8
26/05/2011	85		8	8	8

Colour

The median colour score after two minutes is provided in Table 8 for each product type over time. From this table it can be seen that all product types scored consistently very high. While MAP product appears to exhibit slightly more variability, this is probably due to only single packs being available for assessment.

Table 8: Median scores for colour after two minutes for Vacuum and MAP packed lamb shoulders by storage time.

		MAP		VAC	
Sampling Date	**Storage Time**	**Bone-in**	**Bone-out**	**Bone-in**	**Bone-out**
10/03/2011	8	8		8	7
17/03/2011	15	8	8	8	8
24/03/2011	22	8	8	8	8
31/03/2011	29	8	8	8	8
7/04/2011	36	6		7	8
13/04/2011	42			8	8
21/04/2011	50	8	8	8	8
28/04/2011	57		8	8	8
5/05/2011	64	8		8	8
12/05/2011	71		6.5	8	7
19/05/2011	78	6		6	6
26/05/2011	85		6	6	6

Product that did not receive a colour score of 7 or 8 after two minutes was reassessed three minutes later (total of five minutes after opening). Generally this did not result in a change in the colour score (23 samples), although five samples had a score increase of 1 and four samples had an increase of 2 scores.

pH

Plots of the pH as measured on the surface of freshly exposed lamb and from areas from which a surface slice had been taken are provided in Figure 3 and Figure 4, respectively. From these the following key observations can be made.

- The pH on the surface of packed lamb shoulders changes throughout the storage period, though this change appears to differ between the four different products types. Vacuum packed bone-in product seems to increase in pH over the first 50 days, before stabilising. In contrast vacuum packed bone out product appears stable at first and then drops off at about 50 days. MAP product exhibited an initial drop in pH before returning starting levels.
- Ignoring the changes throughout storage for surface pH (Figure 3), bone-in product was significantly higher than bone-out product (P-value < 0.001) and Vac product was significantly higher than MAP product (P-value = 0.003), on average. The average pH for the four product types was 5.97 (MBI), 5.81 (MBO), 6.07 (VBI) and 5.87 (VBO).

- The high pH of 6.87 on the cut surface of MAP bone-out product was obtained from a product that had barely any residual gas in the pack. For the remaining pH values above 6.5 no special cause could be identified.

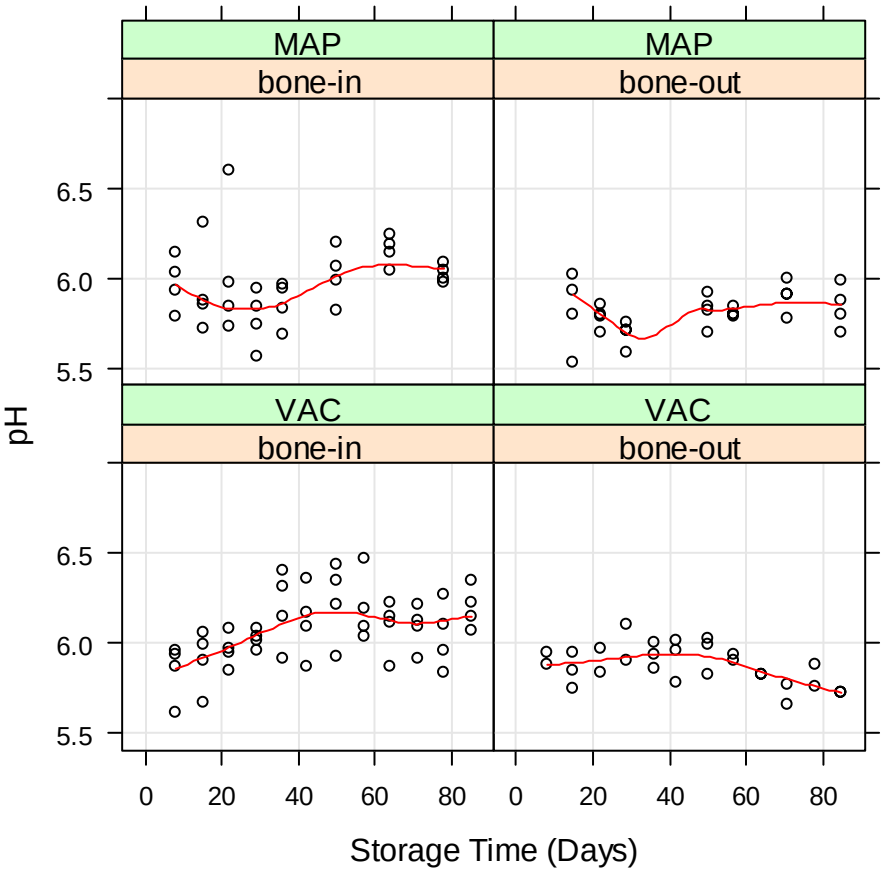

Figure 3: pH of freshly exposed surfaces – the red line indicates a locally weighted regression smoother to indicate the general trend of the data.

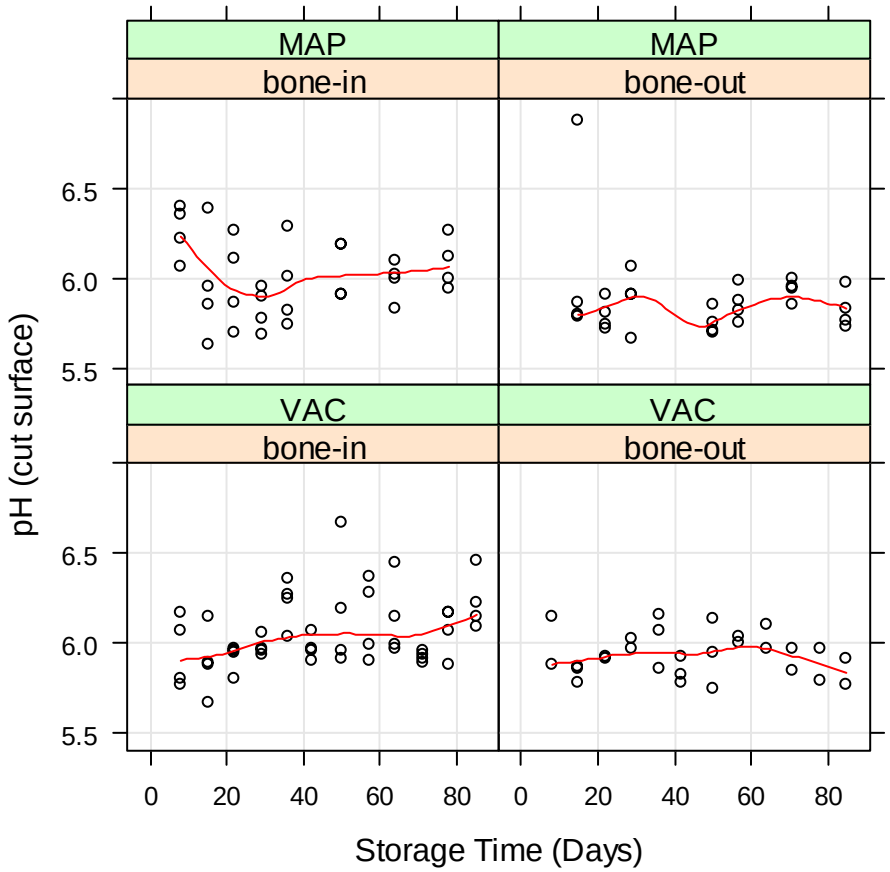

Figure 4: pH of cut surfaces – the red line indicates a locally weighted regression smoother to indicate the general trend of the data.

Microbial

Total Viable Count

A plot of the $\log_{10}$ TVC over time for Vacuum and MAP packed lamb shoulders is shown in Figure 5. From this plot the following observations can be made.

- For MAP product the $\log_{10}$ TVC stays remains fairly constant across the storage period, irrespective of boning status.
- Variability in $\log_{10}$ TVC of MAP product is low early on and reflects that all samples come from the same pack. However, from about 40 days variability between samples seems to increase, which may be due to increased moisture in the pack (see Table 5).
- For VAC product the $\log_{10}$ TVC increases close to linearly, irrespective of boning status. Lag and stationary phases are not clearly apparent.
- For both packaging types variability throughout storage appears greater than immediately prior to packing, despite product being stored under ‘identical’ conditions.

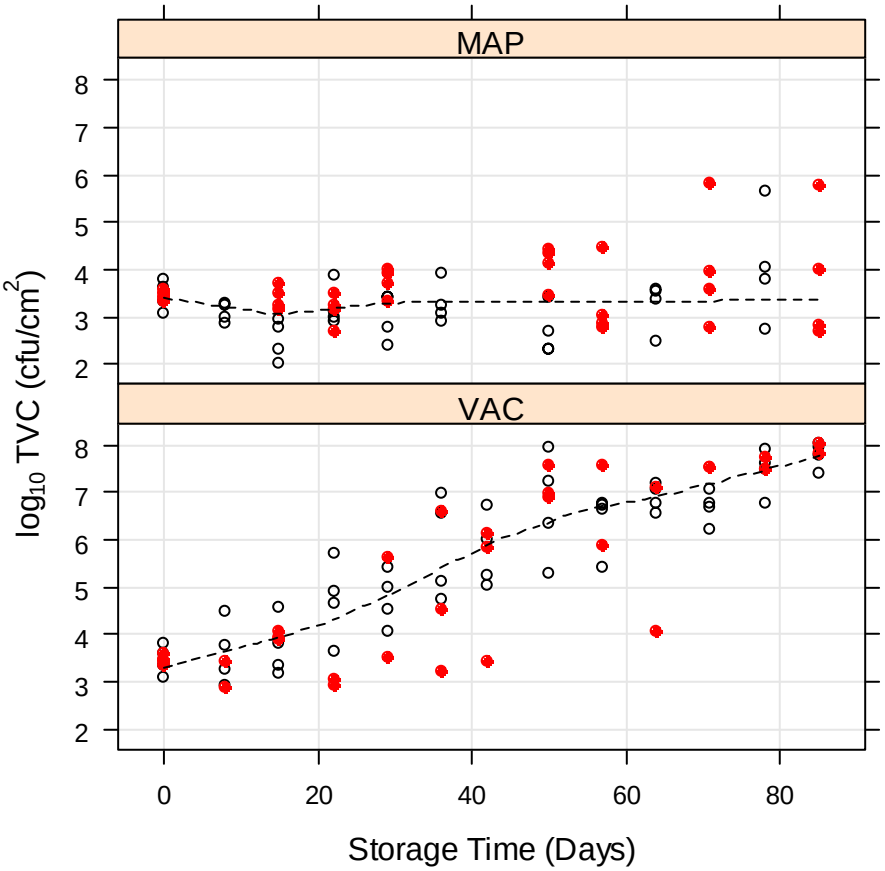

Figure 5: Total Viable Counts for Vacuum and MAP packed bone-in (black circles) and bone-out (red dots) product. The dashed line indicates a smooth curve to show the general trend in the data. Samples shown at time = 0 days are the same for both packaging types as product had not been packed.

