final report

Project code: A.MFS.0147

Prepared by: Mark L Tamplin
University of Tasmania

Date submitted: October 2009

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Ensuring the quality of exported meat primals using a predictive tool for specific spoilage organisms

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

Contents

1 Background

The export of Australian beef contributes over $4 billion per annum to the Australian economy (ABARE, 2007). To maintain the integrity of this very important commodity, exporters require confidence that when a product arrives at a destination, it is of high quality with the desired shelf-life. Refrigeration can extend product shelf-life by lowering the rate of microbial growth, and consequently spoilage. In addition, the growth of aerobic microorganisms, such as pseudomonads, can be markedly reduced by vacuum-packaging the product.

Primal cuts of Australian beef are generally vacuum packaged and stored at temperatures between -1 and 5°C for export. When exported to the United States of America (USA) the total time for shipping and storage can be in excess of 75 days, yet there is insufficient evidence to explain the basis of this desired effect. To increase confidence in the microbiological quality of meat stored for extended periods of time, it is necessary to understand changes in the dominant microflora of beef, the organisms that contribute to spoilage, and to convert these data into an industry tool (ie model).

The microflora of fresh beef is complex and varied, depending on the condition of the animal at slaughter, contamination during slaughter and processing, and the factors (including temperature and time) during storage and distribution (Nottingham, 1982). The microflora of meat plays an important role in the shelf-life of fresh meat (Nychas & Skandamis, 2005). The storage method and temperature impose selective advantages for some organisms and result in changes in community dynamics with time. Organisms which spoil aerobically packaged fresh meat include *Brochothrix thermosphacta*, *Carnobacterium* spp., Enterobacteriaceae, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens* (Borch *et al.*, 1996). When vacuum packaging is used for storage of fresh meat, oxygen is limited and the microflora are dominated by obligate and facultative anaerobic organisms such as *Lactobacillus* spp., *Leuconostoc* spp., *Carnobacterium* spp., and *Brochothrix thermosphacta* (Fontana *et al.*, 2006). Chilling of the meat results in a greater proportion of psychrotrophic bacteria (Richardson, 2001).

Many studies of vacuum packaging and storage of beef primals used culture-dependent methods to identify species and track changes in the microbial flora (Lee & Yoon, 2001; Nissen *et al.*, 1996; Sakala *et al.*, 2002). Complex microbial communities such as those associated with meat contain organisms which are adapted to environments that precede the packing step. Consequently, different species may growth in this new environment and may not be readily cultured (Fleet, 1999). Analysis of such communities may not be feasible considering the range of necessary bacteriological methods and equipment.

Methods based on extraction and amplification of bacterial RNA/DNA are not dependent upon the viability and/or the success of culturing. Terminal-Restriction Fragment Length Polymorphism (t-RFLP) is a method which produces a profile of the bacterial community through known sequences and the sizes of 16s rDNA fragments. As of this study, we were not aware of studies utilising t-RFLP to monitor microbial communities of beef, although it has been reported to for yogurt, cheese and fish (Rademaker *et al.*, 2006; Smith *et al.*, 2007). Some molecular-based studies on beef and other meats have used denaturing gradient gel electrophoresis (DGGE) to monitor microbial communities (Fontana *et al.*, 2006; Li *et al.*, 2006). In this study, we use t-RFLP, in conjunction with clone library, for a more accurate identification of bacterial species.

In addition to knowing which bacterial species predominate on stored meat, it is necessary to understand the kinetics of microbial growth that contributed to high and low product quality. This includes measuring the growth of bacterial populations that are routinely used to measure product quality, such as the total viable count (TVC) and lactic acid bacteria (LAB). Such kinetic data can be converted into predictive models that can help the food industry manage cold chains to maximise product shelf-life.

In this report, we describe the effect of storage temperature and packaging atmosphere on changes in dominant bacteria of beef primals and identify candidate specific spoilage organisms (SSOs). In addition, we measure the kinetics of microbial populations using bacteriological methods employed by importing countries, and compare their performance against the Australian standard method for TVC and LAB. Finally, we produce a predictive model for the viability of TVC and LAB on vacuum-packaged primals and compare model predictions with similar bacterial indicators of vacuum-packaged lamb quality.

2 Objectives

The project had six research objectives:

1. Define the specific spoilage organism(s) affecting primal spoilage
2. Production of draft predictive model for specific spoilage organism(s)
3. Validation of the predictive model against commercial cold chain data
4. Production of model interface
5. Compare methods for bacterial counts on lamb and determine growth of micro organisms at various storage temperatures up to day-35 of storage [extended to product shelf-life]
6. Validate beef TVC and LAB model in vacuum packed lamb

3 Methodologies

3.1 Specific spoilage organism(s) affecting primal spoilage (phase 1)

Sample collection

Primals (striploin) were obtained from two abattoirs, Processor A (large exporter) and Processor B (small domestic producer). Primals were aseptically cut into even-sized portions (5cm x 5cm surface area) and packaged aerobically or anaerobically (vacuum packaged [Technovac T60, Italy; setting - 99.9% vacuum with 10 sec extra vacuum]; O_2 transmission rate=10 to 30 cm^3 m^{-2} atm^{-1} $24h^{-1}$ at 25ºC and 75% relative humidity). Aerobically-packaged meat was stored in the same type of vacuum package bags, but loosely sealed with tape to allow air exchange. All samples were stored at 2ºC and 7ºC in polystyrene boxes to reduce temperature fluctuation. Each box contained a temperature data logger set at 5 min intervals.

Meat surface pH

Meat surface pH was measured with an Orion 250A surface probe at each sampling interval, on at least two samples.

Microbiological studies

At each time interval, one package of meat from each abattoir was opened aseptically. The outer 1 cm thickness was aseptically excised and placed in a filter stomacher bag (BagPage, Interscience). The contents were stomached in 20 ml of 0.1% bacteriological peptone (Oxoid) for 2 min and the stomachate retained for enumeration, clonal library and t-RFLP analysis.

Samples were tested for TVC on tryptone soya agar (TSA) plates and for LAB on de Man, Rogosa and Sharp (MRS) agar. A 0.1 ml portion of stomached sample, and associated dilutions, were spread-plated in duplicate on TSA and MRS. All agars were incubated aerobically at 25ºC for three days. Colony-forming units (CFU) were counted and recorded. The MRS plates were returned to the incubator for an additional three days (total 6 days) at which time they were observed for additional CFU. The CFU counts were transformed into $\log_{10}$ CFU per cm^2 and plotted.

Colonies representing different morphotypes were transferred to storage media (tryptone soya broth (TSB) containing 20% glycerol) and stored at -80ºC for later identification by 16S rRNA sequencing.

DNA was extracted from 1.5 ml of whole stomacher fluid using Qiagen tissue DNA extraction kit. Extracted DNA was stored at -20ºC. The remaining sample fluid was frozen in the event DNA extraction needed to be repeated.

Clone library

Extracted DNA was amplified by PCR with universal 16s rRNA primers 10f and 1492r. The TOPO TA Cloning® kit was used to produce clone libraries. Up to 32 clones from each library were sequenced by Macrogen (Seoul, Korea). A BLAST search of the online database GenBank and CLC sequence viewer were used to determine probable identities of the clones.

3.2 Production of draft predictive model for specific spoilage organism(s) (phase2)

Sample collection

In this phase of the project, new striploins were collected from two export abattoirs located in Processor A and Processor C. Striploins used for validation studies were cut and vacuum-packaged at the abattoir, whereas those that were later irradiated were collected from the abattoir as vacuum-packaged whole striploins and portioned in the laboratory. Processor C meat was collected at approximately 10:00am on 24 September 2008. On site, six striploins were cut transversally into 16 portions of equal size. They were vacuum-packaged on-site and placed in ice. A calibrated temperature probe was inserted into one package which was placed in the centre of all the samples. The samples were then transported to Processor A where similarly cut vacuum-packaged striploins, as well as seven whole striploins to be irradiated, were collected at the Processor A abattoir. These were cut and packaged on 23 September at ~11:00am, and then placed in a blast chiller, set at -12°C, but increased to -5°C at 5:00pm. Product was removed from the blast chiller at 5:00am on 24 September and placed into the holding chiller, set at 0°C, until at ~2:00pm. They were transferred from the cooler at Processor A, placed on ice in two separate coolers, and fitted with a temperature data logger. All samples were then driven to the UTAS laboratory where they were received at ~6:00pm.

Sample preparation

At the time of arrival in the UTAS laboratory, the cut samples (except for two pieces from each abattoir) were removed from ice storage and placed in the test incubators (-1.5, 0, 2, 4 and 7°C) that were equipped with calibrated temperature data loggers.

The seven whole striploins were placed at 2°C for cutting and packaging the following day. On that day, whole striploins were aseptically removed from packages and placed on a sanitised cutting board. Using a sanitised knife, each striploin was cut lengthwise in half and then transversely into a total of 24 pieces. The pieces were vacuum-packaged (Technovac T60, Italy; setting - 99.9% vacuum with 10 sec extra vacuum) in film with the same gas transfer rates as the film used by the abattoirs (O_2 transmission rate=10 to 30 $cm^3\ m^{-2}\ atm^{-1}\ 24h^{-1}$ at 25ºC and 75% relative humidity). The vacuum-packaged samples were placed in a -18°C freezer until 30 September 2008 when they were shipped to a commercial irradiation company in Melbourne, VIC. At the facility, they were treated to a dose of 10 kGy and then shipped back to the UTAS laboratories where they were received frozen on 3 October. Samples were kept frozen until experimental use.

Meat surface pH

At each sample time interval, surface pH was measured on the top and bottom of each meat surface using a calibrated Orion 250A surface probe. Typically, the pH for the separate sites on a single piece of meat varied by less than 0.05 units.

Microbial analyses for validation studies

At each time interval, two packages of meat from each abattoir were removed from the incubator, placed on ice and move to the laboratory for analysis. In instances when lag time was longer than expected and the sampling times needed to be extended beyond the original estimate, single samples from the affect abattoir(s) were tested at later time intervals. The low variation observed between the replicate samples indicated that this approach would not compromise the data. Using an ethanol-flamed scissor, one side of the package was opened. A 5cm by 5cm sterilised metal template was placed on the meat surface and the outer 1 cm thickness excised using an ethanol-flamed scalpel and scissor. The excised piece was placed in a filter stomacher bag (BagPage, Interscience) and 10 ml of 0.1% bacteriological peptone (Oxoid) added. The contents were stomached for 2 min and then diluted in parallel in 0.1% bacteriological peptone and MRS broth. While stomaching, the remaining meat sample was lifted from the package, turned over, and the pH measured.

Samples were tested for TVC and LAB using TVC Petrifilm. One millilitre of a sample dilution was added to Petrifilm in duplicate and incubated aerobically at 25ºC (3d for TVC, 6d for LAB). Colonies were counted, recorded, transformed to $\log_{10}$ values, and growth kinetics and pH values plotted.

All remaining stomachate was frozen for further clone library analyses, as needed.

Sample preparation for meat inoculation studies

Prior to inoculating samples with a defined mixture of spoilage bacteria, the irradiated meat samples were removed from the freezer and thawed at 4ºC for ~2d. A temperature data logger

was placed between meat samples during thawing. During this time, a cocktail of six isolates representing the dominant bacterial genera observed during the phase-1 storage studies was prepared. The isolates were *Brochothrix, Lactococcus, Pseudomonas* (2 strains), *Lactobacillus, Carnobacterium*, and *Lactobacillus*, as determined using 16S RNA sequencing. Isolates were grown in TSB for 21h at 25ºC. A cocktail was made to produce equal numbers of each isolate for a final level of ~10^3 CFU/cm^2 of beef surface.

At the beginning of the experiment, 0.1 ml of inoculum was injected using a sterile syringe through a septum on the bag and the inoculum was massaged over the meat surface. The samples were then transferred to the appropriate incubator and tested at selected time intervals as described in the validation studies above.

Model production

Growth rates were calculated for TVC and LAB counts at each storage temperature for validation and irradiated meat studies. TVC and LAB growth data were recorded in an Excel spreadsheet and then kinetic parameters calculated using DMFit curve-fitting software (Institute of Food Research, Norwich UK). Growth rate (log CFU/h) was transformed to the square root of the specific growth rate (sqrt sgr). Secondary models of sqrt sgr as a function of storage temperature were produced using either a linear regression or the Ratkowsky model in TableCurv 2D software.

3.3 Validation of the predictive model against commercial cold chain data (phase 3)

Independent data for the growth of TVC and LAB were sourced from various sources to compare growth rates with model predictions. Additionally, growth rate data were collected for other bacterial species that are known to affect meat quality, including *Brochothrix thermosphacta*, to again determine the position of growth rates within the model boundaries. Food Science Australia (FSA) provided TVC and LAB counts for commercial samples of striploin and cube roll stored at -1 to 0ºC.

Production of a model interface

A model interface was produced in Excel to predict growth rate and kinetic changes in log CFU using inputs for storage temperature, storage time and prediction interval.

3.4 Comparison of methods for measuring bacterial counts on lamb and determine growth of microorganisms at various storage temperatures up to day-35 of storage [extended to product shelf-life] (phase 4)

Collection and transport of samples

Lamb shoulders were collected at Processor A abattoir, unpacked from cartons into polystyrene boxes and layered with ice. The lamb shoulders were 250mm x 150mm x 50mm in size, vacuum-packaged and transported six to a box. The temperature of the meat remained at -1.5°C±0.5°C throughout the 2h and 45min transport time to the laboratory.

Sampling

At each time interval, two lamb shoulders were removed from the incubator, placed on ice and transported to the laboratory. The package was sprayed with 70% ethanol and opened with alcohol-flamed scissors. The following methods were used to measure microbial levels on lamb shoulders.

Japanese method
Half of the packaging material was lifted off to expose the meat surface. The meat surface was excised to the thickness of approximately 2mm with an alcohol-flamed scalpel and tweezers to collect a total of 25g.

The 25g excised sample was placed in a stomacher bag with 225ml of peptone diluent. The bag was stomached for 2min and placed on ice. Next, 0.5ml was added to 4.5ml diluent, and the same procedure used for further dilutions, as necessary. A 0.1ml aliquot of diluted sample was spread-plated on Bromocresol Purple plate count (BCP) and TVC agars, in duplicate. Plates were incubated at 35°C for 48h, and counts recorded and reported per g excised tissue. All colonies were counted in TVC agar and only yellow colonies on BCP agar. [Note - the total number of excised pieces fit into an area of ~100 cm^2 in three of four samples tested. The fourth sample fit into an area of 75 cm^2. We therefore assumed that the average surface area obtained by tissue excision was 93.7 cm^2 and that there were 3.75 cm^2/g excised tissue. In comparisons of methods, Japanese CFU/g were divided by 3.75 (log 0.574)]

Australian method
A 10cm x10cm plastic template was placed over the sanitised packaging and a scalpel used to outline the area to be sponged. The remaining packaging was removed. A sterile sponge was aseptically cut in half to produce a 4cm x 4cm sponge and then placed in a sterile bag with 10ml of peptone diluent. The sponge was squeezed through the bag to moisten it and to remove excess fluid, lifted out of the bag with flame-sterilised tweezers, and then used to swab a 10cm x 10cm area. The sponge was placed back in the bag and squeezed several times before adding separate aliquots of 0.5ml to 4.5ml of MRS and peptone broth. Additional dilutions were prepared as necessary. One millilitre of each dilution was placed on Petri-film, in duplicate. The MRS step was done in duplicate for both aerobic and anaerobic incubation. The TVC and MRS Petrifilm were both incubated at 25°C. Counts were measured for TVC at 3d and at 6d for MRS. Data were reported per cm^2 meat surface.

Danish method
From the sponge sample and dilutions used in the Australian method, 0.5ml was pour- plated with Plate Count Agar (PCA). PCA plates were incubated at 6.5°C for 10d. Data were reported per cm^2 meat surface.

IDF method
From the sponge sample and dilutions used in the Australian method, 0.5ml was pour- plated on Standard Plate Count Agar (Oxoid) containing 0.1 % skim milk powder (PCAM). PCAM was incubated at 21°C for 48h. Data were reported per cm^2 meat surface.

Enumeration
Agar plates and Petrifilm with between 30 and 300 CFU were counted and data recorded as CFU per cm^2 for the Australian, Danish and IDF methods, or CFU/g for the Japanese method.

Media and reagents

Japanese method

Diluent: Bacteriological Peptone (Oxoid, LP0037, UK) - 0.1%.
Plating media: Bromocresol Purple Plate Count Agar (Eiken, E-MB31, Japan).
Total Viable Count agar was made with 2.5g Yeast Extract (Oxoid, LP0021), 5.0g Bacteriological Peptone (Oxoid), 1g glucose, and 15g agar powder (Gelita, Australia).

Australian method

Diluent: Bacteriological Peptone (Oxoid, LP0037) - 0.1%.
MRS (Oxoid, CM0359) used to the manufacture's recommendations.
Plating media: Petri-film aerobic bacteria total count plates (3M Microbiology products, 3M6400). For anaerobic storage BioMerieux ® GENbag anaer bags with sachets (45534) were used and anaerobic indicators (Oxoid, BR0055B).

Danish method

Plating media: Standard Plate Count Agar (Oxoid, CM0463) was prepared according to the manufacturer's instructions.

IDF method

Diluent: as for the Australian method.
Plating media: Standard Plate Count Agar with 1g per litre of skim milk powder. (HomeBrand, Woolworths) with no added preservative.

Temperature measurements

The temperature of the incubators to be used to store the lamb shoulders was measured for two weeks prior to experimentation, using a glass thermometer and TinyTag® temperature data logger. Logger probes were placed in a container of water for the duration of the experiment. The logger temperature was recorded at 15min intervals. Glass thermometers were calibrated against a certified reference thermometer and also remained in each incubator for a visual check when collecting meat samples.

3.5 Validation of beef TVC and LAB models in vacuum-packaged lamb (phase 5)

Growth rates were calculated for TVC and LAB (for MRS incubated aerobically and anaerobically) counts on vacuum-packaged lamb shoulders and compared to predictions from the model developed from Processor C and Processor A vacuum-packaged beef striploins (phase 2).

4 RESULTS

Define the specific spoilage organism(s) affecting primal spoilage

Meat surface pH

The average pH of beef striploin samples immediately prior to packaging for laboratory storage studies was 6.71 for abattoir I and 6.88 for abattoir II (Fig. 1 & 2).

Aerobically packaged

Meat samples from abattoir II were not tested after 2 wk of aerobic storage at 7ºC as the samples were considered to be grossly spoiled due to white mould covering the entire fat surface. Meat from abattoir I stored at 2 and 7 ºC, and meat from abattoir II stored at 2ºC, were tested over 6 wk at which time they were considered spoiled. The differences between the pH values for the samples tested at 6 wk of storage was 0.39 pH units, the highest being 8.11 for meat from abattoir I at 2ºC with. Meat from abattoir I stored at 7ºC had a final pH of 7.94 and aerobic 2ºC meat from abattoir II had a pH of 7.72, at 6 wk (Fig. 1).

Vacuum packaged

Meat stored at 2ºC was sampled for 14 wk while meat stored at 7ºC was sampled through 12 wk. The final recorded pH of 7ºC meat was 5.97 from abattoir I and 5.33 from abattoir II. For meat stored at 2ºC the final recorded pH was 6.85 and 5.35 for abattoirs I and II, respectively (Fig. 2). The muscle pH at 14wk for vacuum packaged meat stored at 2ºC was 6.46 for abattoir I and 5.34 for abattoir II. The pH of striploins used for the comparison study was measured on both the adipose and lean surfaces. For the whole striploins stored at abattoir I, at 0ºC the pH at 13 wk was 6.42 for adipose and 6.57 for lean. At 16.5 wk these measurements were 5.66 and 5.54. The pH of the 1/3 striploin stored at 2ºC at the laboratory for 13 wk the adipose pH was 5.39 and the lean 5.32.

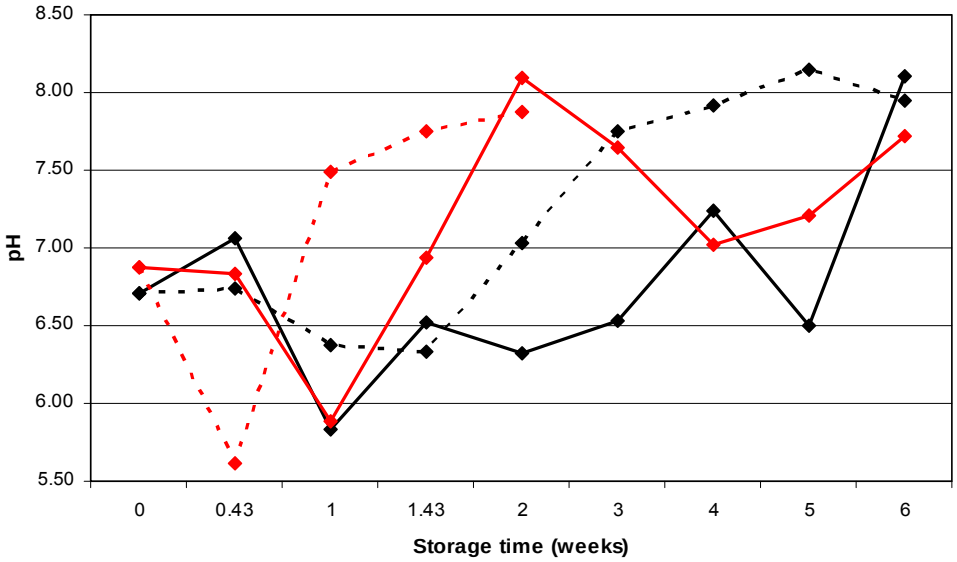


Figure 1. The pH of aerobically packaged meat from abattoir I (black) and abattoir II (red) at 2ºC (—) and 7ºC (---) measured over 6 wk of storage.

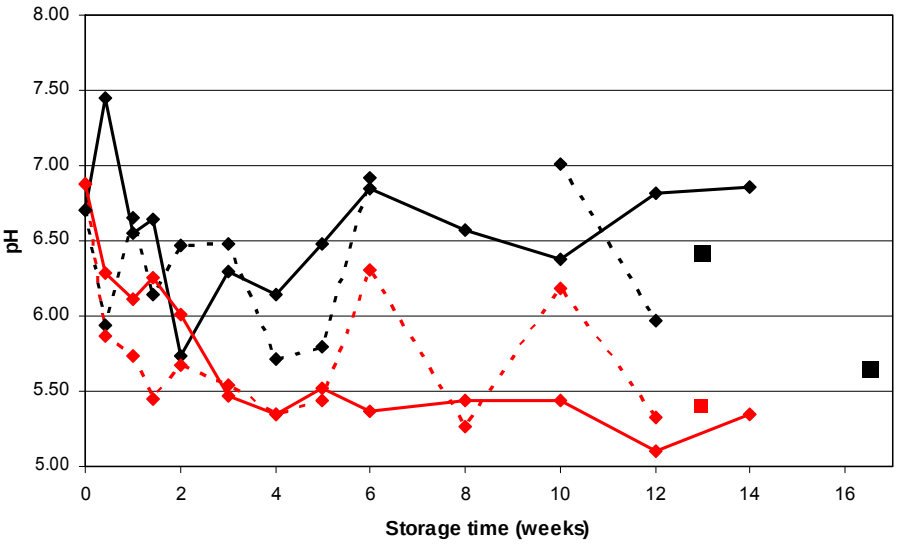


Figure 2. The pH of vacuum packaged meat from abattoir I (black) and abattoir II (red) at 2ºC (—) and 7ºC (---) measured over 14 and 12 wk of storage, respectively. The pH abattoir I (■) and abattoir II (■) industry comparison samples. Abattoir I was 0ºC, abattoir II was 2ºC.

Bacterial enumeration

The initial TVC of meat collected from abattoir I was 3.48 $\log_{10}$ cfu/cm^2 while the LAB was 2.23 $\log_{10}$ cfu/cm^2; for abattoir II the counts were higher at 3.98 and 2.58 $\log_{10}$cfu/cm^2 for TVC and LAB, respectively. Growth rates were measured for TVC and LAB populations. The growth rates were higher for meat stored aerobically at 7°C and lower for vacuum-stored meat at 2ºC (Fig. 3).

The fastest growing microbial community was the LAB population from abattoir II stored aerobically at 7ºC, with a growth rate of 0.04 $\log_{10}$ CFU/h. Conversely, the slowest rate was observed for both the TVC and LAB on the 2ºC vacuum packaged meat from abattoir II (0.005 $\log_{10}$ CFU/h).

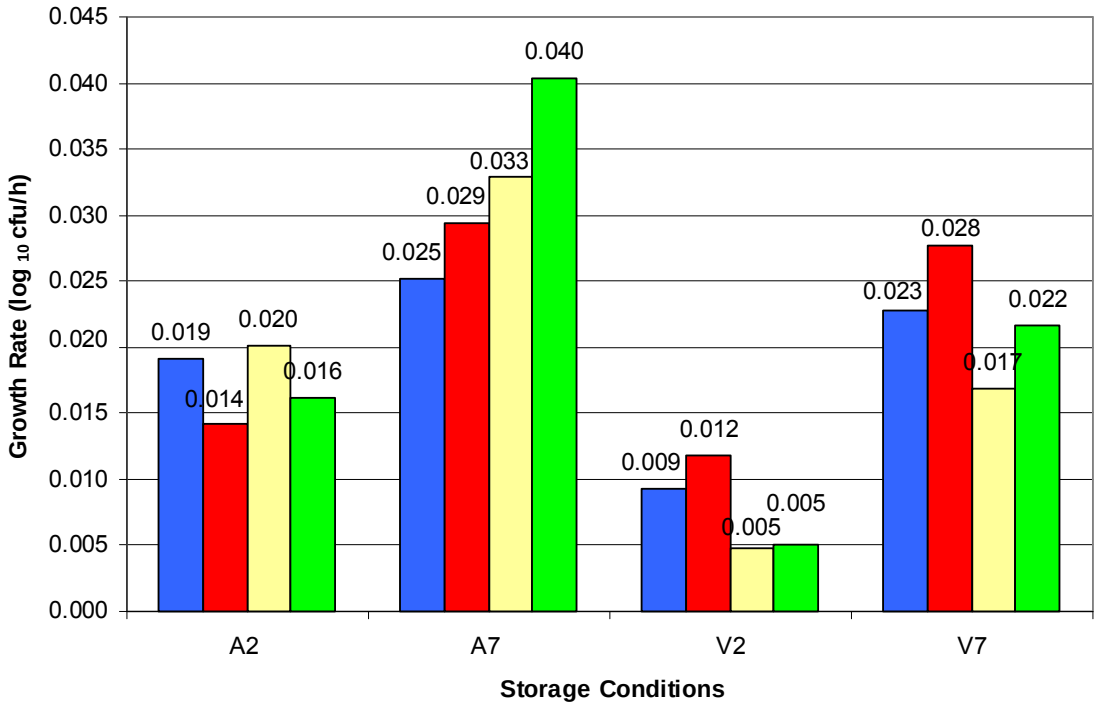


Figure 3. Growth rate of TVC (blue) and LAB (red) from abattoir I and the growth rate of TVC (yellow) and LAB (green) from abattoir II. A = aerobically packaged; V = vacuum packaged; 2 = 2ºC; 7 = 7ºC.

Aerobic packaged

The stationary phase TVC for aerobically stored meat from abattoir I was 9.77 $\log_{10}$ cfu/cm^2 at 2°C and 9.86 $\log_{10}$ cfu/cm^2 at 7°C. The LAB counts were 7.03 and 7.72 $\log_{10}$ cfu/cm^2 for 2 and 7°C, respectively (Fig. 4). For both the TVC and LAB, stationary phase was reached earlier for meat stored at 7°C. The meat collected from abattoir II and stored at 7°C was not sampled after 2 wk due to gross spoilage and therefore the TVC and LAB could not be determined at stationary phase. For meat stored at 2°C from abattoir II, the TVC at stationary phase was 9.96 $\log_{10}$ cfu/cm^2 and the LAB 6.77 $\log_{10}$ cfu/cm^2 (Fig. 5).

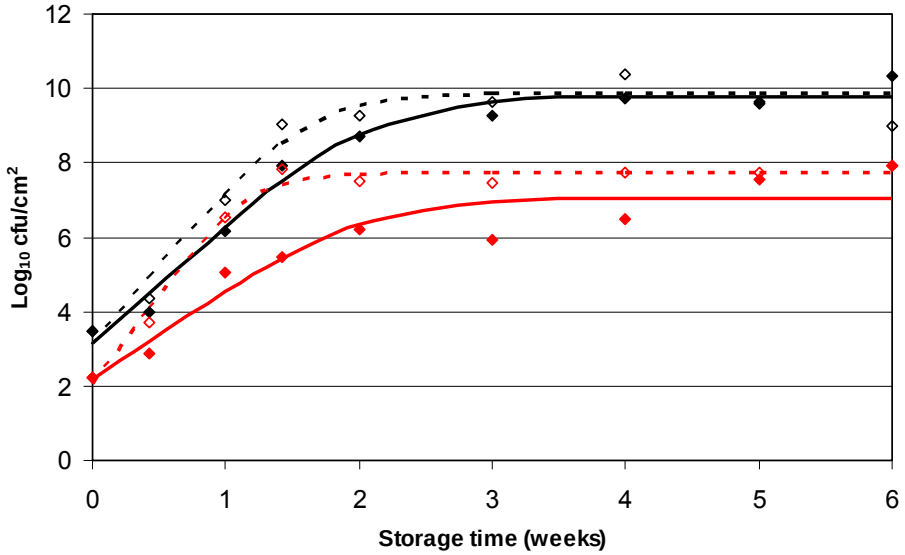


Figure 4. Enumeration of aerobically packaged beef striploin from abattoir I. Primary curve fit (line) and observed (symbol) TVC (black) and LAB (red) counts on beef striploin from abattoir I stored aerobically at 2 (—/♦) and 7ºC (- - -/◊) over 6 wk.

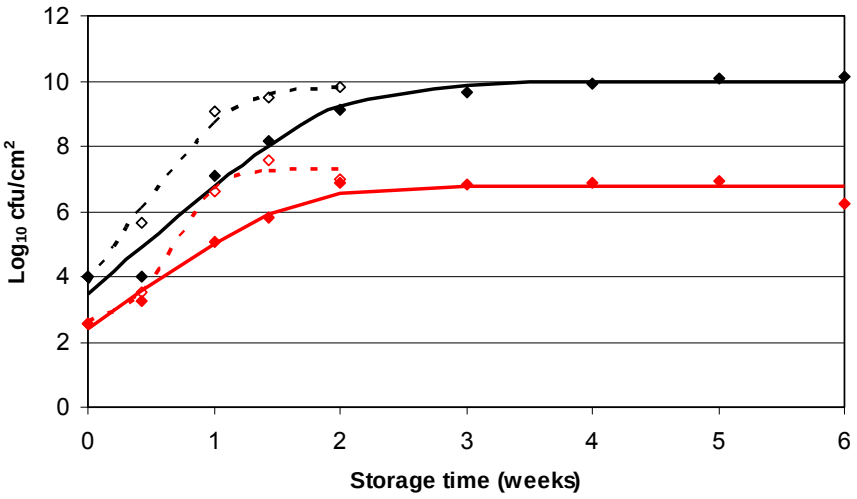


Figure 5. Enumeration of aerobically packaged beef striploin from abattoir II. Primary curve fit (line) and observed (symbol) TVC (black) and LAB (red) counts on beef striploin from abattoir II stored aerobically at 2°C (—/♦) and 7ºC (- - -/◊) over 6 wk.

Vacuum packaged

The stationary phase TVC for vacuum packaged meat was 8.18 $\log_{10}$ cfu/cm^2 for samples collected from abattoir I and stored at 2°C (Fig. 6). The LAB from the same conditions was 7.59 $\log_{10}$ cfu/cm^2. For meat stored at 7°C from abattoir I the TVC was 7.83 $\log_{10}$ cfu/cm^2 and the LAB 7.13 $\log_{10}$ cfu/cm^2. For abattoir II the TVC was 7.38 $\log_{10}$ cfu/cm^2 and 7.76 $\log_{10}$ cfu/cm^2 for

storage at 2 and 7°C, respectively. The stationary phase LAB counts of meat from abattoir II stored at 2 and 7°C were 7.08 $\log_{10}$ cfu/cm^2 and 7.24 $\log_{10}$ cfu/cm^2 (Fig. 7). The counts of meat stored at abattoir I for 13 wk were 7.13 $\log_{10}$ cfu/cm^2 for TVC and 6.32 $\log_{10}$ cfu/cm^2 for LAB (Fig. 6). After 16.5 wk of storage at abattoir I the counts were 6.89 $\log_{10}$ cfu/cm^2 and 5.45 $\log_{10}$ cfu/cm^2 for TVC and LAB. The partial striploin collected from abattoir II and stored at 2°C for 13 wk had a TVC of 7.15 $\log_{10}$ cfu/cm^2 and a LAB count of 6.51 $\log_{10}$ cfu/cm^2 (Fig. 7).

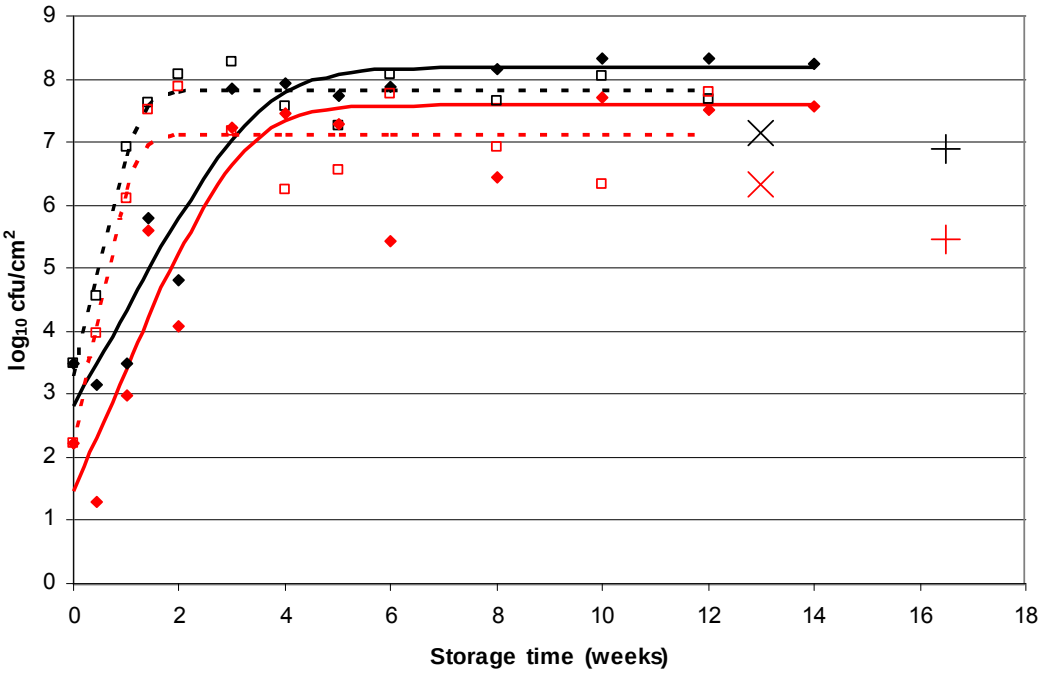


Figure 6. Enumeration of vacuum packaged beef striploin from abattoir I. Predicted and observed (no line) TVC (black) and LAB (red) on beef striploin from abattoir I stored in vacuum package at 2°C (—/♦) and 7ºC (- - -/◊) over 14 wk. Includes observed TVC (black) and LAB (red) for meat stored at abattoir I for 13 wk (X) and 16.5 wk (+).