For vacuum packed product both models fitted the data equally well (Figure 6), though the Gompertz could explain marginally more variability than the Baranyi model (Residual standard errors of 0.8225 [G][2] and 0.8332 [B]). For both models there were no significant differences in the parameters between the two boning types (P-value = 0.28 [G] and 0.40 [B]) and hence a single growth curve can be used to describe the total microbial growth on vacuum packed lamb shoulders. A summary of the estimates of the model parameters is provided in Table 9.

[2] We use the short hand notation [G] to denote the Gompertz model and [B] to denote the Baranyi model.

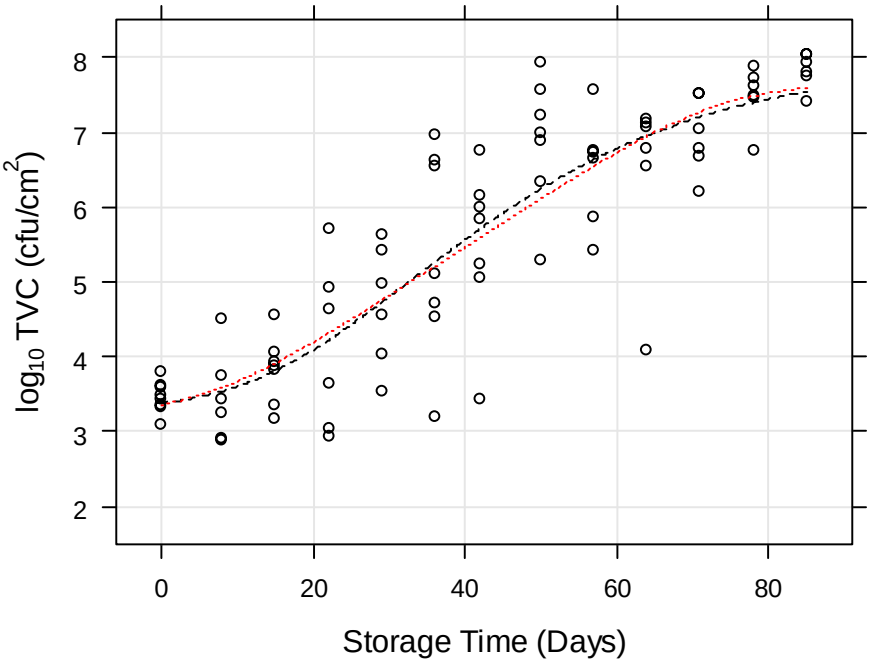

Figure 6: Total Viable Counts for Vacuum packed bone-in and bone-out product. The lines indicate the fitted Gompertz (black dashes) and Baranyi (red dots) growth curve models.

Fitting models to the MAP data (Figure 7) was unsuccessful for both models. This is probably due to the lack of growth, which resulted in the estimate for the lag extending past the last observed time point and subsequently causing the numerical fitting algorithm to fail.

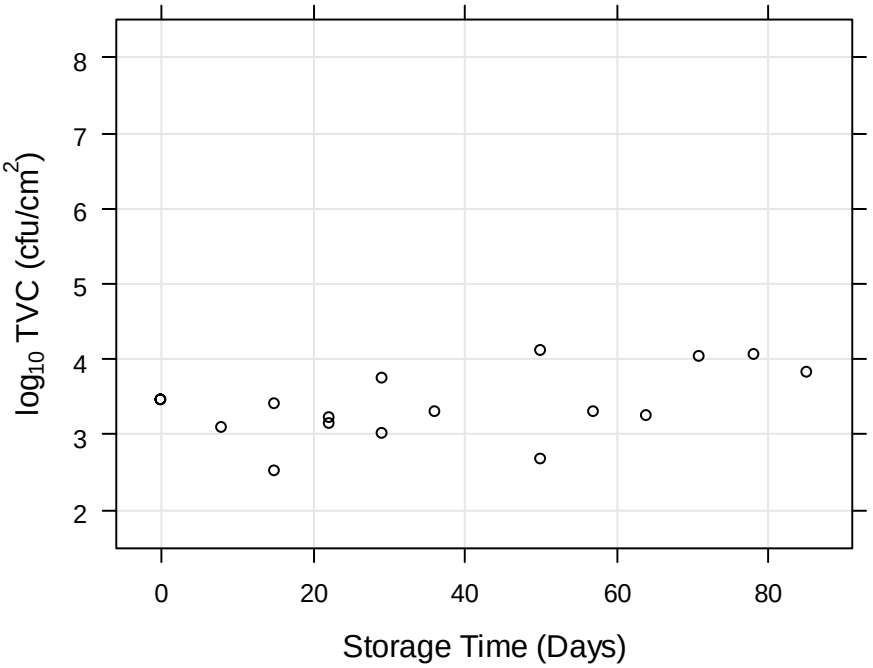

Figure 7: Average Total Viable Counts for MAP packed bone-in and bone-out product (each point represent the average of one sample from each of four shoulders from a single pack).

Table 9: Summary of model parameters for TVC

	MAP		VAC	
Parameter	**Gompertz**	**Baranyi**	**Gompertz**	**Baranyi**
$\log_{10} N_0$ ($\log_{10}$ cfu/g)	-	-	3.33	3.36
$\log_{10} N_m$ ($\log_{10}$ cfu/g)	-	-	7.96	7.66
μ ($\log_{10}$ cfu/g/day)	-	-	0.18	0.15
λ (days)	-	-	10.93	7.89

Lactic Acid Bacteria

A plot of the $\log_{10}$ LAB over time for Vacuum and MAP packed lamb shoulders is shown in Figure 8.

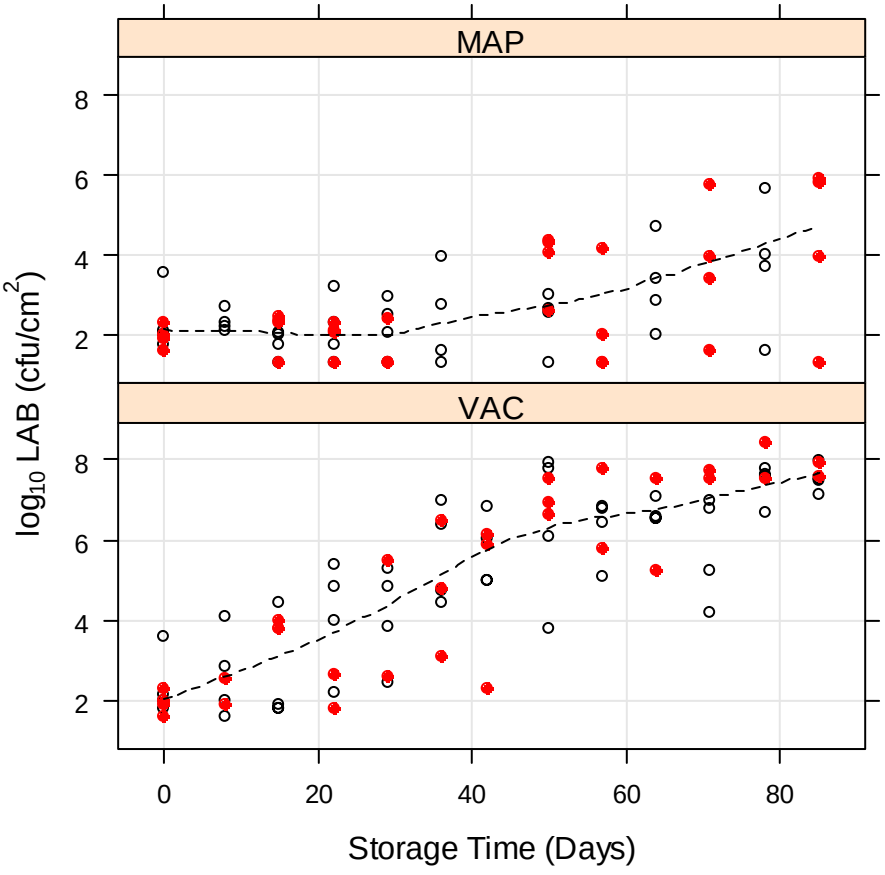

Figure 8: Lactic Acid Bacteria counts for Vacuum and MAP packed bone-in (black circles) and bone-out (red dots) product. The dashed line indicates a smooth curve to show the general trend in the data. Samples shown at time = 0 days are the same for both packaging types as product had not been packed.

From this plot the following observations can be made.

- For MAP product the $\log_{10}$ LAB stays fairly constant for the first 40 days at which point it increases. The variability in samples from a single package also increases.
- For VAC product the $\log_{10}$ LAB increases close to linearly up to about 50 days at which point it appears to slow down, but not quite to a constant level.
- For both packaging types variability throughout storage appears greater than immediately prior to packing, despite product being stored under 'identical' conditions.

For vacuum packed product both models fitted the data equally well (Figure 9), though the Gompertz could again explain marginally more variability than the Baranyi model (Residual standard errors of 1.11 [G] and 1.125 [B]). For both models there were no significant differences in the parameters between the two boning types (P-value = 0.48 [G] and 0.43 [B]). A summary of the model parameter estimates is provided in Table 10.

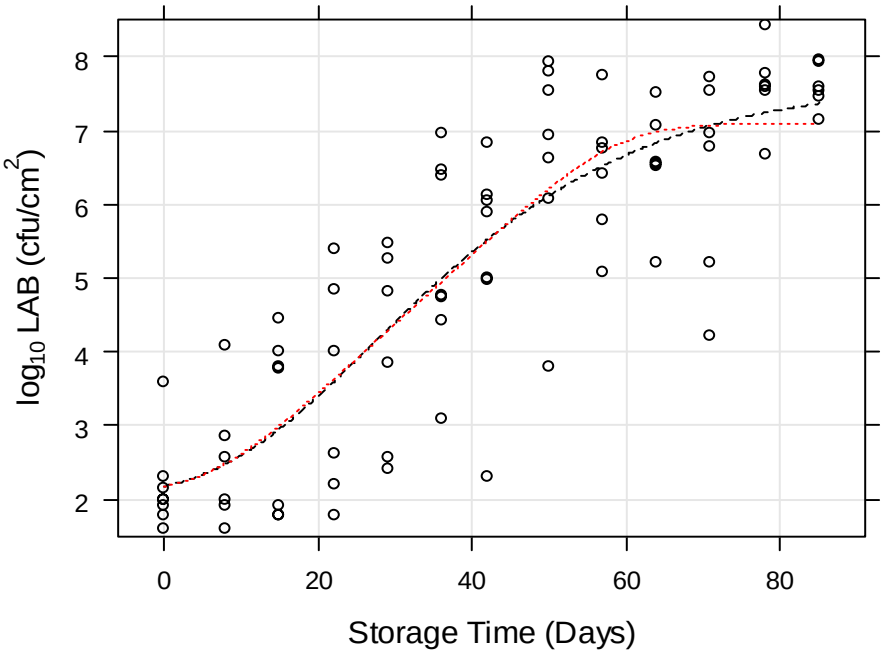

Figure 9: Lactic Acid Bacteria counts for vacuum packed bone-in and bone-out product. The lines indicate the fitted Gompertz (black dashes) and Baranyi (red dots) growth curve models.