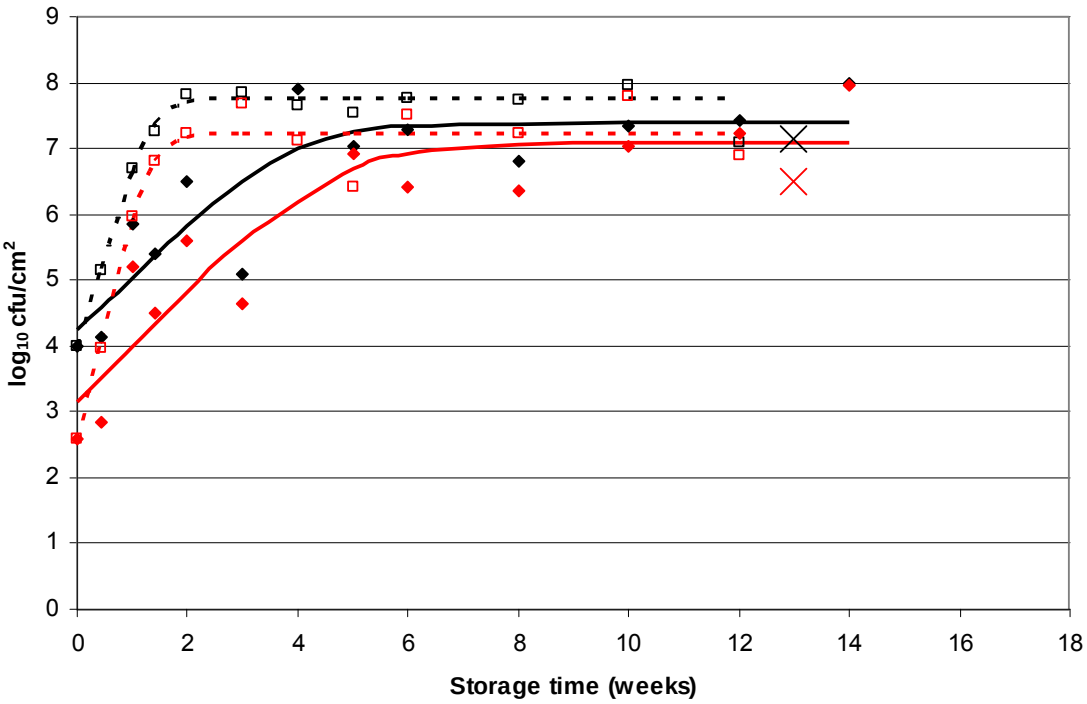


Figure 7. Enumeration of vacuum packaged beef striploin from abattoir II. Predicted and observed (no line) TVC (black) and LAB (red) on beef striploin from abattoir II stored in vacuum package at 2°C (—/♦) and 7ºC (- - -/◊) over 14 wk. Includes meat stored at 2°C for 13 wk (X).

Clone libraries

The sequences obtained by clone libraries aligned with 42 different bacteria genera. The clones from abattoir I at time = 0 aligned to 11 different genera; 19% were most closely aligned to uncultured organisms in the GenBank database (Fig. 8). The clone library of the initial population of beef striploin from abattoir II contained sequences which aligned to 16 genera (Fig. 10). A number of sequences were most closely aligned with "*Aranicola proteolyticus*". As this is not a valid taxonomic classification the sequences were assigned to *Serratia* spp. based on the clustering of these sequenced together in phylogenetic analysis.

Genera analysis-aerobically packaged

The largest proportion of clones were identified as *Pseudomonas* spp. at all times for aerobic storage of meat from abattoir I, initially 25% of clones were identified as most closely related to *Pseudomonas* spp. At 3 and 6 wk of storage aerobically at 2ºC, more than 75% of the community was identified as most closely related to *Pseudomonas* spp. (Fig. 8). For striploin stored at 7ºC *Pseudomonas* spp. constituted 56% of the population at 2 wk and 84% at 6 wk of storage (Fig. 9). The community of striploin collected from abattoir II also comprised large proportions of *Pseudomonas* spp. when stored aerobically. For storage at 2ºC 67% of the clones were *Pseudomonas* spp. at 3 wk and 20% at 6 wk (Fig. 10). *Pseudomonas* made up 83% of the clone library for meat stored at 7ºC for 2 wk (Fig. 11). *Psychrobacter* spp. and *Janthinobacterium* spp. both represented 13% of the clones from meat from abattoir II stored aerobically at 2ºC for 3 wk and at 6 wk both had increased *Janthinobacterium* spp. to 37% and *Psychrobacter* spp. to 27% (Fig. 10). For meat from abattoir I, *Brochothrix* spp. made up 23% of the clone library for meat

stored for 3 wk at 2ºC and 10 and 3% of the 7ºC stored meat clone libraries. For abattoir II, *Brochothrix* spp. was 3 and 10% of the clone libraries at 2ºC and was not detected in the clone library from 7ºC (Fig. 8-11).

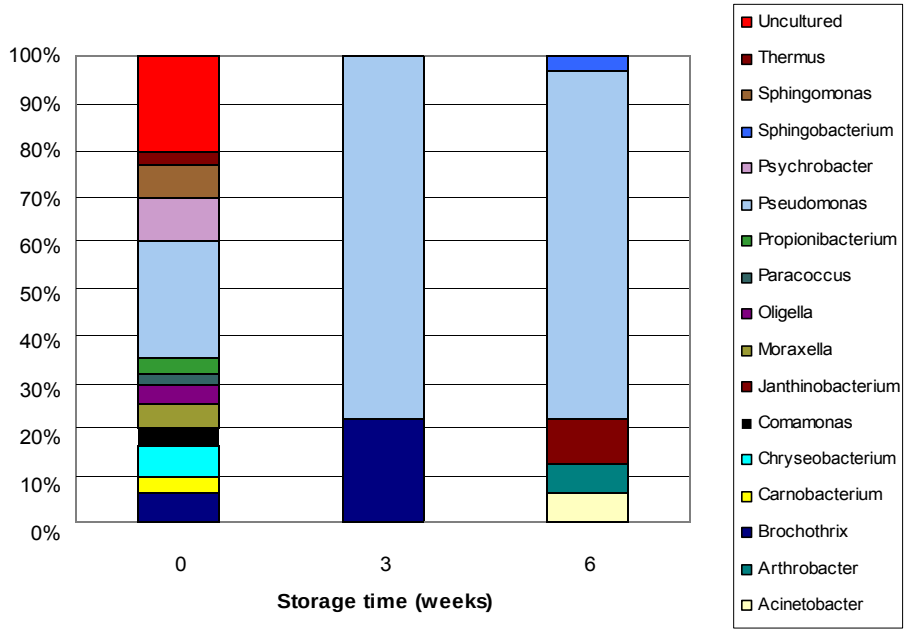


Figure 8. Proportion of bacterial genera on beef striploin from abattoir I at time 0 and after storage aerobically at 2ºC for 3 and 6 wk.

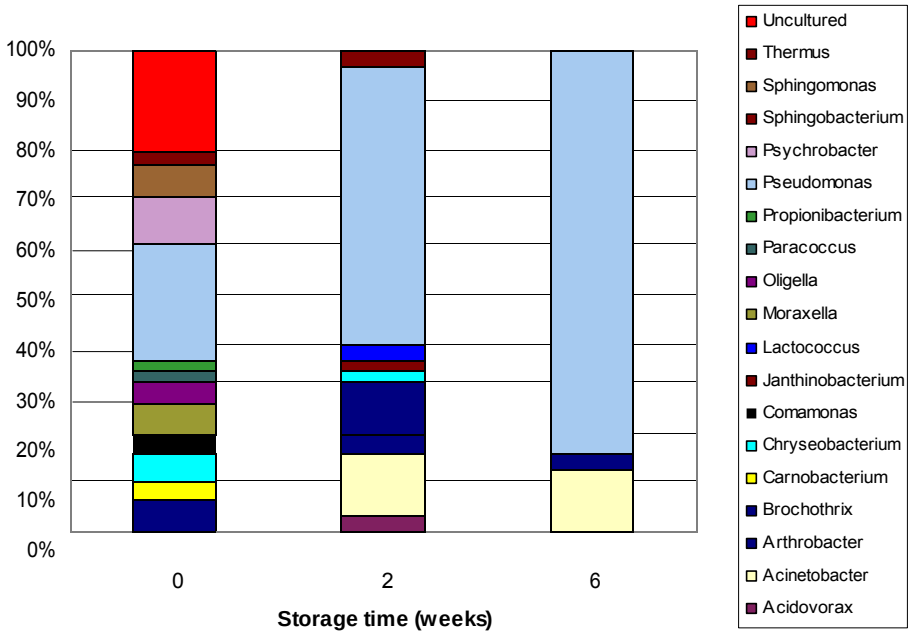


Figure 9. Proportion of bacterial genera on beef striploin from abattoir I at time 0 and after storage aerobically at 7ºC for 2 and 6wk.

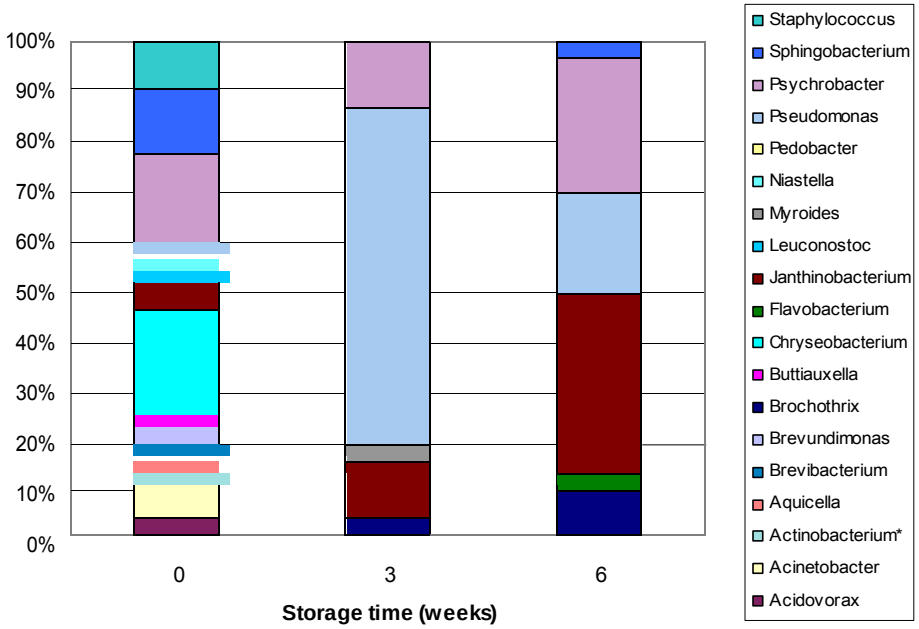


Figure 10. Proportion of bacterial genera on beef striploin from abattoir II at time 0 and after storage aerobically at 2ºC for 3 and 6 wk.

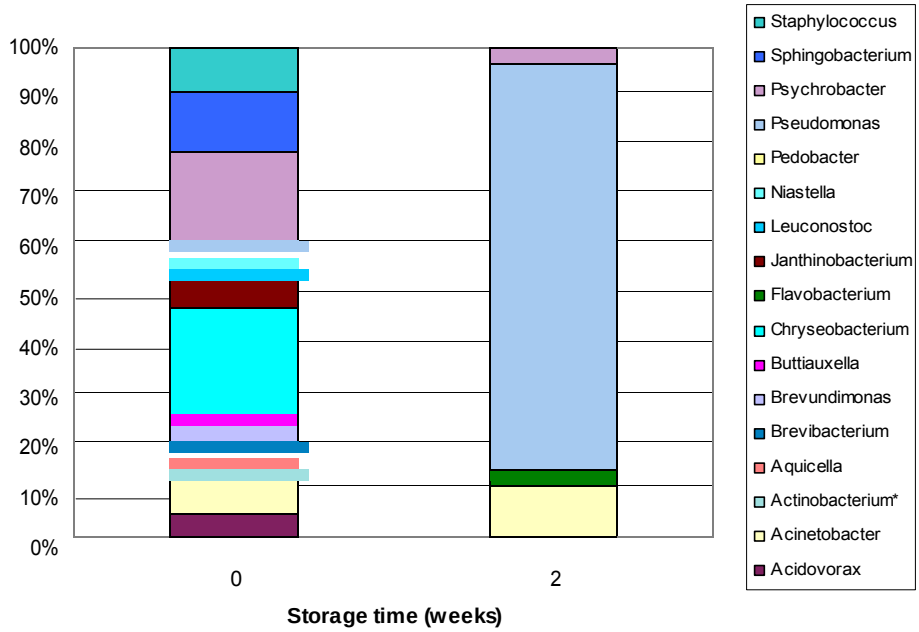


Figure 11. Proportion of bacterial genera on beef striploin from abattoir II at time 0 and after storage aerobically at 7ºC for 2 wk.

Genera analysis-vacuum packaged

There were seven genera identified as dominant in the clone libraries of vacuum packaged beef striploins. These were *Pseudomonas, Clostridium, Carnobacterium, Lactococcus, Brochothrix, Lactobacillus and Buttiauxella*. Sequences aligning with *Serratia* spp. and *Leuconostoc* spp. were also large proportions of the clone library results. The clone libraries produced from meat collected from abattoir I and stored in vacuum packaged at 2ºC (Fig. 12) were completely dominated by *Pseudomonas* spp. at 2 and 6wk. At 2 wk 97% of the clones were identified at *Pseudomonas* spp., this decreased to 48% at 6 wk after which time there were no clones identified as *Pseudomonas* spp. *Brochothrix* spp. were present in the clone libraries at 2, 6 and 10 wk, with a maximum of 25% in the 6 wk clone library. The 10 wk clone library analysis suggested there were large populations of *Serratia* spp., *Lactococcus* spp. and *Carnobacterium* spp. The latter two genera were present in smaller amounts in the 14 wk clone library, which was 84% *Clostridium* spp. A clone library was also produced from the drip of the 14 wk sample. This contained large proportions of *Lactococcus* spp., *Carnobacterium* spp., and *Clostridium* spp. Also clones were identified in the drip as belonging to *Morganella, Providencia* and *Serratia*, which were not in the clones from the adipose sample. Striploin from the same abattoir, stored in vacuum at 7ºC (Fig. 13) was dominated by *Lactococcus* spp. at 1, 3 and 12 wk and the drip from the 12 wk sample. At 10 wk the clone library was dominated by *Clostridium* spp., clones belonging to this genus were not observed in any other clone libraries produced from vacuum packaged striploin from abattoir II stored at 7ºC.

For meat collected from abattoir II and stored in vacuum package at 2ºC (Fig. 14), 82% of the clones at 6 wk of storage were *Pseudomonas* spp., as were 10% of the clones at 2 wk. *Brochothrix* spp. were a dominant genus in the clone libraries for 2 and 10 wk but were absent from the clones of 6 and 14 wk storage. The clone library produced at 10 wk of storage was dominated by *Leuconostoc* spp., *Brochothrix* spp., *Buttiauxella* spp., *Lactobacillus* spp. and *Carnobacterium* spp. While at 14 wk *Lactobacillus* spp. dominated the clone library and *Carnobacterium* spp. and *Buttiauxella* spp. were also present in large amounts. For storage at 7ºC (Fig. 15) *Lactobacillus* spp. dominated the clone library at 1 wk and was also present in large amounts in the 6 and 12 wk clone libraries. *Buttiauxella* spp. represented 49% of the clones at 3 wk and declined to 13 and 3% of the clones at 6 and 12 wk, respectively. The 3 wk clone library was also composed 23% *Carnobacterium* spp. *Lactococcus* spp. were part of the dominant flora of the 3, 6 and 12 wk clone libraries and only 2% of the 1 wk clone library. *Brochothrix* spp. were present in all the clone libraries, constituting 30% of the 1 wk clone library, 12% at 6 wk and 2% at 3 and 12 wk. *Serratia* spp. were in the 3, 6 and 12 wk clone libraries and *Leuconostoc* were in the those from 3 and 12 wk of storage.