For the MAP data, fitting the Baranyi model was unsuccessful. However, the Gompertz model could be fitted. There were no significant differences between the parameter estimates for bone-in and bone-out product (P-value = 0.84). The fit of the model is shown in Figure 10 and the estimates of the model parameters are provided in Table 10.

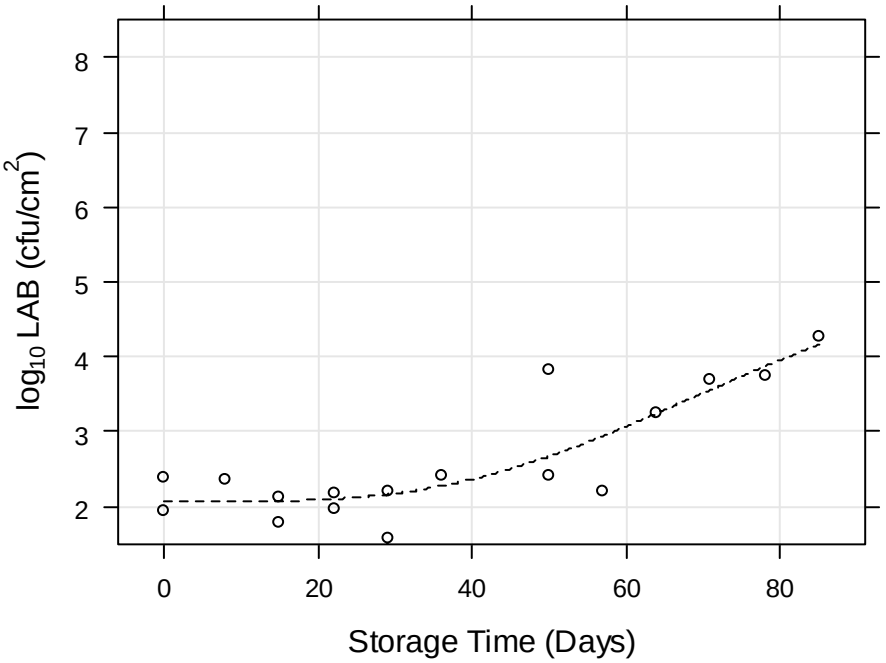

Figure 10: Average Lactic Acid Bacteria counts for MAP packed bone-in and bone-out product (each point represent the average of one sample from each of four shoulders from a single pack). The dashed line indicates the fitted Gompertz growth curve model.

Table 10: Summary of model parameters for LAB

	MAP		VAC	
Parameter	Gompertz	Baranyi	Gompertz	Baranyi
$\log_{10} N_0$ ($\log_{10}$ cfu/g)	2.06	-	2.06	2.17
$\log_{10} N_m$ ($\log_{10}$ cfu/g)	5.83	-	7.68	7.10
μ ($\log_{10}$ cfu/g/day)	0.10	-	0.24	0.22
λ (days)	37.64	-	7.01	6.75

Comparison to previous trial

The TVC and LAB results for vacuum packed bone-out product from this trial (Trial 3) were compared against those obtained from a previous extended storage trial of similar product (Trial 2 – MLA Project A.MFS.0196). The results for $\log_{10}$ TVC and $\log_{10}$ LAB are shown in Figure 11 and Figure 12. From these figures it can be seen that the microbiological results from the two trials agree well, despite the following methodological differences.

- In Trial 2 microbiological analysis were performed in replicate on each of two shoulders, while in Trial 3 single microbiological analyses were performed on four shoulders.
- Shoulders were sourced from different processors for the two trials.

In addition, from current work it is apparent that the results from the previous trial from about 50 day were unusually high and did not indicate stationary phase, as previously expected.

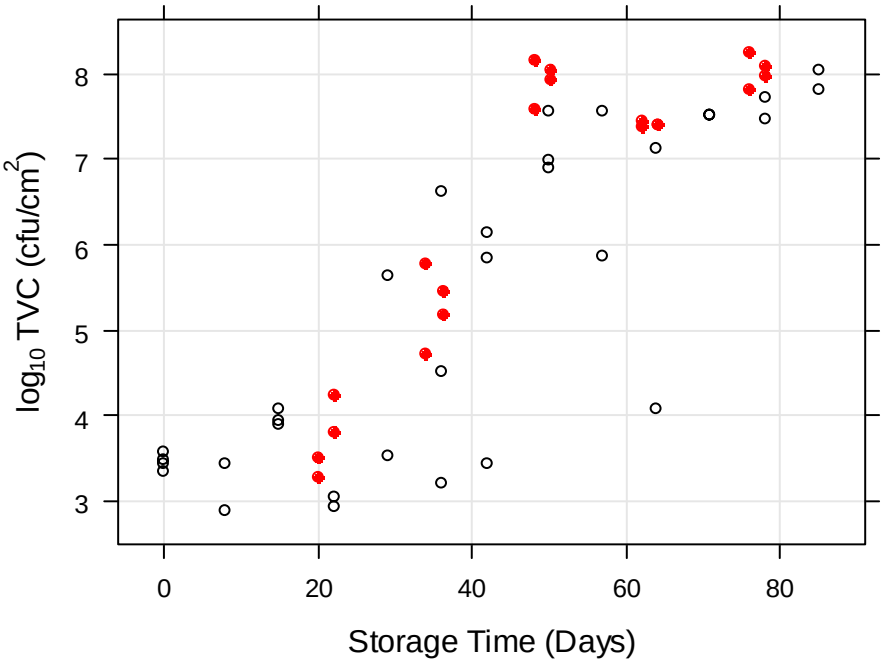

Figure 11: Scatter plot of log10 TVC over time – each red point indicates the mean of two replicate analyses from separately vacuum packed bone-out sample (Trial 2 – A.MFS.0196) and black circles indicate single analyses from separately vacuum packed bone-out samples.

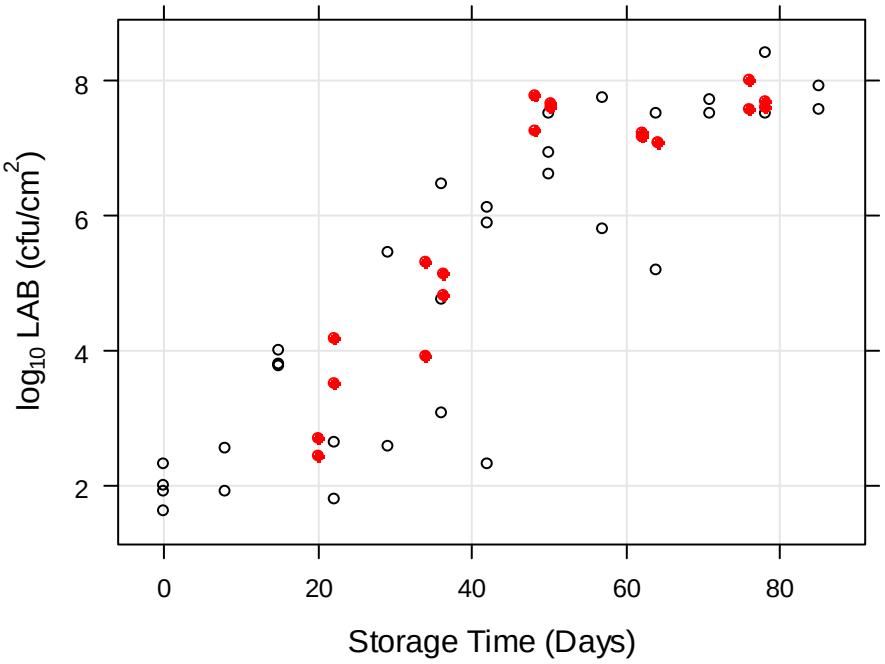

Figure 12: Scatter plot of log10 LAB over time – each red point indicates the mean of two replicate analyses from separately vacuum packed bone-out sample (Trial 2 – A.MFS.0196) and black circles indicate single analyses from separately vacuum packed bone-out samples.

Discussion

In this trial the shelf-life of vacuum packed and modified atmosphere packed lamb shoulders were investigated. For each packaging method bone-in and bone-out product was tested organoleptically and microbiologically.

Based on the data collected in this trial it appears that important sensory quality indicators – colour and odour – scored high throughout the 85 days storage period. This indicates that vacuum packed and MAP lamb shoulders have a shelf-life that can exceed 85 days, provided temperature is well controlled at -0.5 to 0°C. In contrast to the current findings, Gill and Penney (1985) found that packing high pH (>5.9) lamb loins under vacuum or 100% CO_2 resulted in some organoleptic spoilage (strong sweet-putrid odours) between 6 and 9 weeks of storage at -0.5°C. The differences may be due to the microbial ecology, which is being investigated by the University of Tasmania in a separate study.

For vacuum packed shoulders TVCs increased from about 3 to 10 $\log_{10}$ cfu/g over the 85 day storage period. Lactic acid bacteria grew in a similar fashion from about 2 to about 7.5 $\log_{10}$ cfu/g over the same period. This growth could be modelled equally well using the Gompertz or Baranyi growth models. However, it should be noted that despite identical storage on little variability in the raw materials, the variability in TVC and LAB between individually packed shoulders was large. This indicates that consistency of product microbiology may be difficult to control despite good temperature control.

The results obtained in this trial agree with Egan *et al.* (1988) who indicated that vacuum packed meat should have a shelf-life of 6-8 weeks when stored at 0°C, provided that initial counts were 2-3 $\log_{10}$ cfu/cm^2, a low permeability packaging film was used and temperature control was maintained. They also indicated that an increase of about 50% could be achieved at storage of -1°C, though the current trial indicates that storage life can be longer even at -0.5°C.