The clone library produced from the full striploin stored at abattoir I for 13 wk was predominately comprised of sequences belonging to *Carnobacterium* spp., *Clostridium* spp. and *Lactobacillus* spp. (Fig. 16). At 16 wk of storage at abattoir I clone libraries were produced from drip and lean samples also. All three clone libraries were completely dominated by *Carnobacterium* spp., the only other genus represented in the clones was *Serratia* which represented 20% of clones from the drip (Fig. 16).

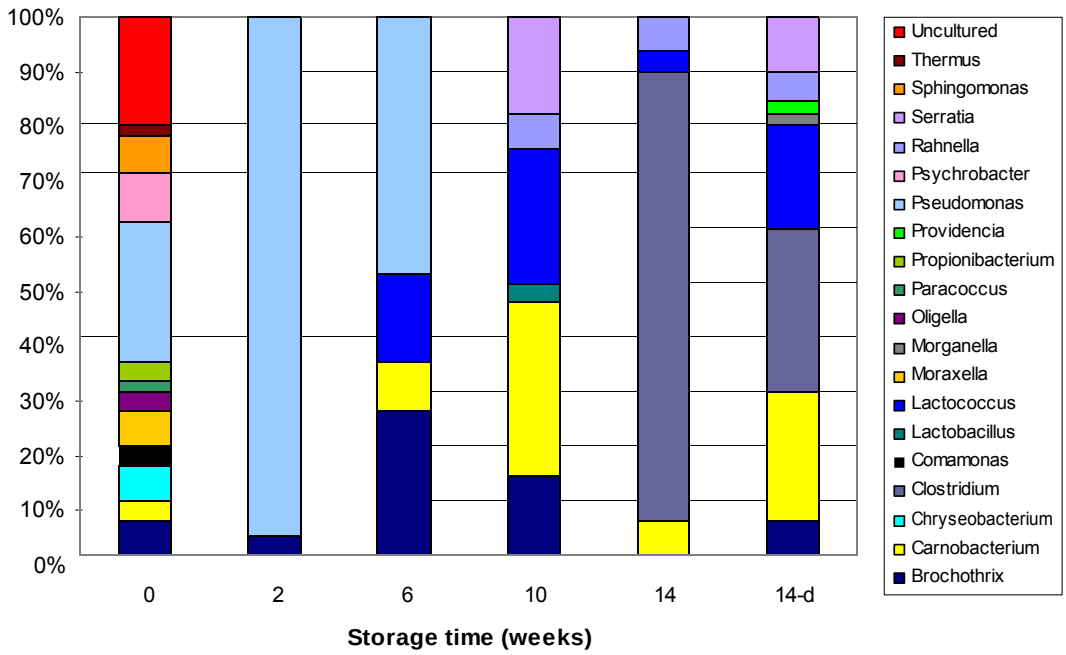


Figure 12. Proportion of bacterial genera on beef striploin from abattoir I at time 0 and after storage in vacuum package at 2ºC for 2, 6, 10 and 14 wk. d = drip sample

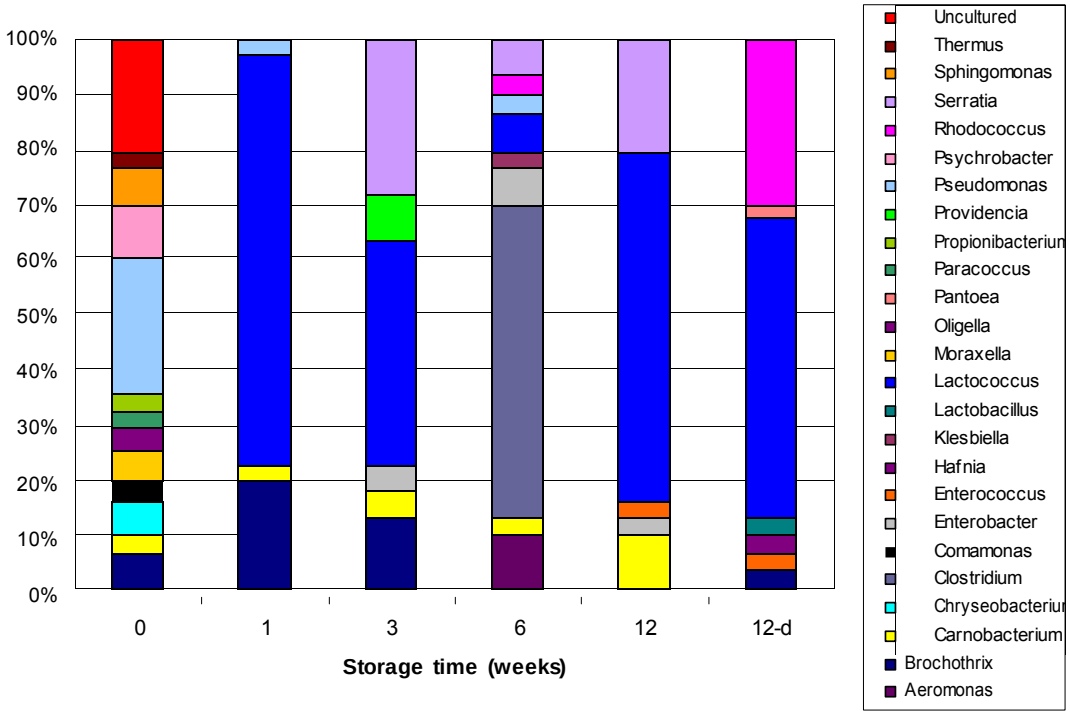


Figure 13. Proportion of bacterial genera on beef striploin from abattoir I at time 0 and after storage in vacuum package at 7ºC for 1, 3, 6, and 12 wk. d = drip sample.

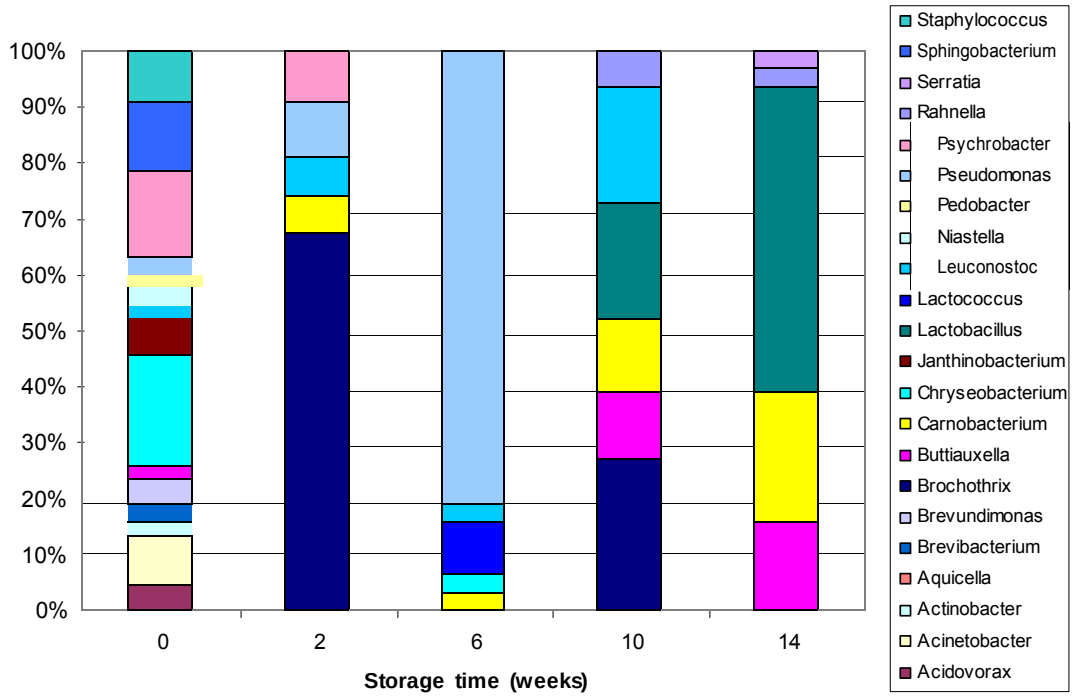


Figure 14. Proportion of bacterial genera on beef striploin from abattoir II at time 0 and after storage in vacuum package at 2ºC for 2, 6, 10 and 14 wk.

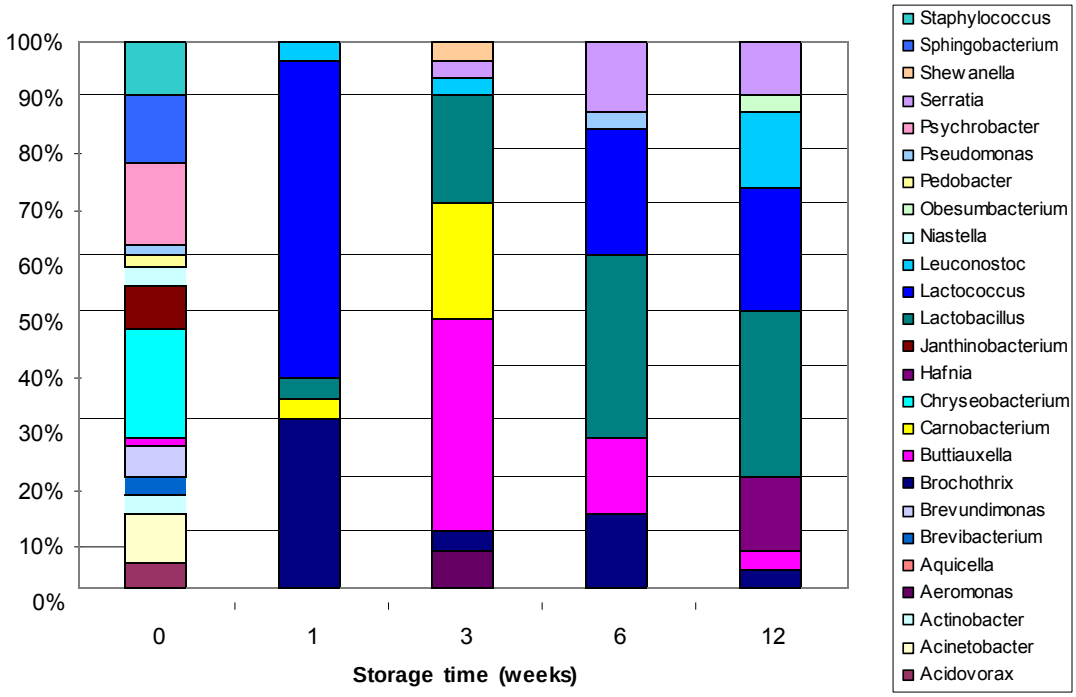


Figure 15. Proportion of bacterial genera on beef striploin from abattoir II at time 0 and after storage in vacuum package at 7ºC for 1, 3, 6, and 12 wk.

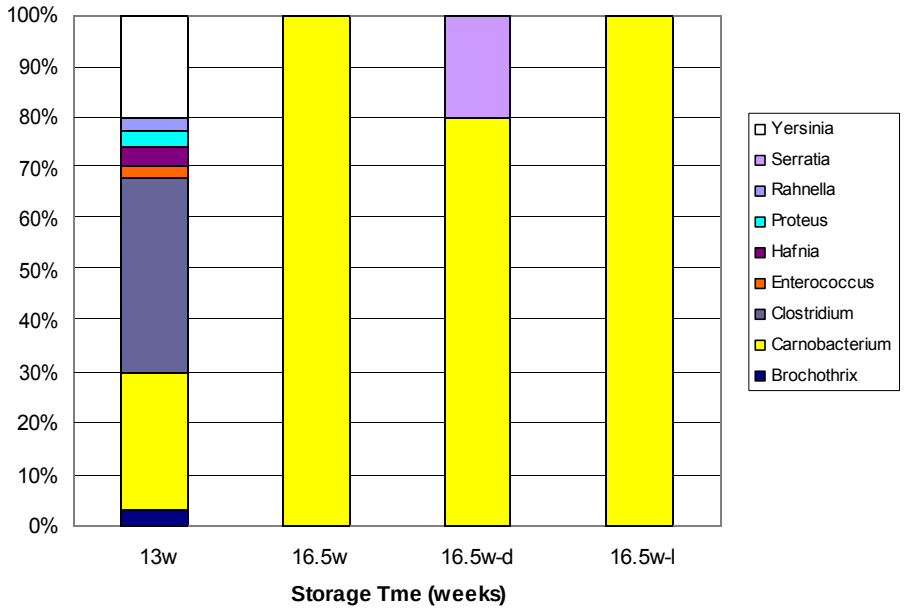


Figure 16. Proportion of genera on vacuum packaged beef striploins stored at abattoir I, at 0ºC for 13 and 16.5 wk. d = drip sample. l = lean sample.

Isolate Identification

All sequences obtained by partial sequencing of the 16S rRNA gene from isolates grown on TSA aligned with previously cultured organisms. A total of 31 different bacterial genera were identified in this section of the work. *Pseudomonas* spp. was identified in all the aerobic stored samples and the piece from abattoir II vacuum packaged at 2°C for 2 wk (Table 1 & 2). *Carnobacterium* spp. were identified on all meat stored in vacuum package at 2 and 7°C and in time 0 for abattoir II and on meat from abattoir I aerobically stored at 7°C for 6 wk. *Lactobacillus* spp. and *Leuconostoc* spp. were not identified in any samples from abattoir I (Table 1) but were isolated from the vacuum stored samples from abattoir II at 2 and 7°C (Table 2). *Lactococcus* spp. was identified in at least one of the vacuum stored samples from both abattoirs. *Brochothrix* spp. was present in samples stored in air and vacuum at 2 and 7°C from both abattoirs (Table 1 & 2).

l

Table 1. Bacterial genera identified by isolate 16S RNA sequencing of meat collected from abattoir I. A = aerobic packaged; V = Vacuum packaged; 2 = 2ºC; 7 = 7ºC.

		A2		A7		V2				V7		
Storage Time (wk)	**0w**	**3w**	**6w**	**2w**	**6w**	**2w**	**6w**	**10w**	**14w**	**3w**	**6w**	**12w**
Acinetobacter	■			■	■							
Aerococcus	■											
Aeromonas											■	
Arthrobacter												
Brochothrix				■		■	■	■				
Burkholderia				■								
Buttiauxella												
Carnobacterium					■	■	■	■	■	■	■	■
Chryseobacterium												
Desemzia	■											
Enterobacter												
Enterococcus	■										■	■
Flavobacterium												
Hafnia												
Janthinobacterium			■									
Klebsiella											■	
Kocuria	■											
Lactobacillus												
Lactococcus					■	■	■	■		■	■	■
Leuconostoc												
Macrococcus	■											
Microbacterium												
Moraxella												
Obesumbacterium												
Pseudomonas		■	■	■	■							
Psychrobacter												
Rahnella									■			
Rhodococcus					■							
Shewanella												
Sphingobacterium												
Staphylococcus	■					■						
Yersinia											■	

Table 2. Bacterial genera identified by isolate 16S RNA sequencing of meat collected from abattoir II. A = aerobic packaged; V = Vacuum packaged; 2 = 2ºC; 7 = 7ºC.

		A2		A7	V2				V7		
Genus	**0**	**3w**	**6w**	**2w**	**2w**	**6w**	**10w**	**14w**	**3w**	**6w**	**12w**
Acinetobacter											
Aerococcus											
Aeromonas											
Arthrobacter											
Brochothrix											
Burkholderia											
Buttiauxella											
Carnobacterium											
Chryseobacterium											
Desemzia											
Enterobacter											
Enterococcus											
Flavobacterium											
Hafnia											
Janthinobacterium											
Klebsiella											
Kocuria											
Lactobacillus											
Lactococcus											
Leuconostoc											
Macrococcus											
Microbacterium											
Moraxella											
Niastella											
Obesumbacterium											
Pseudomonas											
Psychrobacter											
Rahnella											
Rhodococcus											
Shewanella											
Sphingobacterium											
Staphylococcus											
Yersinia											

t-RFLP Analysis

There was no significant difference between the microbial communities as a result of storage temperature (R=0, P=0.464; Fig. 17) or the source abattoir from which the meat was collected (R=0.02, P=0.618; Fig. 18). The atmosphere of storage had a significant effect on the composition of the microbial community (R=0.437, P<0.001; Fig. 19). There was no significant difference on the basis of storage time (Fig. 20) for aerobic or vacuum stored beef striploin. R values for comparison of vacuum packaged meat stored for <6 wk, 6 wk, >6 wk were all less than 0.12 (P=0.222-0.586; Table 3). For aerobic stored meat, storage time of <6 wk was compared to storage for 6 wk with an R value of only 0.11 (P=0.400; Table 3). Comparison of vacuum packaged beef striploin stored at abattoir I with vacuum packaged beef striploin stored at the University of Tasmania showed no significant difference (R=0.196, P=0.131; Fig. 21).

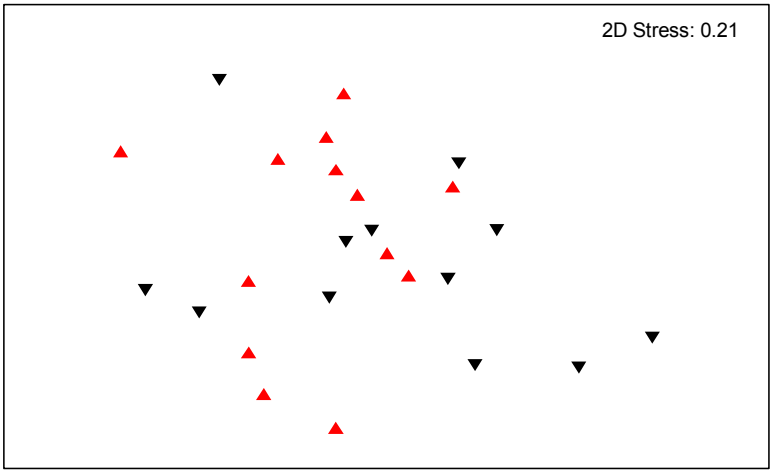


Figure 17. MDS plot of samples on the basis of storage temperature; ▲ 2ºC; ▲ = 7ºC; R stat 0; P value 0.464.