The result from bone-out vacuum packed lamb shoulders align well with those obtained from a previous trial (MLA Project A.MFS.0196). However, since samples were taken at weekly intervals in this trial, the growth and stationary phases in this product can be determined more accurately. This has highlighted that TVC and LAB results from the previous trial were elevated around 50 days and that stationary phase is not reached until about 80 days.

In contrast to vacuum packed product, MAP product did not show much growth in TVC. The average TVC did not increase over the storage period and single shoulders from a bulk pack did not exceed 6 $\log_{10}$ cfu/g. Similarly, Lactic Acid Bacteria did not grow well in MAP product, though some growth could be observed toward the end of the storage period and modelled using the Gompertz growth curve. Based on this model the average LAB after 85 days is not expected to exceed 10^4 cfu/g.

Irrespective of whether vacuum packing or MAP packing was used, no evidence could be found to indicate that bone-in product has a shorter shelf-life than bone-out product.

While MAP product performed well microbiologically and organoleptically, it should be noted that more packaging problems were observed with this product. Out of the 19 packs received four packs were not intact – one had not been properly sealed while three had holes in them. These three bags contained bone-in product and it is likely that the holes were caused by excessive shrinkage which was also observed on other packs, though without the same ill effects. The shrinkage in MAP packs is created as a result of CO_2 being absorbed into the meat and the excessive shrinkage may be due to the low ratio of 1.5 to 1 of CO_2 to meat used by the processor, which agrees with that recommended by Egan *et al.* (1988). However, this ratio is considerably less than the 2.5-3.0 to 1 recommended by MIRINZ in the 1980s and used by

CSIRO in trials in the late 80s and early 90s (pers. comm. I. Eustace). From the problems experienced with the MAP packs, a higher gas to meat ratio may be desirable. Alternatively a small amount of nitrogen, an inert gas, may be used as a filler to help prevent pack collapse and thus prevent subsequent pack damage. The use of gas mixtures has been investigated intensively, though mainly using retail storage conditions (Berruga *et al.*, 2005; Channon *et al.*, 2005; Soldatou *et al.*, 2009). Sheridan *et al*. (1997) packed unspecified lamb primals from low pH (<5.8) lamb carcases using various gas mixtures at a gas to meat ratio of 2:1 and stored them at 0 and 5°C for up to 28 days. For product stored at 0°C they found 6.3 and 6.6 $\log_{10}$ cfu/g of aerobic and anaerobic organisms in vacuum packed product after 28 days. No differences were observed between 100% CO_2 packed and 50% CO_2:50% N_2 packed product – aerobic counts were 3.9 and 4.2 $\log_{10}$ cfu/g and anaerobic counts were 3.8 and 3.9 $\log_{10}$ cfu/g, respectively. In all cases, starting levels of product predicted from a linear regression model were slightly higher (approximately 4 $\log_{10}$ cfu/g) than those observed in the current study. However, they also found slightly lower acceptability, in terms of odour production one hour post pack opening, of vacuum packed product (72% acceptable) than that packed under 100% CO_2 (97% acceptable), though the differences were not statistically significant.

Success in Achieving Objectives

Establish growth curves for Total Viable Counts and Lactic Acid Bacteria counts for vacuum packed and modified atmosphere packed lamb shoulders stored for 84 days at -0.5°C and collect colonies of Total Viable Counts for ecological analysis.

The objectives for this project have been met as:

- Vacuum packed and modified atmosphere packed bone-in and bone-out lamb shoulders were stored at -0.5°C for 85 days;
- Growth curves for TVC and LAB were estimated; and
- Diluent samples and colony picks of Total Viable Count were collected, stored at -80°C, and sent to the University of Tasmania for ecological analysis.

Acknowledgements

We would like to thank staff and management from the participating abattoir for their assistance and cooperation in running the trial. Chris Sentence is thanked for collecting samples from the abattoir and for his assistance with sensory assessments throughout the trial.

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Appendix 1: Sensory Evaluation Form – MAP

Date		**Bone in**		**Boneless**		**Tick one product type**			
	Attribute								
	Residual gas	**Seal**	**Appearance**	**Initial odour**	**Odour after 2 mins**	**Colour after 2 mins**	**Colour after 5 mins**	**pH**	
Sample Number	8=Loose pack with small amount of free gas 6=Loose pack no free gas 4= Slight vacuum 2=Strong vacuum distorting product vacuum 0=Excess gas, tight pack	8=good seal 2=poor seal 0=no seal, leaker	8=no purge, attractive 6=minimal purge 4=moderate purge 2=significant purge 0=excess purge, unattractive	8=no odour 6=v sl sour odour 4=moderate sour odour 2=strong sour odour 0=off odour	8=no odour 6=v sl sour odour 4=moderate sour odour 2=strong sour odour 0=off odour	8=Full bloom to red 6=Bloom to light red 4=Poor bloom, some greyness 2=V poor bloom, grey colour 0=Other colour eg green	*(only if 2 min check is 6 or less)* 8=Full bloom to red 6=Bloom to light red 4=Poor bloom, some greyness 2=V poor bloom, grey colour 0=Other colour eg green	Untouched lean surface	New lean surface

Appendix 2: Sensory Evaluation Form – VAC

Date		**Bone in**		**Boneless**		**Tick one product type**			
	Attribute								
	Vacuum	**Seal/shrink**	**Appearance**	**Initial odour**	**Odour after 2 mins**	**Colour after 2 mins**	**Colour after 5 mins**	**pH**	
Sample Number	8=complete vacuum, tight package adhesion 6=good vacuum 4=moderate vacuum 2=poor vacuum 0=no vacuum, leaker	8=good seal, tight shrink 6=good seal, average shrink 4=good seal, poor shrink 2=poor seal 0=no seal, leaker	8=no purge, attractive 6=minimal purge 4=moderate purge 2=significant purge 0=excess purge, unattractive	8=no odour 6=v sl sour odour 4=moderate sour odour 2=strong sour odour 0=off odour	8=no odour 6=v sl sour odour 4=moderate sour odour 2=strong sour odour 0=off odour	8=Full bloom to red 6=Bloom to light red 4=Poor bloom, some greyness 2=V poor bloom, grey colour 0=Other colour eg green	(only reqd if 2 min check is 6 or less) 8=Full bloom to red 6=Bloom to light red 4=Poor bloom, some greyness 2=V poor bloom, grey colour 0=Other colour eg green	Untouched lean surface	New lean surface

Appendix 3: Data

labnum	tested	rep	tvc	lab	product	packaging	pack	vac.res.gas	seal	appearance	odour.init	odour.2min	colour.2min	colour.5min	ph	ph.cut	use	age
FS11-0038	2/03/2011 18:00	1	4100	60	bone-in	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0038a	2/03/2011 18:00	1	4100	60	bone-in	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0039	2/03/2011 18:00	2	1200	140	bone-in	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0039a	2/03/2011 18:00	2	1200	140	bone-in	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0040	2/03/2011 18:00	3	2100	100	bone-in	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0040a	2/03/2011 18:00	3	2100	100	bone-in	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0041	2/03/2011 18:00	4	6200	3800	bone-in	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0041a	2/03/2011 18:00	4	6200	3800	bone-in	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0042	2/03/2011 18:00	1	3800	100	bone-out	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0042a	2/03/2011 18:00	1	3800	100	bone-out	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0043	2/03/2011 18:00	2	2200	80	bone-out	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0043a	2/03/2011 18:00	2	2200	80	bone-out	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0044	2/03/2011 18:00	3	2700	40	bone-out	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0044a	2/03/2011 18:00	3	2700	40	bone-out	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0045	2/03/2011 18:00	4	3000	200	bone-out	MAP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0045a	2/03/2011 18:00	4	3000	200	bone-out	VAC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0
FS11-0089	10/03/2011 14:00	1	1900	220	bone-in	MAP	1	8	8	8	8	8	8	NA	6.14	6.35	1	8
FS11-0090	10/03/2011 14:00	2	980	140	bone-in	MAP	1	NA	NA	NA	8	8	8	NA	5.93	6.06	1	8
FS11-0091	10/03/2011 14:00	3	710	160	bone-in	MAP	1	NA	NA	NA	8	8	8	NA	6.03	6.39	1	8
FS11-0092	10/03/2011 14:00	4	1800	540	bone-in	MAP	1	NA	NA	NA	8	8	8	NA	5.78	6.22	1	8
FS11-0093	10/03/2011 14:00	1	1800	700	bone-in	VAC	1	8	6	8	6	8	8	NA	5.61	5.8	1	8
FS11-0094	10/03/2011 14:00	2	810	40	bone-in	VAC	2	8	6	8	6	8	8	NA	5.95	6.07	1	8
FS11-0095	10/03/2011 14:00	3	31000	12000	bone-in	VAC	3	8	6	8	6	8	8	NA	5.93	6.17	1	8

labnum	tested	rep	tvc	lab	product	packaging	pack	vac.res.gas	seal	appearance	odour.init	odour.2min	colour.2min	colour.5min	ph	ph.cut	use	age
FS11-0096	10/03/2011 14:00	4	5600	100	bone-in	VAC	4	8	6	8	6	8	8	NA	5.86	5.76	1	8
FS11-0097	10/03/2011 14:00	1	750	80	bone-out	VAC	1	8	6	8	6	8	6	8	5.88	6.14	1	8
FS11-0098	10/03/2011 14:00	2	2700	360	bone-out	VAC	2	8	6	8	6	8	8	NA	5.94	5.88	1	8
FS11-0099	17/03/2011 14:00	1	900	60	bone-in	MAP	1	2	8	6	8	8	8	NA	6.31	6.38	1	15
FS11-0100	17/03/2011 14:00	2	600	120	bone-in	MAP	1	NA	NA	NA	NA	8	6	8	5.72	5.95	1	15
FS11-0101	17/03/2011 14:00	3	200	100	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	5.87	5.63	1	15
FS11-0102	17/03/2011 14:00	4	100	20	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	5.85	5.85	1	15
FS11-0103	17/03/2011 14:00	1	1700	20	bone-out	MAP	1	6	8	6	6	8	8	NA	6.02	6.87	1	15
FS11-0104	17/03/2011 14:00	2	3300	240	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.93	5.86	1	15
FS11-0105	17/03/2011 14:00	3	1500	200	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.53	5.78	1	15
FS11-0106	17/03/2011 14:00	4	5300	300	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.8	5.8	1	15
FS11-0107	17/03/2011 14:00	1	2200	60	bone-in	VAC	1	8	6	6	6	8	8	NA	5.9	6.14	1	15
FS11-0108	17/03/2011 14:00	2	6500	60	bone-in	VAC	2	8	6	6	6	8	8	NA	6.05	5.66	1	15
FS11-0109	17/03/2011 14:00	3	37000	28000	bone-in	VAC	3	8	6	6	6	8	8	NA	5.99	5.88	1	15
FS11-0110	17/03/2011 14:00	4	1500	80	bone-in	VAC	4	8	6	6	6	8	8	NA	5.67	5.89	1	15
FS11-0111	17/03/2011 14:00	1	8500	5800	bone-out	VAC	1	8	8	6	6	8	8	NA	5.84	5.87	1	15
FS11-0112	17/03/2011 14:00	2	7700	6400	bone-out	VAC	2	8	6	4	6	8	8	NA	5.94	5.85	1	15
FS11-0113	17/03/2011 14:00	3	11600	10000	bone-out	VAC	3	8	8	6	6	8	8	NA	5.74	5.78	1	15
FS11-0133	24/03/2011 13:30	1	1300	20	bone-in	MAP	1	2	8	6	8	8	8	NA	5.84	6.11	1	22
FS11-0134	24/03/2011 13:30	2	7200	1700	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	6.59	5.86	1	22
FS11-0135	24/03/2011 13:30	3	960	220	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	5.97	6.26	1	22
FS11-0136	24/03/2011 13:30	4	790	60	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	5.73	5.7	1	22
FS11-0137	24/03/2011 13:30	1	1400	120	bone-out	MAP	1	6	8	6	8	8	8	NA	5.8	5.81	1	22
FS11-0138	24/03/2011 13:30	2	3200	220	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.78	5.91	1	22
FS11-0139	24/03/2011 13:30	3	520	20	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.85	5.72	1	22
FS11-0140	24/03/2011 13:30	4	1710	140	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.7	5.74	1	22
FS11-0141	24/03/2011 13:30	1	81000	68000	bone-in	VAC	1	8	6	6	6	8	8	NA	5.84	5.94	1	22

labnum	tested	rep	tvc	lab	product	packaging	pack	vac.res.gas	seal	appearance	odour.init	odour.2min	colour.2min	colour.5min	ph	ph.cut	use	age
FS11-0142	24/03/2011 13:30	2	520000	250000	bone-in	VAC	2	8	6	6	6	8	8	NA	5.96	5.95	1	22
FS11-0143	24/03/2011 13:30	3	4400	160	bone-in	VAC	3	8	6	6	6	8	8	NA	6.08	5.97	1	22
FS11-0144	24/03/2011 13:30	4	43000	10000	bone-in	VAC	4	8	6	6	6	8	8	NA	5.94	5.8	1	22
FS11-0145	24/03/2011 13:30	1	850	60	bone-out	VAC	1	8	8	6	6	8	8	NA	5.83	5.92	1	22
FS11-0146	24/03/2011 13:30	2	1100	420	bone-out	VAC	2	8	8	6	8	8	8	NA	5.96	5.91	1	22
FS11-0266	31/03/2011 14:00	1	9700	20	bone-out	MAP	1	4	8	NA	6	8	8	NA	5.71	5.91	1	29
FS11-0267	31/03/2011 14:00	2	8500	260	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.71	5.91	1	29
FS11-0268	31/03/2011 14:00	3	2200	20	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.58	6.06	1	29
FS11-0269	31/03/2011 14:00	4	4900	20	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.75	5.66	1	29
FS11-0270	31/03/2011 14:00	1	35000	260	bone-in	VAC	1	8	8	4	6	8	8	NA	6.01	6.05	1	29
FS11-0271	31/03/2011 14:00	2	270000	180000	bone-in	VAC	2	8	8	4	6	8	8	NA	5.95	5.97	1	29
FS11-0272	31/03/2011 14:00	3	11000	7200	bone-in	VAC	3	8	8	4	6	8	8	NA	6.08	5.95	1	29
FS11-0273	31/03/2011 14:00	4	93000	66000	bone-in	VAC	4	8	8	6	6	8	8	NA	6.03	5.93	1	29
FS11-0274	31/03/2011 14:00	1	430000	290000	bone-out	VAC	1	8	8	4	6	8	8	NA	5.9	6.02	1	29
FS11-0275	31/03/2011 14:00	2	3400	380	bone-out	VAC	2	8	8	4	6	8	8	NA	6.1	5.97	1	29
FS11-0276	31/03/2011 14:00	1	260	20	bone-in	MAP	2	4	8	4	6	8	8	NA	5.84	5.95	1	29
FS11-0277	31/03/2011 14:00	2	630	120	bone-in	MAP	2	NA	NA	NA	NA	8	8	NA	5.56	5.68	1	29
FS11-0278	31/03/2011 14:00	3	2500	900	bone-in	MAP	2	NA	NA	NA	NA	8	8	NA	5.74	5.89	1	29
FS11-0279	31/03/2011 14:00	4	2500	320	bone-in	MAP	2	NA	NA	NA	NA	8	8	NA	5.94	5.77	1	29
FS11-0329	7/04/2011 11:30	1	8300	9200	bone-in	MAP	1	3	8	5	7	8	6	6	5.96	5.74	1	36
FS11-0330	7/04/2011 11:30	2	1800	20	bone-in	MAP	1	NA	NA	NA	NA	NA	6	6	5.68	5.82	1	36
FS11-0331	7/04/2011 11:30	3	790	560	bone-in	MAP	1	NA	NA	NA	NA	NA	6	6	5.94	6.01	1	36
FS11-0332	7/04/2011 11:30	4	1200	40	bone-in	MAP	1	NA	NA	NA	NA	NA	6	6	5.83	6.28	1	36
FS11-0333	7/04/2011 11:30	1	9200000	9400000	bone-in	VAC	1	0	4	8	6	8	6	6	6.4	6.35	1	36
FS11-0334	7/04/2011 11:30	2	52000	27000	bone-in	VAC	2	8	6	4	6	8	6	8	6.31	6.03	1	36
FS11-0335	7/04/2011 11:30	3	130000	54000	bone-in	VAC	3	8	6	6	6	8	8	NA	5.91	6.26	1	36
FS11-0336	7/04/2011 11:30	4	3500000	2400000	bone-in	VAC	4	8	6	4	6	8	8	NA	6.14	6.24	1	36

labnum	tested	rep	tvc	lab	product	packaging	pack	vac.res.gas	seal	appearance	odour.init	odour.2min	colour.2min	colour.5min	ph	ph.cut	use	age
FS11-0337	7/04/2011 11:30	1	4100000	2900000	bone-out	VAC	1	8	8	8	6	8	8	NA	6	6.15	1	36
FS11-0338	7/04/2011 11:30	2	1600	1200	bone-out	VAC	2	8	8	4	6	8	8	NA	5.85	5.85	1	36
FS11-0339	7/04/2011 11:30	3	33000	58000	bone-out	VAC	3	8	8	6	6	8	8	NA	5.93	6.06	1	36
FS11-0348	13/04/2011 13:30	1	170000	94000	bone-in	VAC	1	8	6	5	6	8	8	NA	5.87	5.9	1	42
FS11-0349	13/04/2011 13:30	2	970000	1100000	bone-in	VAC	2	8	6	5	6	8	8	NA	6.16	6.07	1	42
FS11-0350	13/04/2011 13:30	3	5500000	6800000	bone-in	VAC	3	8	6	6	6	8	8	NA	6.35	5.96	1	42
FS11-0351	13/04/2011 13:30	4	110000	100000	bone-in	VAC	4	8	6	5	6	8	8	NA	6.09	5.95	1	42
FS11-0352	13/04/2011 13:30	1	2700	200	bone-out	VAC	1	8	8	8	6	8	8	NA	6.01	5.82	1	42
FS11-0353	13/04/2011 13:30	2	700000	760000	bone-out	VAC	2	8	8	4	6	8	8	NA	5.78	5.78	1	42
FS11-0354	13/04/2011 13:30	3	1400000	1300000	bone-out	VAC	3	8	8	3	6	8	8	NA	5.95	5.92	1	42
FS11-0419	21/04/2011 13:30	1	200	380	bone-in	MAP	1	2	8	2	6	8	8	NA	5.82	5.91	1	50
FS11-0420	21/04/2011 13:30	2	500	20	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	6.06	6.18	1	50
FS11-0421	21/04/2011 13:30	3	2700	1100	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	5.98	6.18	1	50
FS11-0422	21/04/2011 13:30	4	200	480	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	6.2	5.91	1	50
FS11-0423	21/04/2011 13:30	1	23000	20000	bone-out	MAP	1	4	8	4	6	8	8	NA	5.82	5.71	1	50
FS11-0424	21/04/2011 13:30	2	14000	11000	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.69	5.75	1	50
FS11-0425	21/04/2011 13:30	3	2900	400	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.84	5.69	1	50
FS11-0426	21/04/2011 13:30	4	27000	24000	bone-out	MAP	1	NA	NA	NA	NA	8	8	NA	5.92	5.85	1	50
FS11-0427	21/04/2011 13:30	1	2100000	1200000	bone-in	VAC	1	8	6	6	6	8	8	NA	6.43	6.66	1	50
FS11-0428	21/04/2011 13:30	2	190000	6200	bone-in	VAC	2	8	6	4	6	8	8	NA	6.21	5.95	1	50
FS11-0429	21/04/2011 13:30	3	86000000	62000000	bone-in	VAC	3	8	6	4	4	8	8	NA	6.34	6.19	1	50
FS11-0430	21/04/2011 13:30	4	17000000	86000000	bone-in	VAC	4	8	6	6	6	8	8	NA	5.92	5.91	1	50
FS11-0431	21/04/2011 13:30	1	37000000	34000000	bone-out	VAC	1	8	8	4	6	8	8	NA	6.02	5.94	1	50
FS11-0432	21/04/2011 13:30	2	7800000	4200000	bone-out	VAC	2	8	8	6	5	8	8	NA	5.99	6.13	1	50
FS11-0433	21/04/2011 13:30	3	9800000	8800000	bone-out	VAC	3	8	8	4	5	8	8	NA	5.82	5.74	1	50
FS11-0444	28/04/2011 12:30	1	29000	15000	bone-out	MAP	1	4	8	4	4	8	8	NA	5.8	5.75	1	57
FS11-0445	28/04/2011 12:30	2	760	100	bone-out	MAP	1	NA	NA	NA	NA	NA	8	NA	5.79	5.87	1	57