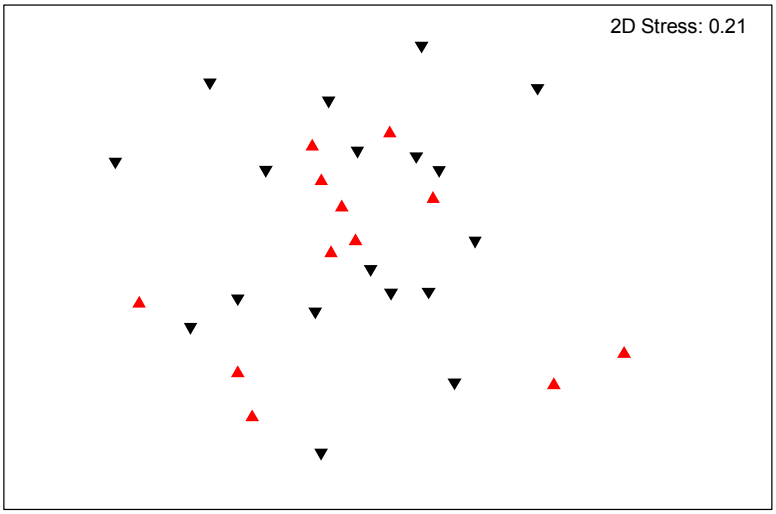


Figure 18. MDS plot of all samples on the basis of abattoir; ▲ = abattoir I; ▲ = abattoir II; R stat 0.02; P value 0.618.

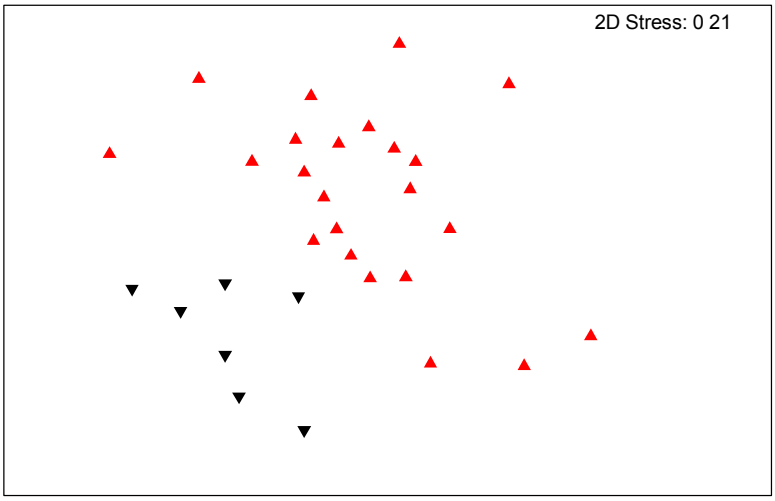


Figure 19. MDS plot of all sample on the basis of packaging atmosphere; ▲ = aerobic packaged; ▲ = vacuum packaged; R stat 0.437; P value 0.001.

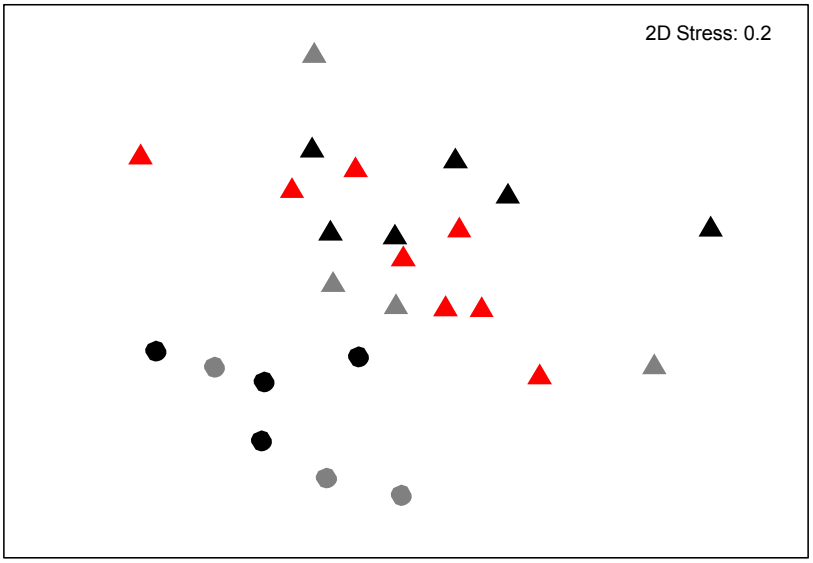


Figure 20. MDS plot of all samples on the basis of atmosphere and time; R stat 0.296; P value 0.020. ▲vacuum packaged < 6 wk; ▲ vacuum packaged 6 wk; ▲ vacuum packaged > 6 wk; ● aerobic packaged < 6 wk; ● aerobic packaged 6 wk.

Table 3. ANOSIM Pairwise Tests of combined storage atmosphere and time. V = vacuum packaged; A = aerobically packaged; <6 = samples stored for less than 6 wk; 6= samples stored for 6 wk; >6 = samples stored for more than 6 wk. Statistically significant results are highlighted yellow.

Groups	R Statistic	P value
V>6, V<6	0.011	0.401
V>6, V6	0.114	0.222
V<6, V6	-0.056	0.586
V>6, A<6	0.496	0.006
V>6, A6	0.661	0.006
V<6, A<6	0.472	0.005
V<6, A6	0.556	0.024
A<6, V6	0.323	0.029
A6, V6	0.259	0.143
A<6, A6	0.111	0.400

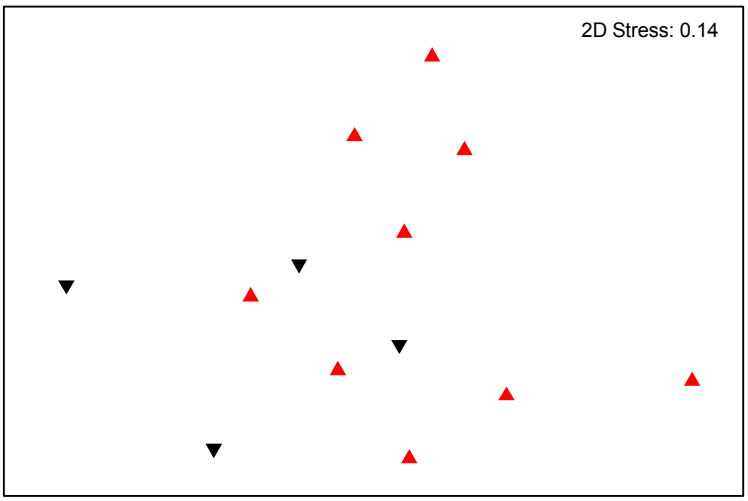


Figure 21. MDS plot of vacuum packaged samples stored for 10 or more wk on the basis of storage location R stat 0.196; P value 0.131. ▲ = University of Tasmania; ▲ = abattoir I.

Production of draft predictive model for specific spoilage organism(s)

Figures 22 through 31 display the growth profiles of TVC and LAB, as well as surface pH, for Processor A and Processor C samples at -1.5, 0, 2, 4, and 7ºC. TVC and LAB counts were similar for the majority of time intervals. These data were used to measure lag time, growth rate and maximum population density with DMFit curve-fitting software.

Figures 32 through 36 show growth profiles for the defined cocktail added to irradiated meat. Growth rates for the inoculum were markedly higher than that observed for endogenous flora on commercial primals (Fig. 37).

A predictive model was produced to estimate TVC and LAB levels on vacuum-packaged beef primals from -1.5 to 7ºC. The model forms were very similar and are:

TVC square root of the specific growth rate = 0.112 x temperature (ºC) + 0.089

LAB square root of the specific growth rate = 0.111 x temperature (ºC) + 0.089

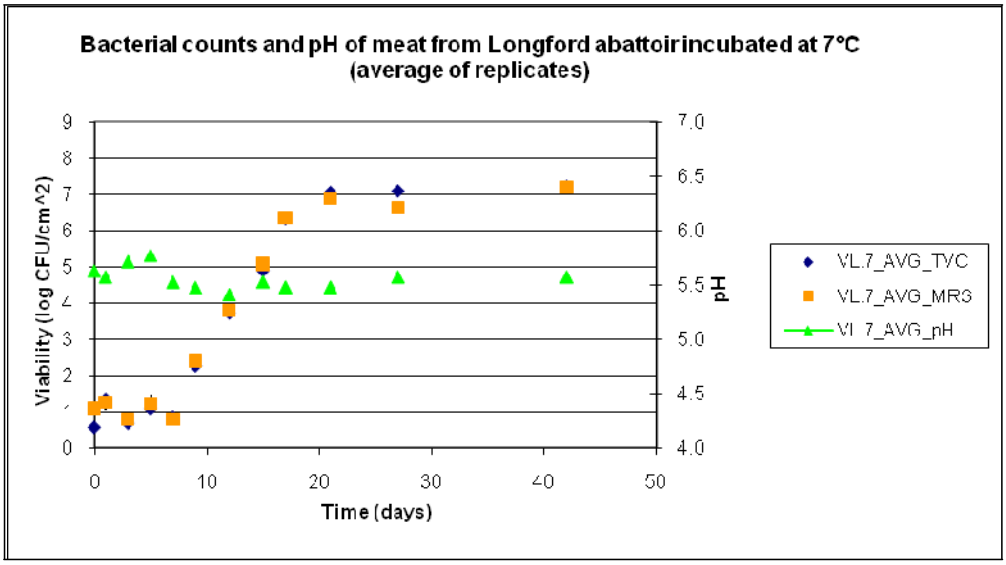


Figure 22. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor A samples stored at 7ºC.

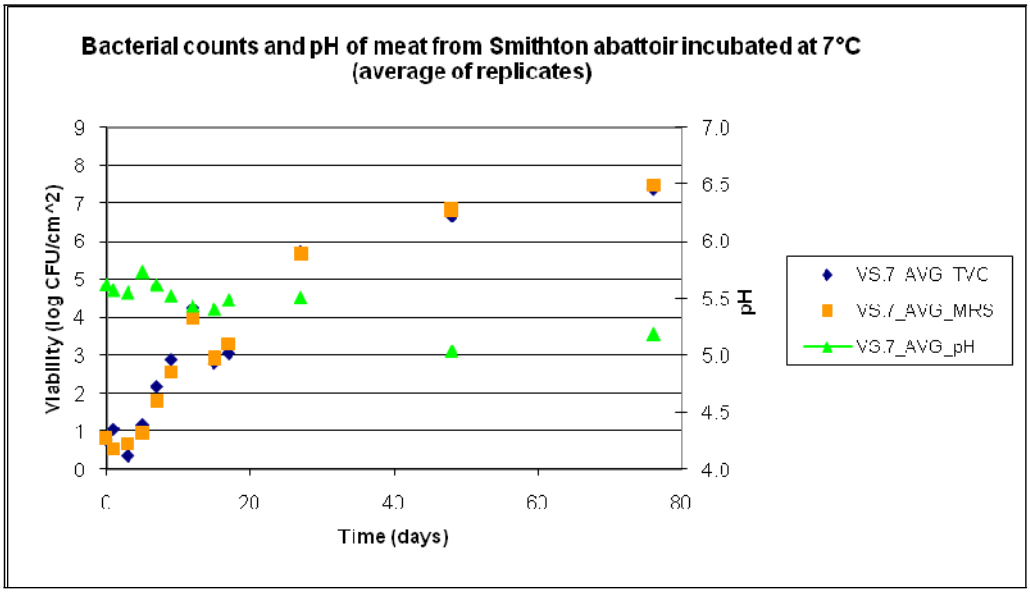


Figure 23. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor C samples stored at 7ºC.

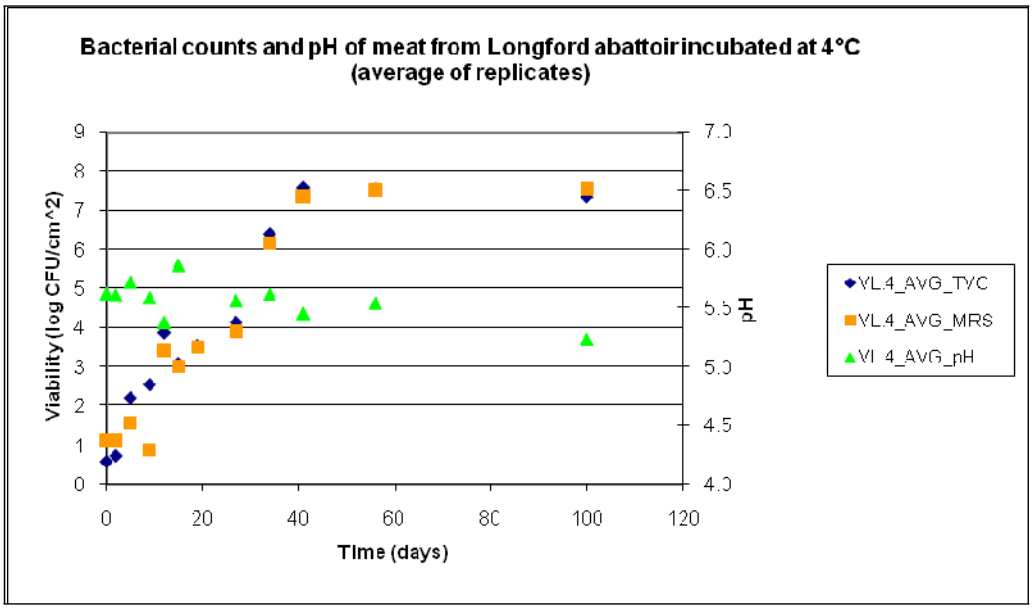


Figure 24. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor A samples stored at 4ºC.

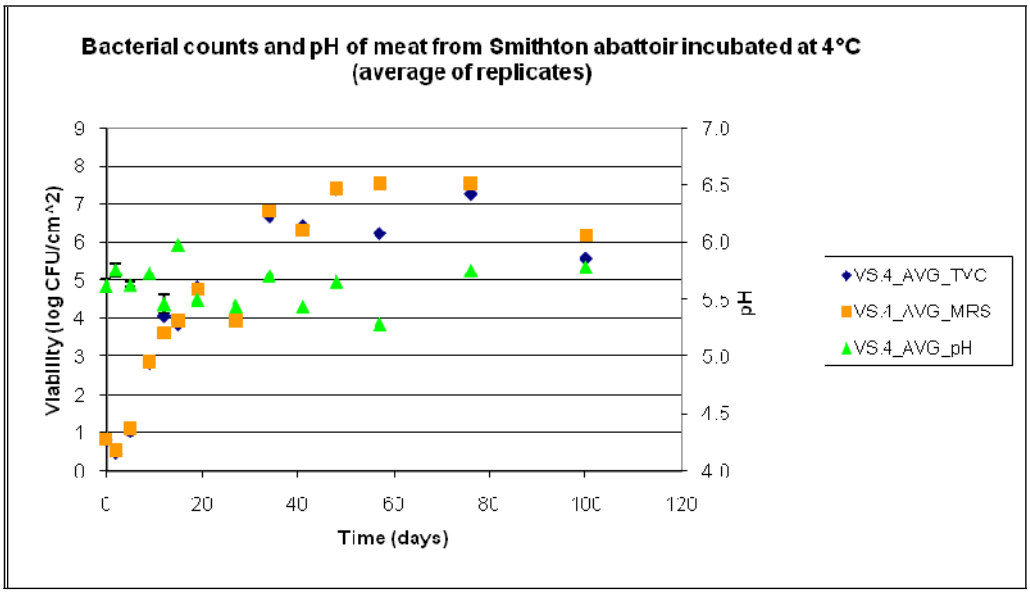


Figure 25. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor C samples stored at 4ºC.

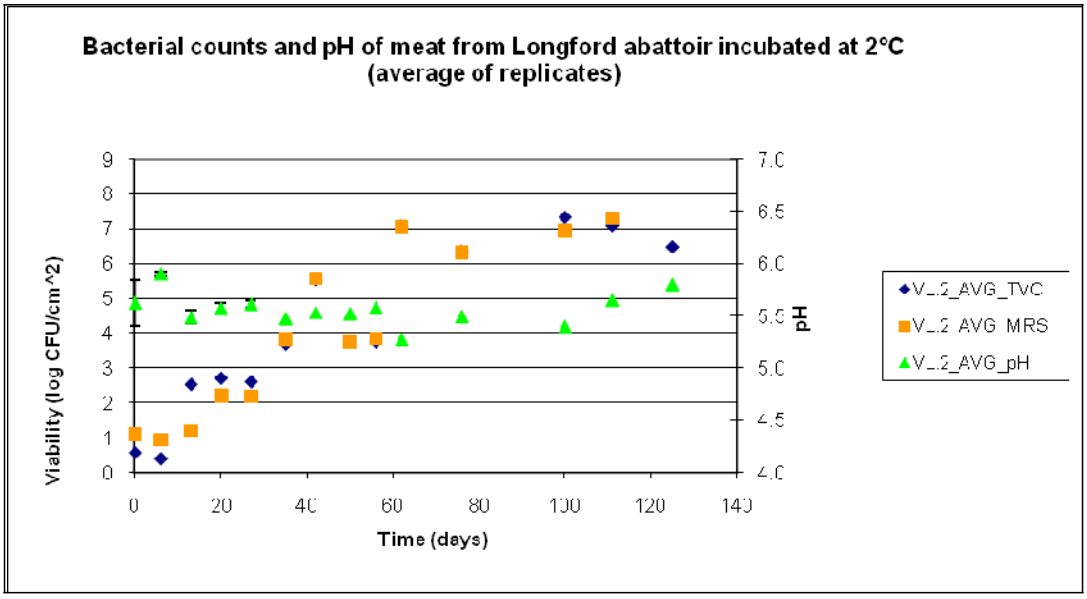


Figure 26. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor A samples stored at 2ºC.