labnum	tested	rep	tvc	lab	product	packaging	pack	vac.res.gas	seal	appearance	odour.init	odour.2min	colour.2min	colour.5min	ph	ph.cut	use	age
FS11-0446	28/04/2011 12:30	3	1100	20	bone-out	MAP	1	NA	NA	NA	NA	NA	8	NA	5.78	5.819	1	57
FS11-0447	28/04/2011 12:30	4	590	20	bone-out	MAP	1	NA	NA	NA	NA	NA	8	NA	5.84	5.98	1	57
FS11-0448	28/04/2011 12:30	1	5800000	2600000	bone-in	VAC	1	8	8	2	6	6	8	NA	6.03	5.9	1	57
FS11-0449	28/04/2011 12:30	2	5400000	6600000	bone-in	VAC	2	8	8	2	6	8	8	NA	6.09	5.99	1	57
FS11-0450	28/04/2011 12:30	3	4400000	5800000	bone-in	VAC	3	8	8	2	6	8	8	NA	6.46	6.28	1	57
FS11-0451	28/04/2011 12:30	4	260000	120000	bone-in	VAC	4	8	8	2	6	8	8	NA	6.19	6.36	1	57
FS11-0452	28/04/2011 12:30	1	37000000	56000000	bone-out	VAC	1	8	8	2	6	8	8	NA	5.93	6	1	57
FS11-0453	28/04/2011 12:30	2	720000	620000	bone-out	VAC	2	8	8	4	6	8	8	NA	5.9	6.03	1	57
FS11-0535	5/05/2011 13:30	1	2300	760	bone-in	MAP	1	2	8	4	8	8	8	NA	6.18	6.02	1	64
FS11-0536	5/05/2011 13:30	2	3400	2600	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	6.04	6	1	64
FS11-0537	5/05/2011 13:30	3	4000	50000	bone-in	MAP	1	NA	NA	NA	NA	4	8	NA	6.14	5.83	1	64
FS11-0538	5/05/2011 13:30	4	300	100	bone-in	MAP	1	NA	NA	NA	NA	8	8	NA	6.24	6.09	1	64
FS11-0539	5/05/2011 13:30	1	3400000	3200000	bone-in	VAC	1	8	6	4	6	6	8	NA	5.87	6.44	1	64
FS11-0540	5/05/2011 13:30	2	12000000	3800000	bone-in	VAC	2	8	6	4	8	8	8	NA	6.22	6.14	1	64
FS11-0541	5/05/2011 13:30	3	6000000	3400000	bone-in	VAC	3	8	6	4	8	8	8	NA	6.14	5.99	1	64
FS11-0542	5/05/2011 13:30	4	15000000	12000000	bone-in	VAC	4	8	6	4	6	8	8	NA	6.11	5.97	1	64
FS11-0543	5/05/2011 13:30	1	13000000	32000000	bone-out	VAC	1	8	8	8	8	8	8	NA	5.82	6.1	1	64
FS11-0544	5/05/2011 13:30	2	12000	160000	bone-out	VAC	2	8	8	4	6	8	8	NA	5.82	5.97	1	64
FS11-0567	12/05/2011 10:30	1	9000	9600	bone-out	MAP	1	6	8	2	6	8	6	7	5.91	5.99	1	71
FS11-0568	12/05/2011 10:30	2	580	40	bone-out	MAP	1	NA	NA	NA	NA	8	7	7	5.91	5.94	1	71
FS11-0569	12/05/2011 10:30	3	4000	2700	bone-out	MAP	1	NA	NA	NA	NA	8	6	7	6	5.95	1	71
FS11-0570	12/05/2011 10:30	4	630000	580000	bone-out	MAP	1	NA	NA	NA	NA	8	7	NA	5.77	5.85	1	71
FS11-0571	12/05/2011 10:30	1	4700000	160000	bone-in	VAC	1	8	6	4	6	7	6	7	6.09	5.93	1	71
FS11-0572	12/05/2011 10:30	2	1600000	16000	bone-in	VAC	2	8	6	4	6	8	8	NA	6.12	5.89	1	71
FS11-0573	12/05/2011 10:30	3	6000000	6000000	bone-in	VAC	3	6	6	4	6	8	8	NA	5.91	5.91	1	71
FS11-0574	12/05/2011 10:30	4	11000000	9000000	bone-in	VAC	4	8	8	4	6	8	8	NA	6.21	5.95	1	71
FS11-0575	12/05/2011 10:30	1	32000000	34000000	bone-out	VAC	1	8	8	4	7	7	8	NA	5.76	5.96	1	71

labnum	tested	rep	tvc	lab	product	packaging	pack	vac.res.gas	seal	appearance	odour.init	odour.2min	colour.2min	colour.5min	ph	ph.cut	use	age
FS11-0576	12/05/2011 10:30	2	32000000	52000000	bone-out	VAC	2	8	8	2	7	8	6	7	5.65	5.84	1	71
FS11-0603	19/05/2011 10:30	1	560	40	bone-in	MAP	1	2	8	4	8	8	6	6	5.99	6.12	1	78
FS11-0604	19/05/2011 10:30	2	11000	10000	bone-in	MAP	1	NA	NA	NA	NA	8	6	6	6.04	5.94	1	78
FS11-0605	19/05/2011 10:30	3	430000	480000	bone-in	MAP	1	NA	NA	NA	NA	8	6	6	6.08	5.99	1	78
FS11-0606	19/05/2011 10:30	4	6200	5000	bone-in	MAP	1	NA	NA	NA	NA	8	6	6	5.97	6.26	1	78
FS11-0607	19/05/2011 10:30	1	42000000	42000000	bone-in	VAC	1	8	6	4	6	8	8	NA	5.83	6.16	1	78
FS11-0608	19/05/2011 10:30	2	5600000	4600000	bone-in	VAC	2	8	6	4	6	8	6	6	6.1	5.88	1	78
FS11-0609	19/05/2011 10:30	3	77000000	58000000	bone-in	VAC	3	8	6	4	4	8	6	6	6.26	6.16	1	78
FS11-0610	19/05/2011 10:30	4	30000000	38000000	bone-in	VAC	4	8	6	4	6	8	6	7	5.95	6.07	1	78
FS11-0611	19/05/2011 10:30	1	29000000	34000000	bone-out	VAC	1	8	8	4	8	8	6	6	5.88	5.96	1	78
FS11-0612	19/05/2011 10:30	2	53000000	270000000	bone-out	VAC	2	8	8	3	6	8	6	6	5.75	5.79	1	78
FS11-0626	26/05/2011 11:30	1	520	660000	bone-out	MAP	1	8	8	3	4	8	6	8	5.98	5.97	1	85
FS11-0627	26/05/2011 11:30	2	620000	860000	bone-out	MAP	1	NA	NA	NA	NA	8	6	6	5.79	5.73	1	85
FS11-0628	26/05/2011 11:30	3	670	20	bone-out	MAP	1	NA	NA	NA	NA	8	6	6	5.87	5.83	1	85
FS11-0629	26/05/2011 11:30	4	9800	9200	bone-out	MAP	1	NA	NA	NA	NA	8	6	6	5.7	5.76	1	85
FS11-0630	26/05/2011 11:30	1	110000000	92000000	bone-in	VAC	1	8	8	4	6	8	6	6	6.34	6.45	1	85
FS11-0631	26/05/2011 11:30	2	85000000	28000000	bone-in	VAC	2	8	8	4	6	8	6	6	6.14	6.14	1	85
FS11-0632	26/05/2011 11:30	3	26000000	14000000	bone-in	VAC	3	8	8	6	6	8	6	6	6.07	6.09	1	85
FS11-0633	26/05/2011 11:30	4	57000000	34000000	bone-in	VAC	4	8	8	3	6	8	6	6	6.22	6.22	1	85
FS11-0634	26/05/2011 11:30	1	110000000	84000000	bone-out	VAC	1	8	8	4	4	8	6	6	5.72	5.76	1	85
FS11-0635	26/05/2011 11:30	2	64000000	38000000	bone-out	VAC	2	8	8	2	6	8	6	6	5.72	5.91	1	85