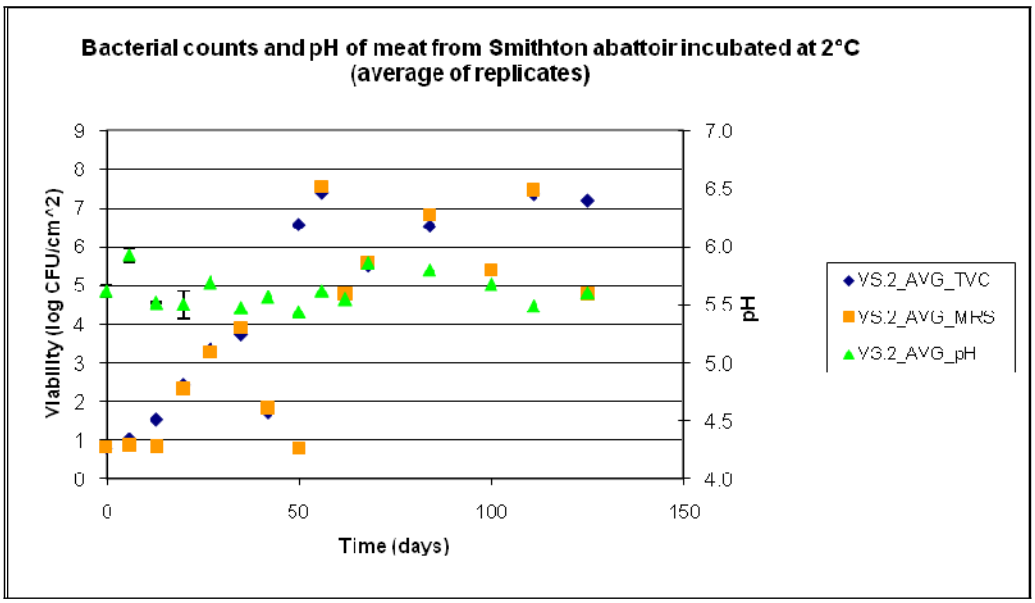


Figure 27. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor A samples stored at 2ºC.

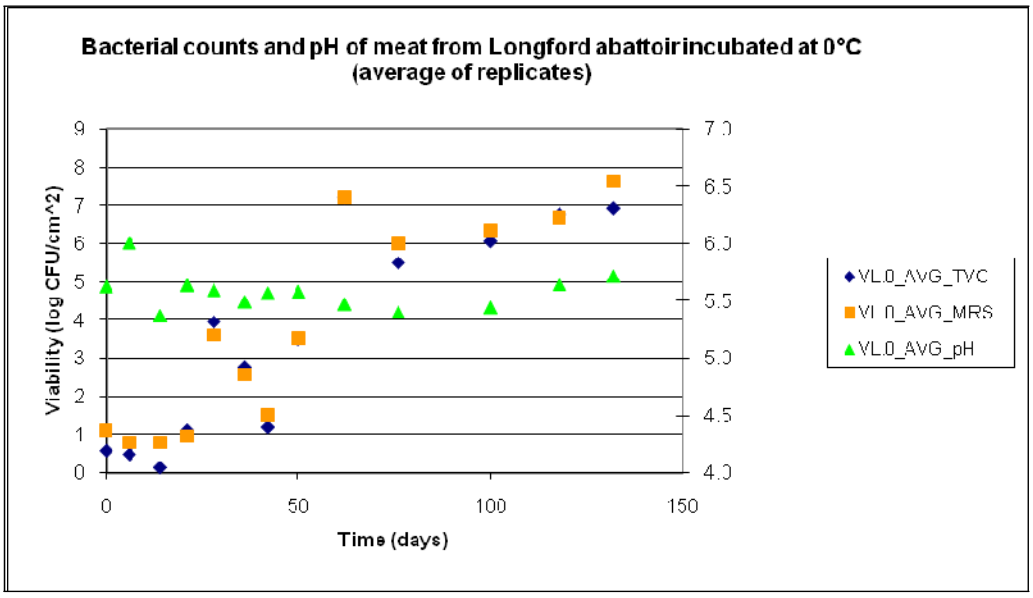


Figure 28. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor A samples stored at 0ºC.

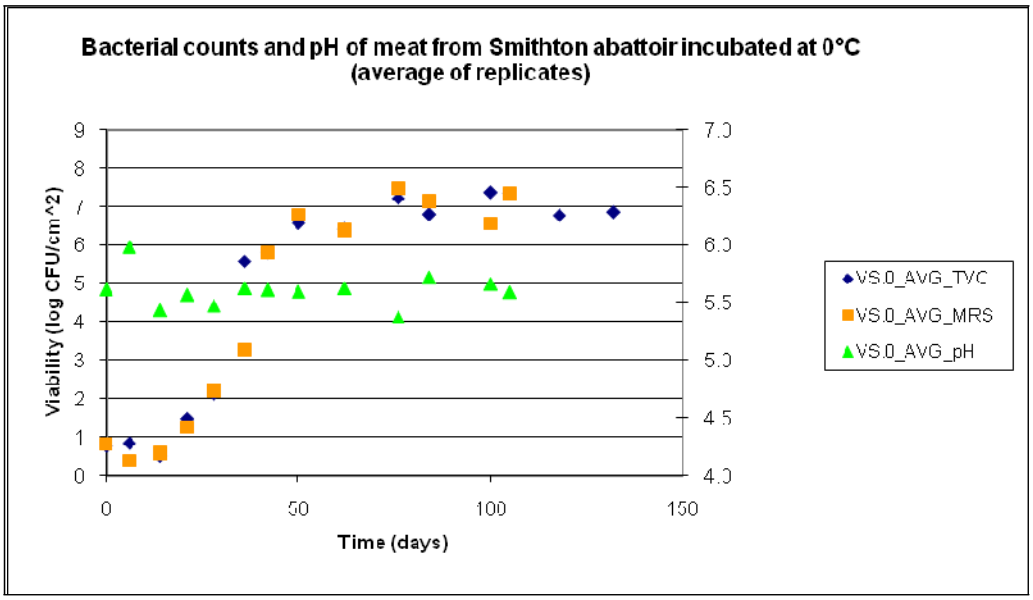


Figure 29. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor C samples stored at 0ºC.

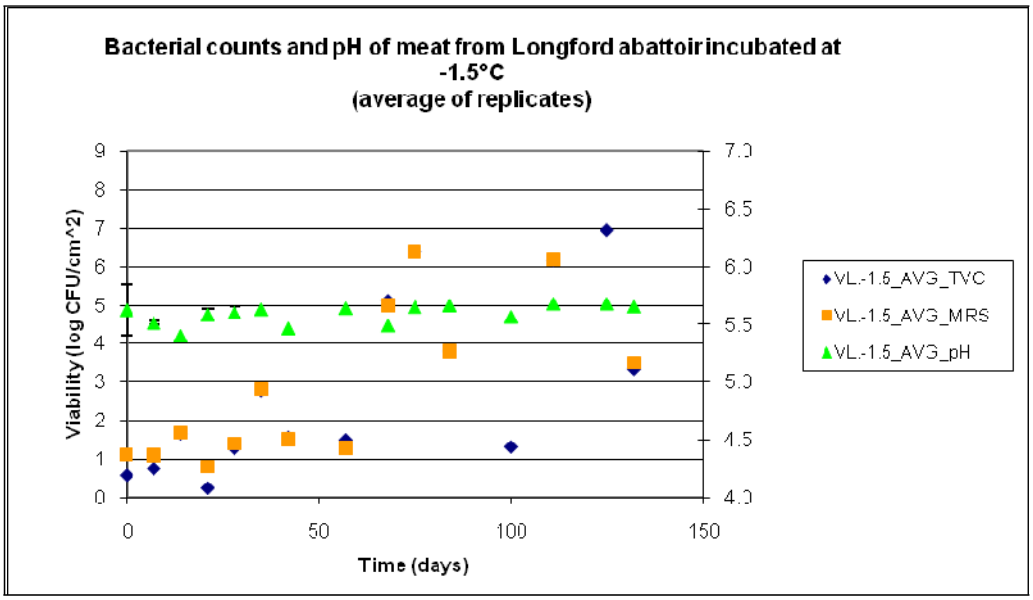


Figure 30. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor A samples stored at -1.5ºC.

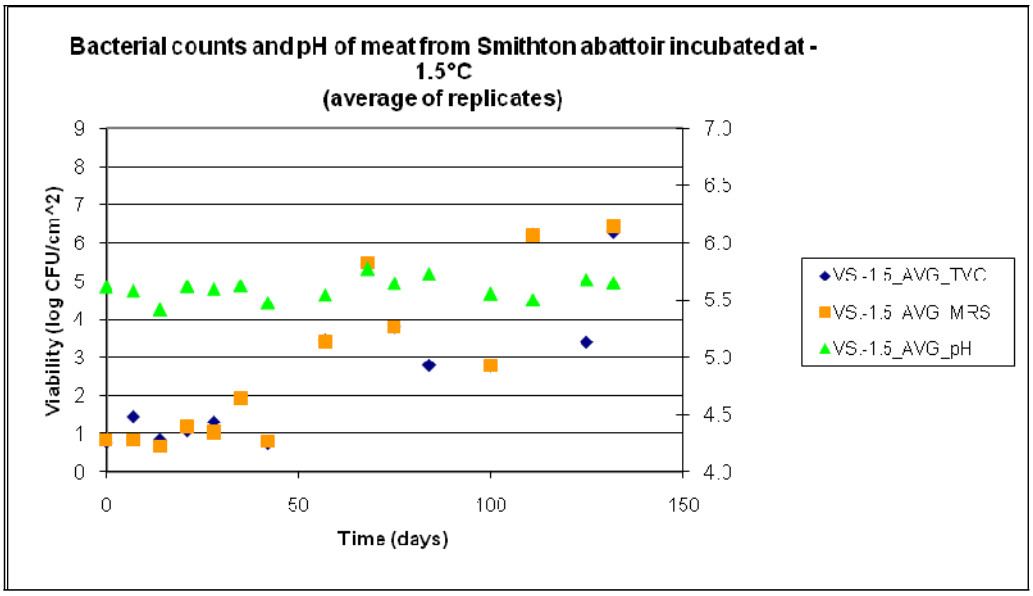


Figure 31. Kinetics of TVC and LAB (MRS) bacteria, and pH, for Processor C samples stored at -1.5ºC.

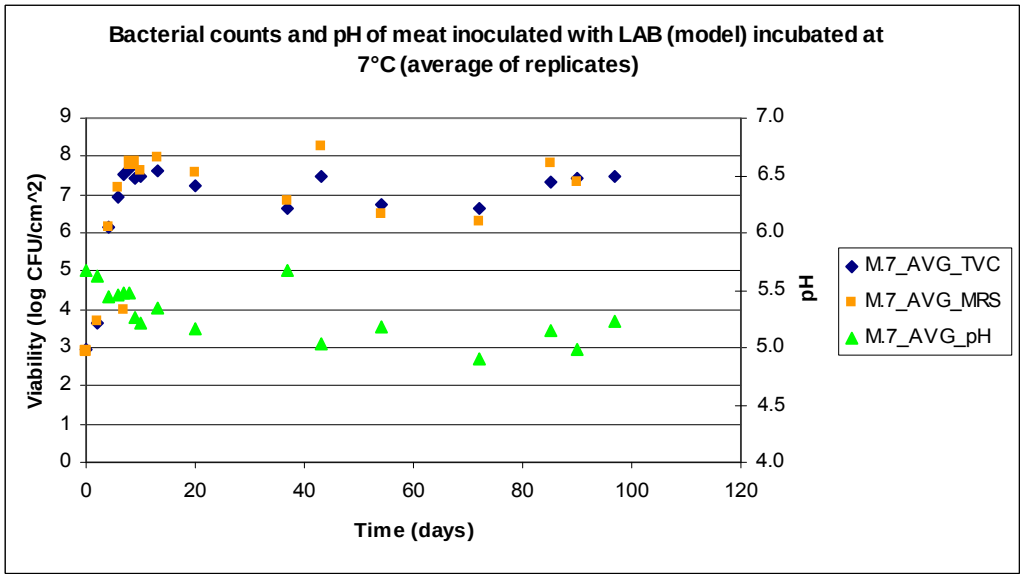


Figure 32. Kinetics of TVC and LAB (MRS) bacteria, and pH, on irradiated and inoculated samples stored at 7ºC.

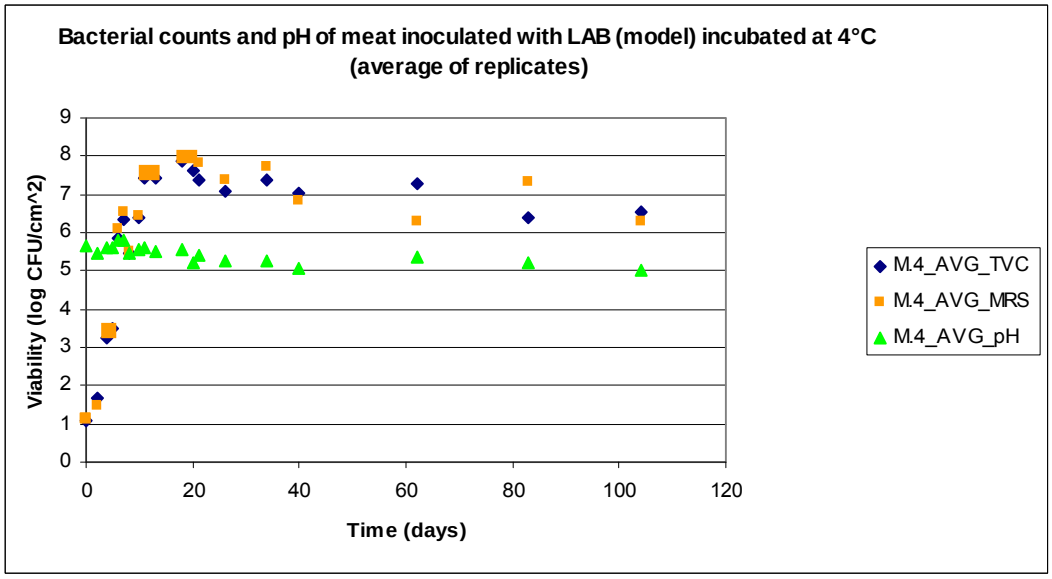


Figure 33. Kinetics of TVC and LAB (MRS) bacteria, and pH, on irradiated and inoculated samples stored at 4ºC.

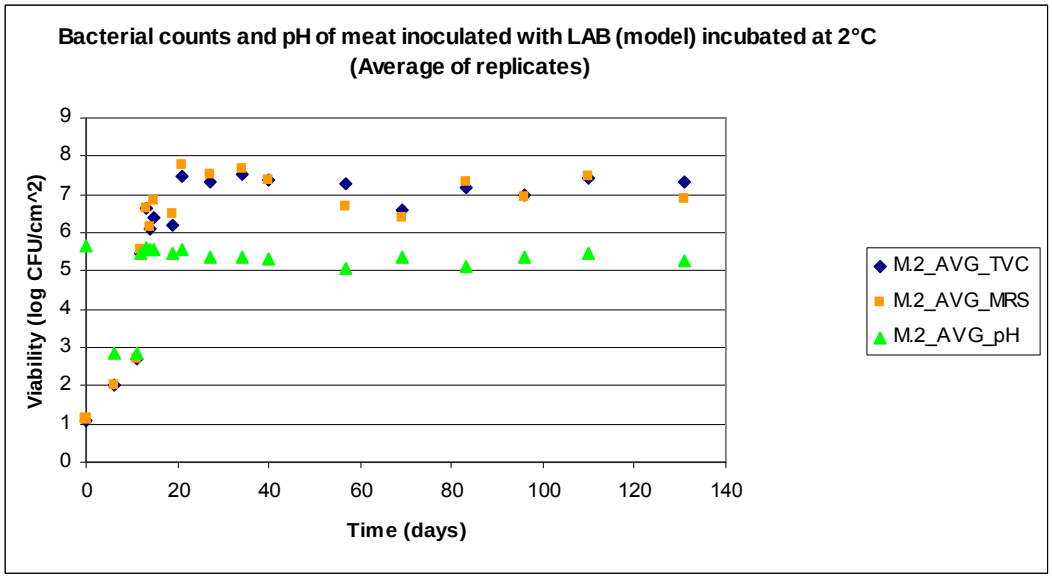


Figure 34. Kinetics of TVC and LAB (MRS) bacteria, and pH, on irradiated and inoculated samples stored at 2ºC.