Appendix 4: Statistical Analysis

```
## ------------------------------------------------------------------------
## Required Packages:
## ------------------------------------------------------------------------
require(car)
require(nlme)
require(nlstools)

## Model formulations for fitting growth curves
## A=asymptote and C=constant
## gompertz.zw uses the parameterisation of Zwietering et al 1990, but slightly
## better to tease out the asymptote and constant as per nlstools/ Toldra (2009)
gompertz.zw <-
  deriv(~ C + (A-C)*exp(-exp(mu*exp(1)*(lam - x)/((A-C)*log(10)) + 1)),
        c("C","A","mu","lam"), function(x, C, A, mu, lam){})

## Baranyi model based on the corresponding function in nlstools/Toldra (2009)
baranyi.m <-
  deriv(~ A + log((-1 + exp(mu * lam) + exp(mu * x))/
                    (exp(mu * x) - 1 + exp(mu * lam) * 10^(A - C)), base=10),
        c("C","A","mu","lam"), function(x, C, A, mu, lam){} )

## ------------------------------------------------------------------------
## Data Import and manipulation:
## ------------------------------------------------------------------------
## Import the sensory data
sensory <- read.csv("../data/sensory.csv",header=T, as.is=TRUE)
sensory <- transform(sensory,
                     date=as.Date(date, "%d/%m/%Y"),
                     product=factor(product),
                     packaging=factor(packaging))

## Import the micro data
micro <- read.csv("../data/micro.csv",header=T, as.is=TRUE)
micro$lab[micro$lab=="<200"]=200
micro$lab[micro$lab=="<20"]=20
micro <- transform(micro,
                   date.collected=as.Date(date.collected, "%d/%m/%Y"),
                   ## collected=as.Date(collected, "%d/%m/%Y %H:%M"),
                   date.received=as.Date(date.received, "%d/%m/%Y"),
                   ## received=as.Date(received, "%d/%m/%Y %H:%M"),
                   date.tested=as.Date(date.tested, "%d/%m/%Y"),
                   ## tested=as.Date(tested, "%d/%m/%Y %H:%M"),
                   lab=as.numeric(lab),
                   details5=NULL)
micro$age <- as.numeric(micro$date.tested - as.Date("2011-03-02"))
micro$date.collected <- NULL
micro$time.collected <- NULL
micro$date.received <- NULL
micro$time.received <- NULL
micro$date.tested <- NULL
micro$time.tested <- NULL

## merge the sensory and micro data by laboratory number
lamb <- merge(micro,sensory,by=c("labnum"),all.x=TRUE)
lamb$date <- NULL
lamb$age <- as.numeric(lamb$age.x)
lamb$age.x <- NULL
lamb$age.y <- NULL
lamb.all <- lamb
lamb.all$collected <- NULL
lamb.all$received <- NULL
lamb.all$details3 <- NULL
lamb.all$details4 <- NULL
lamb.all$comment.x <- NULL
lamb.all$comment.y <- NULL

## Restrict data to those packs where nothing went wrong (use=1), i.e. no holes
lamb.all <- subset(lamb.all, use==1)
write.csv(lamb.all, file="../data/lamb_all.csv", row.names=FALSE)

## Some intergrity checks - these should all come back with 0 rows - they do
subset(lamb, packaging=="VAC" & substring(lamb$details4, 1, 1) != "V")
subset(lamb, packaging=="MAP" & substring(lamb$details4, 1, 1) != "M")
subset(lamb, product=="bone-in" & substring(lamb$details4, 3, 3) != "I")
subset(lamb, product=="bone-out" & substring(lamb$details4, 3, 3) != "O")
```

```
## How many observations did we have per time point and product type
with(lamb.all, table(product, packaging))
with(lamb.all, table(age, product, packaging))

## import previous micro data (from the second trial)
micro2 <- read.csv("../data/micro2.csv", header=TRUE, as.is=TRUE)
micro2$collect.date <- NULL
micro2$collect.time <- NULL
micro2$collect <- NULL
micro2$test.date <- NULL
micro2$test.time <- NULL

## Keep only the data on pieces - these were collected from whole shoulders
## after opening, whereas slices were packed in overwrap trays and tested to
## assess retail storage. Also average over the repeat analytical samples as
## part of the current trial we only have a single analytical sample from each
## shoulder.
micro2.pieces <- subset(micro2, sample.type=="pieces")
micro2.pieces$sample.type <- NULL
micro2.pieces$storage <- NULL
micro2.pieces <- transform(micro2.pieces,
                       sample=factor(sample, levels=c("gold", "white", "silver","orange")))
micro2.sum <-  aggregate(apc ~ sample + age, data=micro2.pieces,
                       FUN=function(el){10^mean(log10(el))})
micro2.sum <-  cbind(micro2.sum,
                     lab=aggregate(lab ~ sample + age, data=micro2.pieces,
                       FUN=function(el){10^mean(log10(el))})$lab)

## Combine Trial 2 & 3 data for Vacuum packed bone-out product to compare them.
## trial2 <- subset(micro, details4=="VBO")[,c("age","tvc","lab")]
trial2 <- subset(lamb.all, packaging=="VAC" & product=="bone-out")[,c("age","tvc","lab")]
trial3 <- micro2.sum[,c("age","apc","lab")]
names(trial3)[2] <- "tvc"
trials23 <- data.frame(trial=factor(rep(c(3,2), c(nrow(trial3), nrow(x=trial2)))),
                  rbind(trial3, trial2))

## ------------------------------------------------------------------------
## Sensory Analysis:
## ------------------------------------------------------------------------
## Summary tables of the median for each sensory characteristic
with(lamb.all, tapply(vac.res.gas, list(age,product,packaging), median, na.rm=TRUE))
with(lamb.all, tapply(seal, list(age,product,packaging), median, na.rm=TRUE))
with(lamb.all, tapply(appearance, list(age,product,packaging), median, na.rm=TRUE))
with(lamb.all, tapply(odour.init, list(age,product,packaging), median, na.rm=TRUE))
with(lamb.all, tapply(odour.2min, list(age,product,packaging), median, na.rm=TRUE))
with(lamb.all, tapply(colour.2min, list(age,product,packaging), median, na.rm=TRUE))

## How big were the changes in colour after 5 minutes, when colour after two
## minutes wasn't 7 or 8.
table(with(subset(lamb.all, !is.na(colour.5min)), colour.5min-colour.2min))

## ------------------------------------------------------------------------
## pH Analysis:
## ------------------------------------------------------------------------
## Plot pH of surfaces prior to sampling
ph.xyplot <-
  xyplot(ph ~ age|product*packaging, data=lamb.all,
         type=c("p"), col="black",
         ylim=c(5.4, 7),
         layout=c(2,2), as.table=TRUE,
         ylab="pH", xlab="Storage Time (Days)",
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
           panel.loess(x,y, span=0.5, col="red")
         })
win.metafile(filename="../graphics/pH_xyplot.wmf", width=5, height=5)
print(ph.xyplot)
dev.off()
```

```
## Plot pH of freshly exposed surfaces (after sampling for micro)
ph.xyplot.cut <-
  xyplot(ph.cut ~ age|product*packaging, data=lamb.all,
         type=c("p"), col="black",
         ylim=c(5.4, 7),
         layout=c(2,2), as.table=TRUE,
         ylab="pH (cut surface)", xlab="Storage Time (Days)",
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
           panel.loess(x,y, span=0.5, col="red")
         })
win.metafile(filename="../graphics/pH_xyplot_cut.wmf", width=5, height=5)
print(ph.xyplot.cut)
dev.off()

## Ignoring the changes over time, does the pH differ between the four product
## types?
aov.ph1 <- aov(ph ~ product*packaging, data=lamb.all)
Anova(aov.ph1) ## Interaction not significant - remove
aov.ph2 <- update(aov.ph1, .~. - product:packaging)
Anova(aov.ph2)

## Despite the changes in pH over time the diagnostic plots look good.
plot(aov.ph2)
qqPlot(resid(aov.ph1))
qqPlot(resid(aov.ph2))
model.tables(aov.ph1, type="means")
model.tables(aov.ph2, type="means")

## ------------------------------------------------------------------------
## Graphics: TVC
## ------------------------------------------------------------------------
TVC.xyplot <-
  xyplot(log10(tvc)~ age|product*packaging, data=lamb.all,
         type=c("p"), col="black",
         layout=c(2,2), as.table=TRUE,
         xlab="Storage Time (Days)",
         ylab=expression(paste("log"[10]," TVC (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
         })
win.metafile(file="../graphics/TVC_xyplot.wmf", width=5, height=5)
print(TVC.xyplot)
dev.off()

TVC.xyplot2 <-
  xyplot(log10(tvc)~ age|packaging, groups=product, data=lamb.all,
         type=c("p"), col=c("black", "red"), pch=c(1,19),
         layout=c(1,2), as.table=TRUE,
         xlab="Storage Time (Days)",
         ylab=expression(paste("log"[10]," TVC (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
           panel.loess(x,y,span=0.5, lty=2, ...)
         })
win.metafile(file="../graphics/TVC_xyplot2.wmf", width=5, height=5)
print(TVC.xyplot2)
dev.off()

## ------------------------------------------------------------------------
## Graphics: LAB
## ------------------------------------------------------------------------
LAB.xyplot <-
  xyplot(log10(lab)~ age|product*packaging, data=lamb.all,
         type=c("p"), col="black",
         layout=c(2,2), as.table=TRUE,
         xlab="Storage Time (Days)",
         ylab=expression(paste("log"[10]," LAB (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
         })
```

```
win.metafile(file="../graphics/LAB_xyplot.wmf", width=5, height=5)
print(LAB.xyplot)
dev.off()

LAB.xyplot2 <-
  xyplot(log10(lab)~ age|packaging, groups=product, data=lamb.all,
         type=c("p"), col=c("black", "red"), pch=c(1,19),
         layout=c(1,2), as.table=TRUE,
         xlab="Storage Time (Days)",
         ylab=expression(paste("log"[10]," LAB (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
           panel.loess(x,y,span=0.5, lty=2, ...)
         })
win.metafile(file="../graphics/LAB_xyplot2.wmf", width=5, height=5)
print(LAB.xyplot2)
dev.off()

## ------------------------------------------------------------------------
## Compare the results between the two trials
TVC.xyplot.trials <-
  xyplot(log10(tvc)~ age, groups=trial, data=trials23,
         col=c("black", "red"), pch=c(1,19),
         xlab="Storage Time (Days)",
         ylab=expression(paste("log"[10]," TVC (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
         })
win.metafile(file="../graphics/TVC_xyplot_trials.wmf", width=5, height=4)
print(TVC.xyplot.trials)
dev.off()

LAB.xyplot.trials <-
  xyplot(log10(lab)~ age, groups=trial, data=trials23,
         col=c("black", "red"), pch=c(1,19),
         xlab="Storage Time (Days)",
         ylab=expression(paste("log"[10]," LAB (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
         })
win.metafile(file="../graphics/LAB_xyplot_trials.wmf", width=5, height=4)
print(LAB.xyplot.trials)
dev.off()

## ------------------------------------------------------------------------
## Analysis: TVC in Vacuum packed product
## ------------------------------------------------------------------------
## Use the code from nlstools to try and find initial values that might be close
## to what we need. This should give good starting points
gc.vac <- subset(lamb.all, packaging=="VAC")[,c("tvc","age")]
names(gc.vac) <- c("LOG10N","t")
gc.vac[,1] <- log10(gc.vac[,1])
preview(formula=gompertzm, data=gc.vac, variable=2,
        start=list(LOG10N0=2, LOG10Nmax=8, mumax=0.3, lag=10))
test <- nls(gompertzm, data=gc.vac,
            start=list(LOG10N0=2, LOG10Nmax=8, mumax=0.3, lag=10))