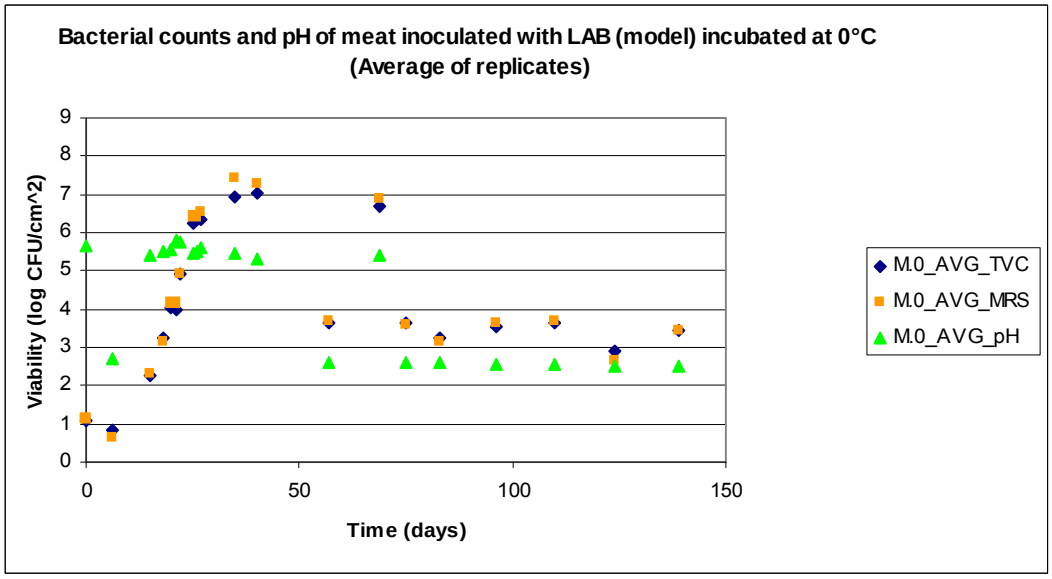


Figure 35. Kinetics of TVC and LAB (MRS) bacteria, and pH, on irradiated and inoculated samples stored at 0ºC.

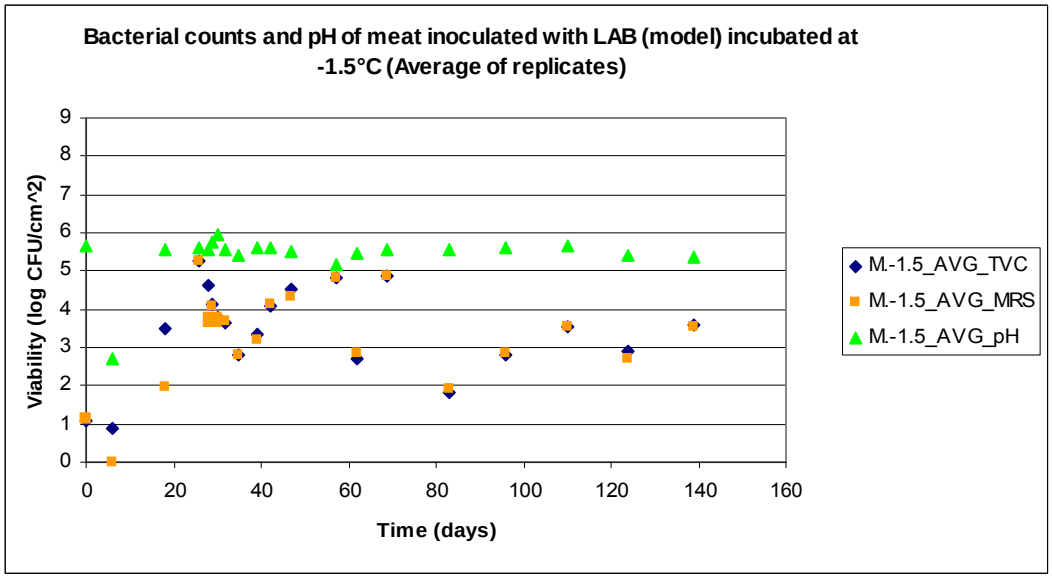


Figure 36. Kinetics of TVC and LAB (MRS) bacteria, and pH, on irradiated and inoculated samples stored at -1.5ºC.

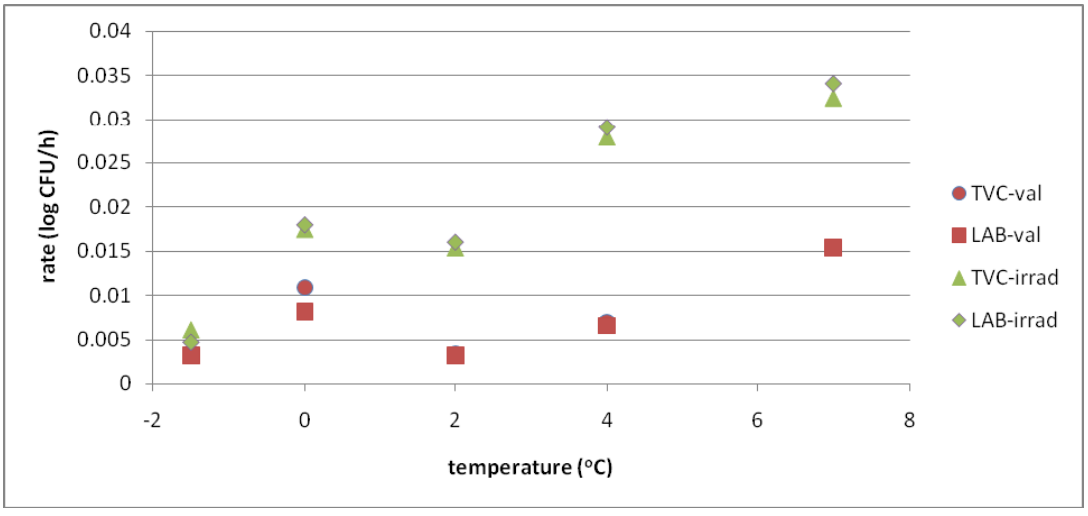


Figure 37. Growth rates of TVC and LAB in irradiated (irrad) and commercial (val) primal at storage temperatures of -1.5 to 7ºC.

Validation of the predictive model against commercial cold chain data

Figure 38 shows the model based on TVC growth in irradiated striploin inoculated with the 5-strain cocktail of spoilage organisms (described in Milestone report 3). Lines represent the predicted growth rate (pink), 95% prediction limits (dashed), and growth rates (symbols) for TVC in Processor C (!)- and Processor A (!)-packaged meat. Model predictions are compared to TVC growth rates observed in striploins packaged at Sm and Ln, showing general over-prediction by the model.

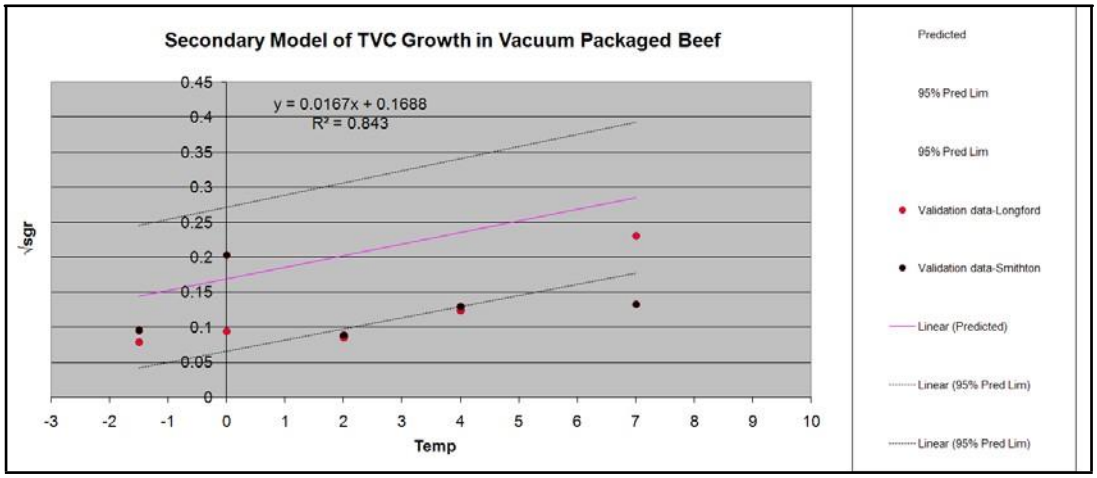


Figure 38. Prediction of TVC growth rate in vacuum packaged striploin. The model is based on TVC rates observed in irradiated striploins inoculated with a 5-strain cocktail of spoilage bacteria. Predicted growth rate is compared to rates observed in commercially packaged striploins.

Further comparisons were made for TVC and *Brochothrix thermosphacta* growth rates reported in other studies of vacuum packaged Australian beef primals, sourced from reports by FSA and Simmons (phase 1). FSA data were collected at 0°C and Simmons at 2 and 7°C (Fig. 39).

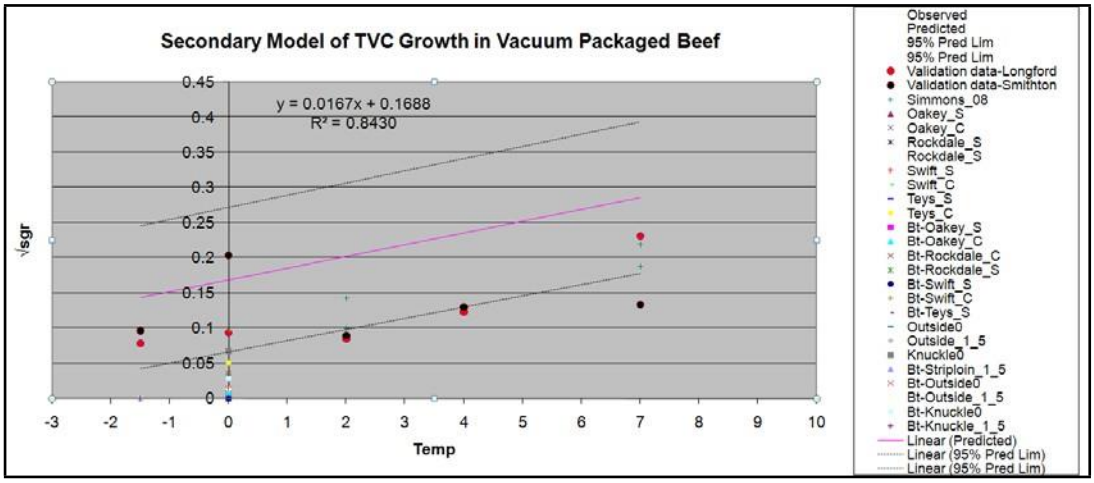


Figure 39. Similar to Fig. 38, except predicted TVC growth rate is also compared to TVC and *B. thermosphacta* rates reported in studies by FSA, and TVC rates by Simmons.

Further comparisons were made with data collected from ComBase and from the peer reviewed literature (Fig.40).

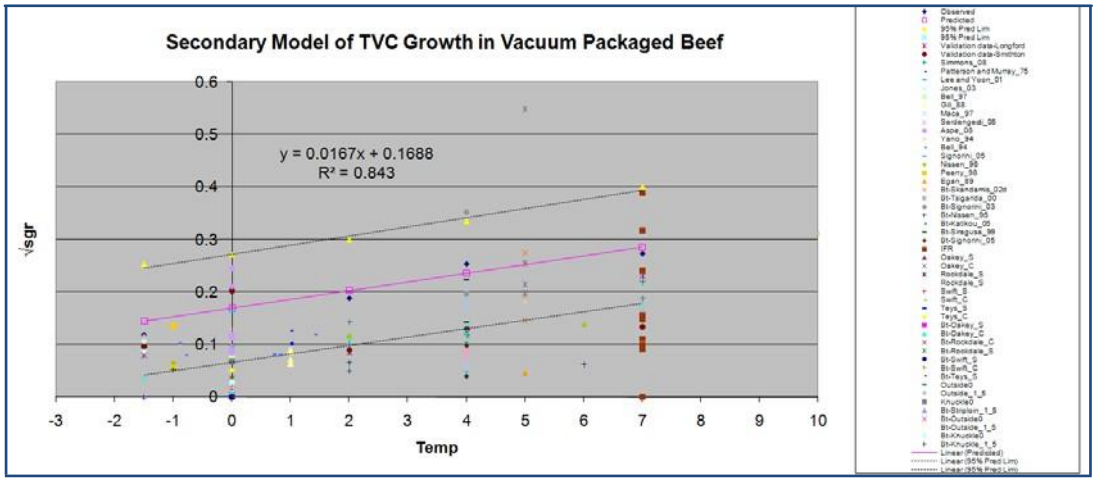


Figure 40. Similar to Fig. 39, except predicted growth rate is also compared to TVC rates calculated from data in ComBase and published literature.

As shown in Figures 38-40, predicted TVC rates from -1.5 to 7°C were consistently greater than rates observed in commercial product.

Figures 41-43 show information analogous to Figures 1-3 except for LAB growth rate. Again, there was a trend of model over-prediction as observed for TVC.

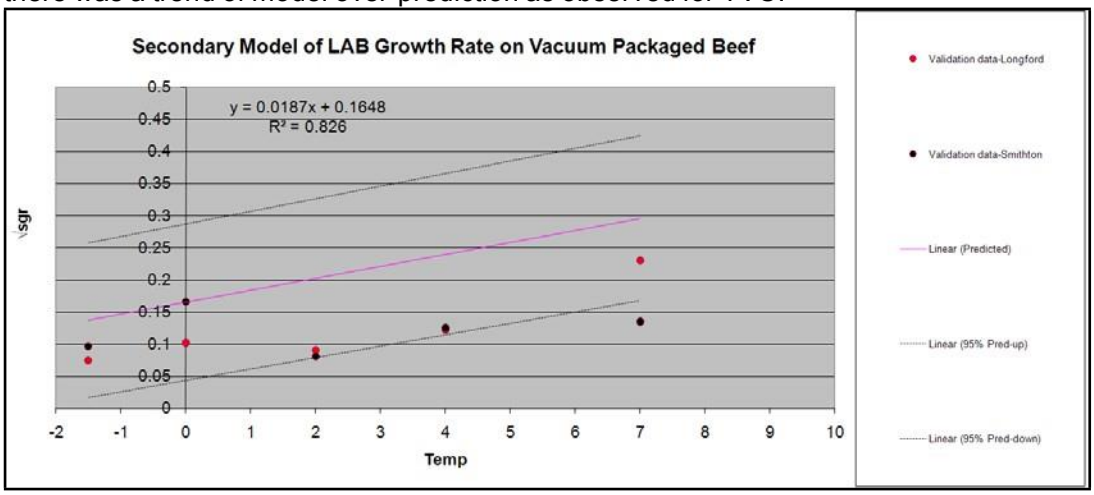


Figure 41. Prediction of LAB growth rate in vacuum packaged striploins. The model is based on LAB rates observed in irradiated striploins inoculated with a 5-strain cocktail of spoilage bacteria. Predicted growth rates are compared to rates observed in commercially packaged striploins.

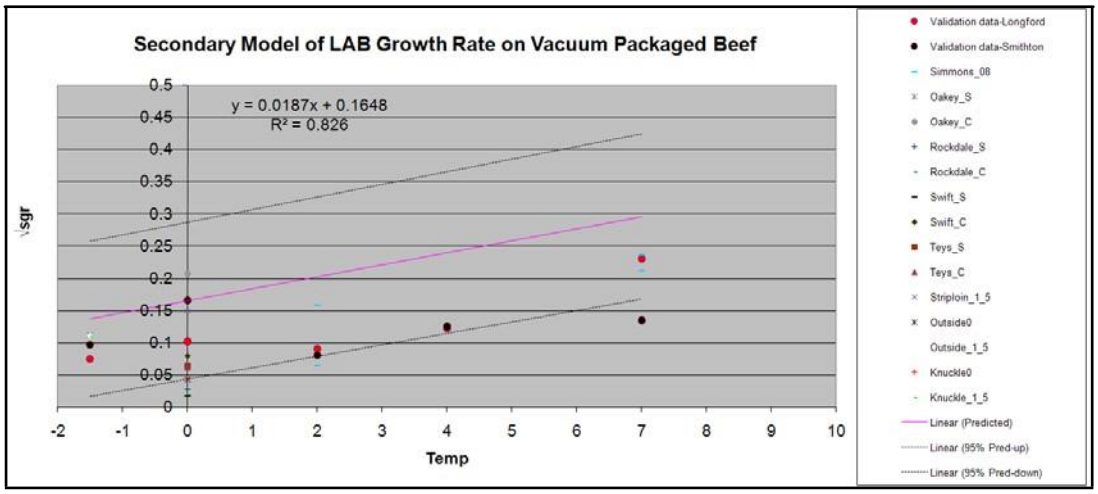


Figure 42. Similar to Fig. 41, except predicted LAB growth rate is also compared to LAB rates reported in studies by FSA and Simmons.