plotfit(test, smoot=TRUE)

## ------------------------------------------------------------------------
## Fit the Gompertz model
## Start off with a single model for both bone-in and bone-out
nlm.vac.tvc1 <- nls(log10(tvc) ~ gompertz.zw(age, C, A, mu, lam),
                start=list(C=3.5, A=8, mu=0.3, lam=20),
                data=subset(lamb.all, packaging=="VAC"), trace=TRUE)
summary(nlm.vac.tvc1)
plot(nlm.vac.tvc1)          ## A couple of low points ... which we know
qqPlot(resid(nlm.vac.tvc1)) ## Not too bad at all.

## Now allow for separate models for bone-in and bone-out
nlm.vac.tvc1a <- gnls(log10(tvc) ~ gompertz.zw(age, C, A, mu, lam),
                  start=list(C=c(3.5,3.5), A=c(8,8), mu=c(0.2,0.2), lam=c(10,10)),
                  param=list(C ~ product - 1, A ~ product - 1,
                    mu ~ product - 1, lam ~ product - 1),
                  data=subset(lamb.all, packaging=="VAC"))
```

```
## And test if there is a significant difference bewteen the two of them using
## the likelihood ratio test. The answer is no there isn't. So stick with a
## single model for bone-in and bone-out.
anova(nlm.vac.tvc1a, nlm.vac.tvc1)

## ------------------------------------------------------------------------
## Fit the baranyi model
## Start off with a single model for both bone-in and bone-out
nlm.vac.tvc2 <- nls(log10(tvc) ~ baranyi.m(age, C, A, mu, lam),
                start=list(C=3.35, A=7.66, mu=0.15, lam=8),
                data=subset(lamb.all, packaging=="VAC"), trace=TRUE)
summary(nlm.vac.tvc2)
plot(nlm.vac.tvc1)
qqPlot(resid(nlm.vac.tvc2)) ## Not too bad at all.

## Then try it using separate models for bone-in and bone-out
nlm.vac.tvc2a <- gnls(log10(tvc) ~ baranyi.m(age, C, A, mu, lam),
                  start=list(C=c(3.35, 3.35), A=c(7.66, 7.66), mu=c(0.15,0.15), lam=c(8,8)),
                  param=list(C ~ product - 1, A ~ product - 1,
                    mu ~ product - 1, lam ~ product - 1),
                  data=subset(lamb.all, packaging=="VAC"))

## And test if there is a significant difference bewteen the two of them using
## the likelihood ratio test. The answer is no there isn't. So stick with a
## single model.
anova(nlm.vac.tvc2a, nlm.vac.tvc2)

## And overall the Gompertz model fits slightly better, but let's plot both.
TVC.xyplot.vac.fit <-
  xyplot(log10(tvc)~ age, data=subset(lamb.all, packaging=="VAC"),
         col="black", pch=1,
         xlab="Storage Time (Days)",
         ylim=c(1.5,8.5),
         ylab=expression(paste("log"[10]," TVC (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
           x <- seq(0,85, by=0.2)
           y1 <- predict(nlm.vac.tvc1, newdata=data.frame(age=x))
           panel.lines(x, y1, col="black", lty=2)
           y2 <- predict(nlm.vac.tvc2, newdata=data.frame(age=x))
           panel.lines(x, y2, col="red", lty=3)
         })
win.metafile(file="../graphics/TVC_xyplot_vac_fit.wmf", width=5, height=4)
print(TVC.xyplot.vac.fit)
dev.off()

## ------------------------------------------------------------------------
## Analysis: TVC in MAP product
## ------------------------------------------------------------------------
## MAP product was four shoulders (=samples) per pack. So we average them to fit
## the growth curves.
map.tvc <-  aggregate(tvc ~ product + age, data=lamb.all, subset=packaging=="MAP",
                      FUN=function(el){10^mean(log10(el))})

## What does it look like?
xyplot(log10(tvc) ~ age, data=map.tvc)

## Use the code from nlstools to try and find initial values that might be close
## to what we need. This should give good starting points
gc.map <- map.tvc[,c(3,2)]
names(gc.map) <- c("LOG10N","t")
gc.map[,1] <- log10(gc.map[,1])

preview(formula=gompertzm, data=gc.map, variable=2,
        start=list(LOG10N0=3.2, LOG10Nmax=3.7, mumax=0.05, lag=30))
test <- nls(gompertzm, data=gc.map,
            start=list(LOG10N0=3.2, LOG10Nmax=3.7, mumax=0.05, lag=30), trace=TRUE)
## Doesn't work numerically ... the lag looks like it's going past the end.

preview(formula=baranyi, data=gc.map, variable=2,
        start=list(LOG10N0=3.2, LOG10Nmax=3.7, mumax=0.05, lag=30))
test <- nls(baranyi, data=gc.map,
            start=list(LOG10N0=3.2, LOG10Nmax=3.7, mumax=0.05, lag=30), trace=TRUE)
## Doesn't work numerically ... the lag looks like it's going past the end.
```

```
TVC.xyplot.map.fit <-
  xyplot(log10(tvc)~ age, data=map.tvc,
         col="black", pch=1,
         xlab="Storage Time (Days)",
         ylim=c(1.5,8.5),
         ylab=expression(paste("log"[10],"  TVC (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
         })
win.metafile(file="../graphics/TVC_xyplot_map_fit.wmf", width=5, height=4)
print(TVC.xyplot.map.fit)
dev.off()

## ------------------------------------------------------------------------
## Analysis: LAB in Vacuum packed product
## ------------------------------------------------------------------------
## Use the code from nlstools to try and find initial values that might be close
## to what we need. This should give good starting points
gc.vac <- subset(lamb.all, packaging=="VAC")[,c("lab","age")]
names(gc.vac) <- c("LOG10N","t")
gc.vac[,1] <- log10(gc.vac[,1])
preview(formula=gompertzm, data=gc.vac, variable=2,
        start=list(LOG10N0=2, LOG10Nmax=8, mumax=0.3, lag=7))
test <- nls(gompertzm, data=gc.vac,
            start=list(LOG10N0=2, LOG10Nmax=8, mumax=0.3, lag=10))
plotfit(test, smooth=TRUE)

## ------------------------------------------------------------------------
## Fit the Gompertz model
## Start off with a single model for both bone-in and bone-out
nlm.vac.lab1 <- nls(log10(lab) ~ gompertz.zw(age, C, A, mu, lam),
                start=list(C=2, A=7.5, mu=0.23, lam=7),
                data=subset(lamb.all, packaging=="VAC"), trace=TRUE)
summary(nlm.vac.lab1)
plot(nlm.vac.lab1)          ## Again three points that are low.
qqPlot(resid(nlm.vac.lab1)) ## Pretty good.

## Now allow for separate models for bone-in and bone-out
nlm.vac.lab1a <- gnls(log10(lab) ~ gompertz.zw(age, C, A, mu, lam),
                  start=list(C=c(2,2), A=c(7.6,7.6), mu=c(0.23,0.23), lam=c(7,7)),
                  param=list(C ~ product - 1, A ~ product - 1,
                    mu ~ product - 1, lam ~ product - 1),
                  data=subset(lamb.all, packaging=="VAC"))

## And test if there is a significant difference bewteen the two of them using
## the likelihood ratio test. The answer is no there isn't. So stick with a
## single model.
anova(nlm.vac.lab1a, nlm.vac.lab1)

## ------------------------------------------------------------------------
## Fit the baranyi model
## Start off with a single model for both bone-in and bone-out
nlm.vac.lab2 <- nls(log10(lab) ~ baranyi.m(age, C, A, mu, lam),
                start=list(C=2, A=7.5, mu=0.23, lam=7),
                data=subset(lamb.all, packaging=="VAC"), trace=TRUE)
summary(nlm.vac.lab2)
plot(nlm.vac.lab1)
qqPlot(resid(nlm.vac.lab2)) ## Also not bad. Some points outside.

## Then try it using separate models for bone-in and bone-out
nlm.vac.lab2a <- gnls(log10(lab) ~ baranyi.m(age, C, A, mu, lam),
                  start=list(C=c(2,2), A=c(7.6,7.6), mu=c(0.23,0.23), lam=c(7,7)),
                  param=list(C ~ product - 1, A ~ product - 1,
                    mu ~ product - 1, lam ~ product - 1),
                  data=subset(lamb.all, packaging=="VAC"))

## And test if there is a significant difference bewteen the two of them using
## the likelihood ratio test. The answer is no there isn't. So stick with a
## single model.
anova(nlm.vac.lab2a, nlm.vac.lab2)
```

```
LAB.xyplot.vac.fit <-
  xyplot(log10(lab)~ age, data=subset(lamb.all, packaging=="VAC"),
         col=c("black"), pch=1, ylim=c(1.5,8.5),
         xlab="Storage Time (Days)", ylab=expression(paste("log"[10],"  LAB (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
           x <- seq(0,85, by=0.2)
           y1 <- predict(nlm.vac.lab1, newdata=data.frame(age=x))
           panel.lines(x, y1, col="black", lty=2)
           y2 <- predict(nlm.vac.lab2, newdata=data.frame(age=x))
           panel.lines(x, y2, col="red", lty=3)
         })
win.metafile(file="../graphics/LAB_xyplot_vac_fit.wmf", width=5, height=4)
print(LAB.xyplot.vac.fit)
dev.off()

## ------------------------------------------------------------------------
## Analysis: LAB in MAP product
## ------------------------------------------------------------------------
map.lab <-  aggregate(lab ~ product + age, data=lamb.all, subset=packaging=="MAP",
                      FUN=function(el){10^mean(log10(el))})
xyplot(log10(lab) ~ age, data=map.lab)

## Use the code from nlstools to try and find initial values that might be close
## to what we need. This should give good starting points
gc.map <- map.lab[,c(3,2)]
names(gc.map) <- c("LOG10N","t")
gc.map[,1] <- log10(gc.map[,1])

preview(formula=gompertzm, data=gc.map, variable=2,
        start=list(LOG10N0=2, LOG10Nmax=4, mumax=0.1, lag=30))
test <- nls(gompertzm, data=gc.map,
            start=list(LOG10N0=2, LOG10Nmax=4, mumax=0.1, lag=30), trace=TRUE)
plotfit(test, smooth=TRUE)

preview(formula=baranyi, data=gc.map, variable=2,
        start=list(LOG10N0=2, LOG10Nmax=5, mumax=0.1, lag=37))
test <- nls(baranyi, data=gc.map,
            start=list(LOG10N0=2, LOG10Nmax=5, mumax=0.1, lag=37), trace=TRUE)
## Doesn't work numerically. The asymptote and the lag seems to be going off.

## ------------------------------------------------------------------------
## Fit the Gompertz model
## Start off with a single model for both bone-in and bone-out
nlm.map.lab1 <- nls(log10(lab) ~ gompertz.zw(age, C, A, mu, lam),
                start=list(C=2, A=5.8, mu=0.1, lam=37),
                data=map.lab, trace=TRUE)
summary(nlm.map.lab1)
plot(nlm.map.lab1)          ## Hard to tell given the few data points
qqPlot(resid(nlm.map.lab1)) ## Not great - there are some extreme points.

## Now allow for separate models for bone-in and bone-out
nlm.map.lab1a <- gnls(log10(lab) ~ gompertz.zw(age, C, A, mu, lam),
                  start=list(C=c(2,2), A=c(5.8,5.8), mu=c(0.1,0.1), lam=c(37,37)),
                  param=list(C ~ product - 1, A ~ product - 1,
                    mu ~ product - 1, lam ~ product - 1),
                  data=map.lab)

## And test if there is a significant difference bewteen the two of them using
## the likelihood ratio test. The answer is no there isn't. So stick with a
## single model.
anova(nlm.map.lab1a, nlm.map.lab1)

LAB.xyplot.map.fit <-
  xyplot(log10(lab)~ age, data=map.lab,
         col="black", pch=1, ylim=c(1.5,8.5),
         xlab="Storage Time (Days)", ylab=expression(paste("log"[10],"  LAB (cfu/cm"^2,")")),
         scales=list(alternating=FALSE),
         panel=function(x,y,...){
           panel.grid(h=-1,v=-1)
           panel.xyplot(x,y,...)
           x <- seq(0,85, by=0.2)
           y1 <- predict(nlm.map.lab1, newdata=data.frame(age=x))
           panel.lines(x, y1, col="black", lty=2)
         })
win.metafile(file="../graphics/LAB_xyplot_map_fit.wmf", width=5, height=4)
print(LAB.xyplot.map.fit)
dev.off()
```