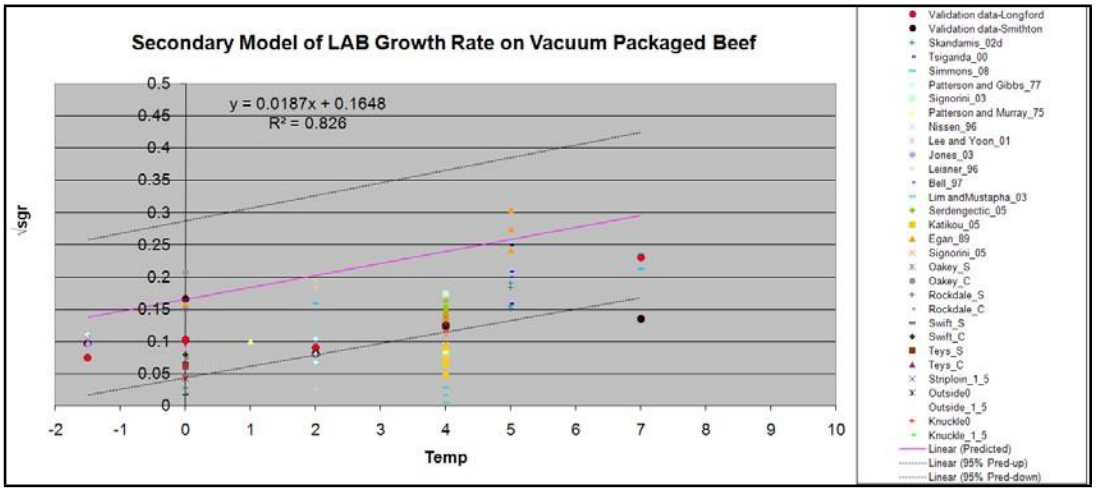


Figure 43. Similar to Fig. 42, except predicted growth rate is also compared to LAB rates calculated from data in ComBase and published literature.

Based on the trend of over-prediction of TVC and LAB growth rate, separate models were produced for TVC and LAB based on growth profiles measured in Sm and Ln packaged striploins (Fig. 44 and 45). Observations of growth rate were, in general, within the 95% prediction limits. LAB growth rates calculated from the data of Egan et al. (1989) were above of the 95% prediction interval (Fig. 44; see [>]). Unlike the other studies that examined natural TVC and LAB flora, these authors inoculated beef with a *Lactobacillus saki* strain and supplemented beef with glucose.

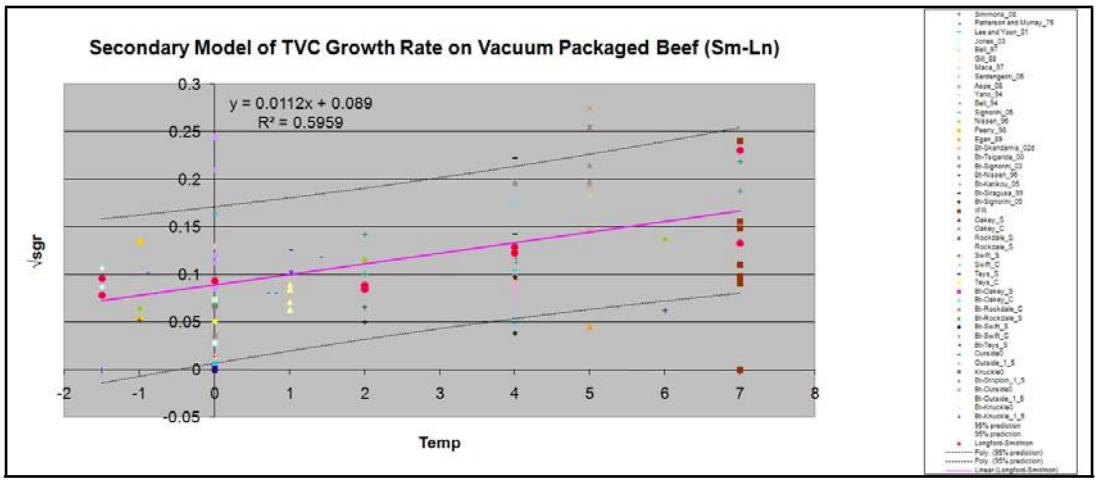


Figure 44. Prediction of TVC growth rate in vacuum packaged striploins. The model is based on TVC rates observed in commercially packaged striploins (Sm and Ln). Growth rates are compared to TVC rates from FSA, Simmons, ComBase and published literature data.

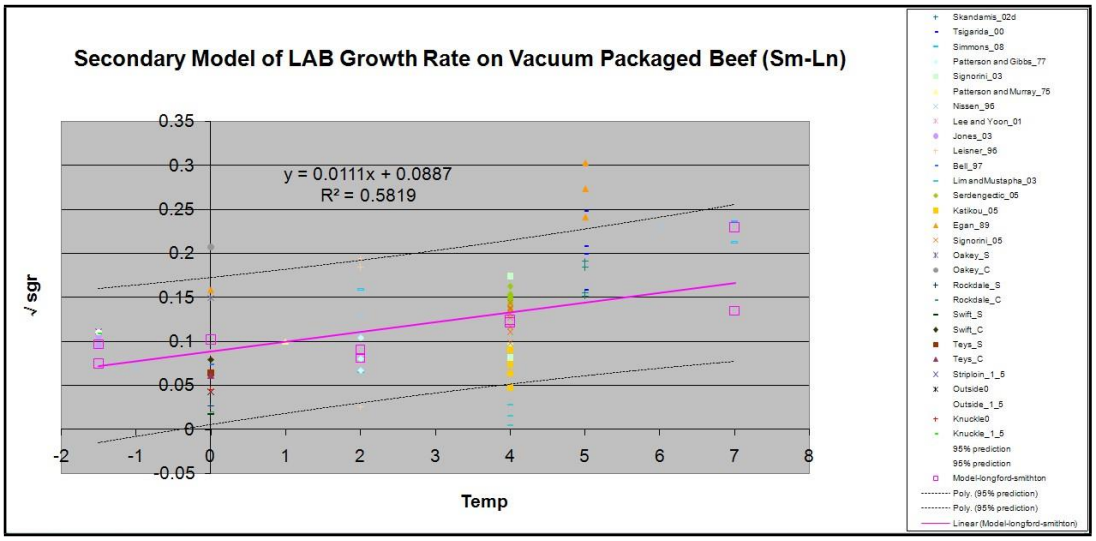


Figure 45. Prediction of LAB growth rate in vacuum packaged striploins. The model is based on LAB rates observed in commercially packaged striploins (Sm and Ln). Growth rates are compared to LAB rates from FSA, Simmons, ComBase and published literature data.

CSIRO (Canon Hill) collected two meat primals (striploin, cube roll) from six abattoirs, which were stored at ~ -0.5ºC for 30 weeks. Growth rates were calculated for TVC and LAB (Table 4). Rates (log CFU/h) ranged from 0.0001 to 0.0008. In contrast, the beef model prediction was higher at 0.003 log CFU/h.

Table 4. Growth rates for TVC and LAB on vacuum-packaged lamb shoulders stored at ~ -0.5ºC. TS=TVC on striploin, TC=TVC on cube roll, LAS=LAB on striploin measured with MRS agar incubated aerobically, LAC=LAB on cube roll measured with MRS agar incubated aerobically, LNS=LAB on striploin measured with MRS agar incubated anaerobically, LNC=LAB on cube roll measured with MRS agar incubated anaerobically).

Abattoir	Test Method					
	TS	TC	LAS	LAC	LNS	LNC
AB1	0.0008	0.0004	0.0007	0.0005	0.0008	0.0005
AB2	0.0006	0.0008	0.0005	0.0006	0.0005	0.0008
AB3	0.0006	0.0006	0.0005	0.0001	0.0003	0.0000
AB4	0.0008	0.0005	0.0010	0.0004	0.0007	0.0005
AB5	0.0005	0.0006	0.0002	0.0003	0.0006	0.0007
AB6	0.0004	0.0002	0.0003	0.0001	0.0006	0.0004
Prediction	0.0030					

Production of model interface
A model interface for estimating TVC and LAB growth rates and kinetic changes in microbial levels from -1.5 to 7ºC was produced.

Comparison of methods for measuring bacterial counts on lamb and determine growth of micro organisms at various storage temperatures up to day-35 of storage [extended to end of shelf-life]
Vacuum-packaged lamb shoulders were stored at -1, 2 and 7ºC. Kinetic profiles of TVC and LAB counts, and comparisons among the different test methods are shown in Figures 46-48.

In general, regardless of temperature, TVC counts were highest by the Japanese method, followed by the Australian, Danish and IDF (Fig. 46-48, panel A). When Japanese counts were adjusted to CFU/cm^2 they more closely approached Australian counts, but were generally higher (Fig. 46-48, panel C). These differences are better illustrated in Figures 46-48 in panel E, where the difference in TVC count (from the perspective of the Australian method) among the Japanese, Danish and IDF methods are plotted. It should be noted that counts by the Japanese method were adjusted to CFU/cm^2 for the latter comparisons. Closer examination of panel E shows that at the early phase of storage, Japanese counts were usually higher, and the IDF lower, than the Australian method. Both of these methods appeared to converge to the Australian count with extended storage time, although the Japanese method produced lower counts after 35-40d.

For LAB counts, the Japanese method (ie BCP agar) generally displayed higher counts than MRS agar at less than 35d, regardless of whether MRS was incubated aerobically or anaerobically (Panel B, Fig. 46-48). Panel D shows Australian MRS counts with Japanese counts adjusted to CFU/cm^2. Panel F compares each method to the Australian MRS anaerobic method. At 2 and 7°C, the BCP method showed a downward trend in over-prediction with increasing storage time. Few differences were observed between counts measured on MRS incubated aerobically or anaerobically.

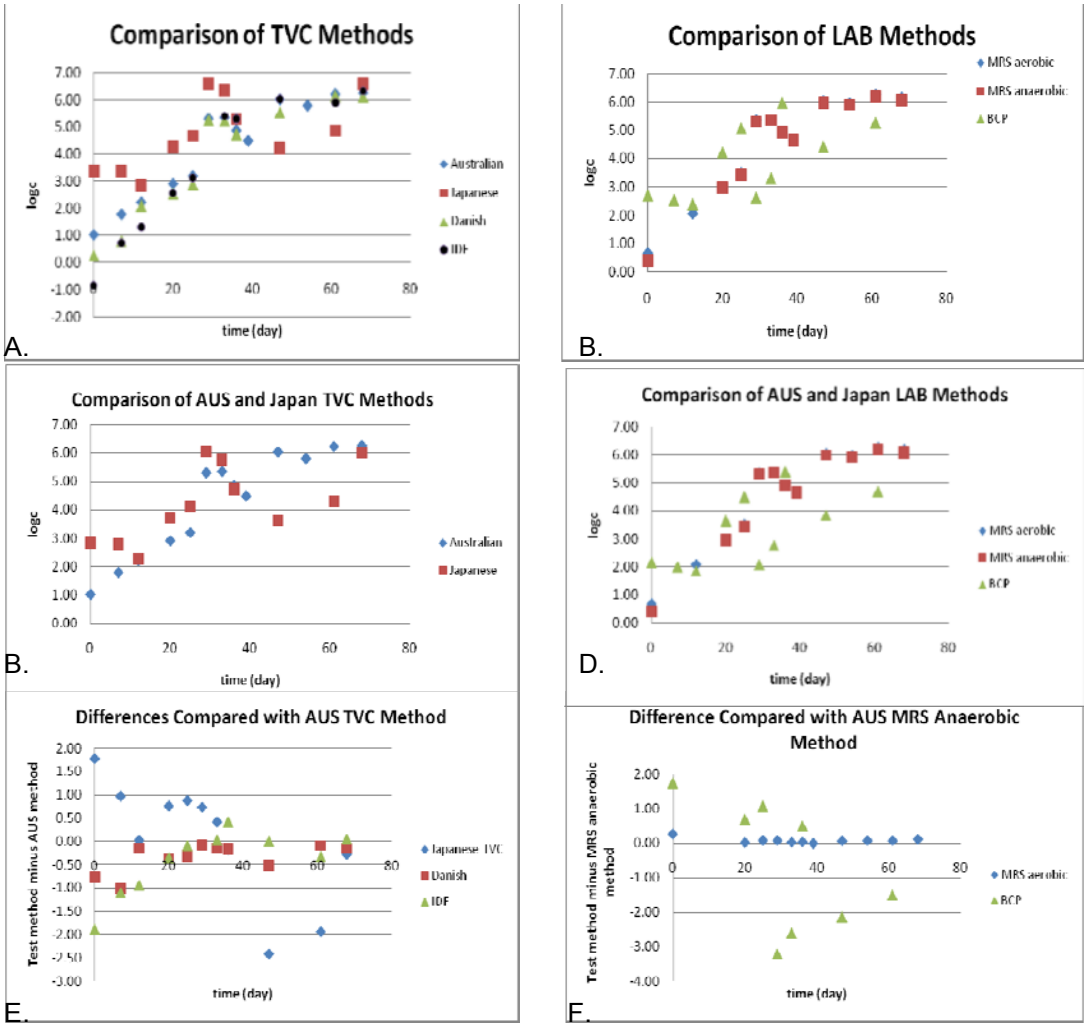


Figure 46. Comparison of TVC (A, C, E) and LAB (B, D, F) counts at -1°C among all methods (A, B) and Australian versus adjusted Japanese method (C, D). In addition, the difference in $\log_{10}$ counts among all TVC methods was compared to the Australian method (E) and LAB counts compared to the Australian MRS anaerobic method (F).

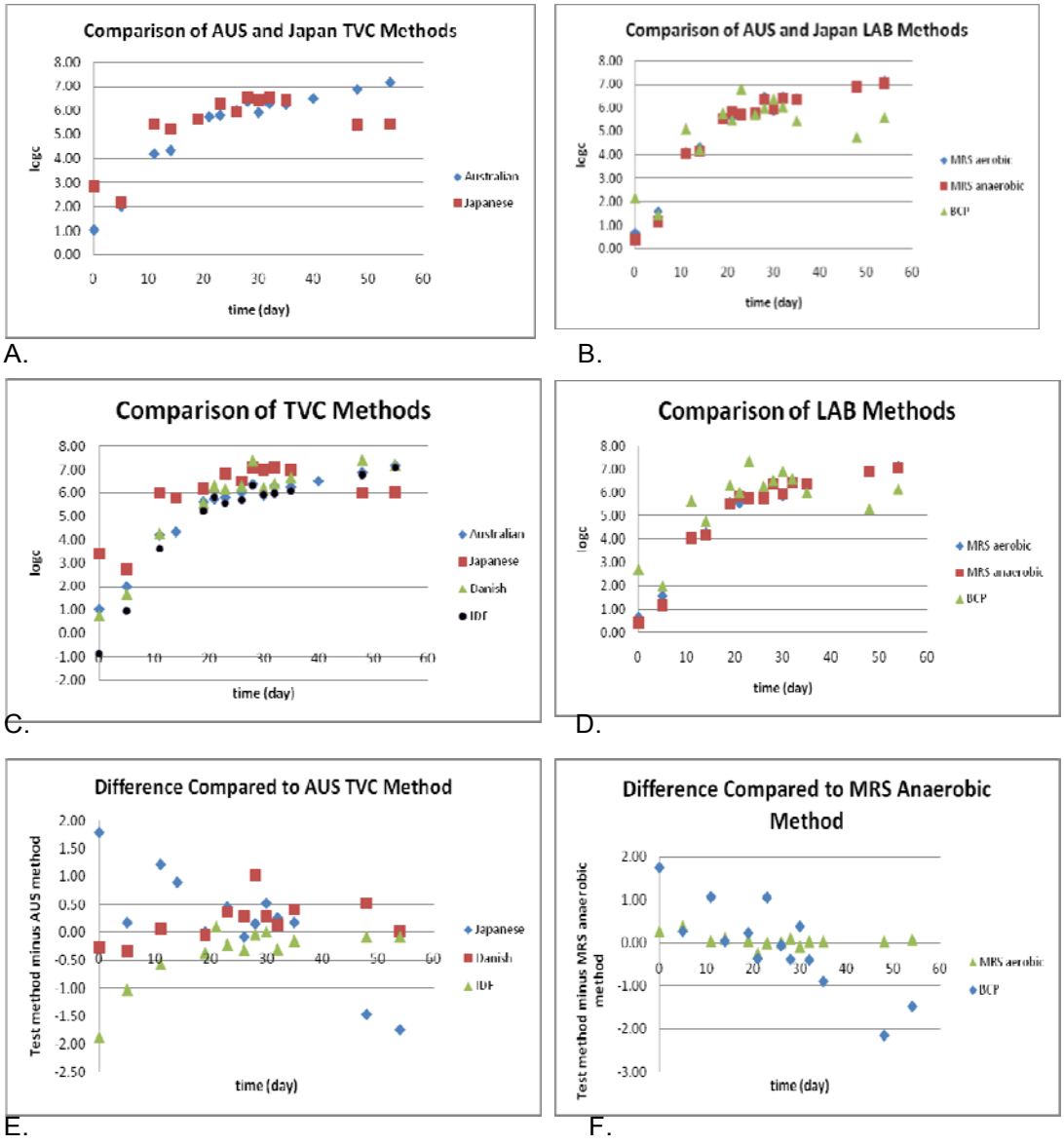


Figure 47. Comparison of TVC (A, C, E) and LAB (B, D, F) counts at 2°C among all methods (A, B) and Australian versus adjusted Japanese method (C, D). In addition, the difference in $\log_{10}$ counts among all TVC methods was compared to the Australian method (E) and LAB counts compared to the Australian MRS anaerobic method (F).

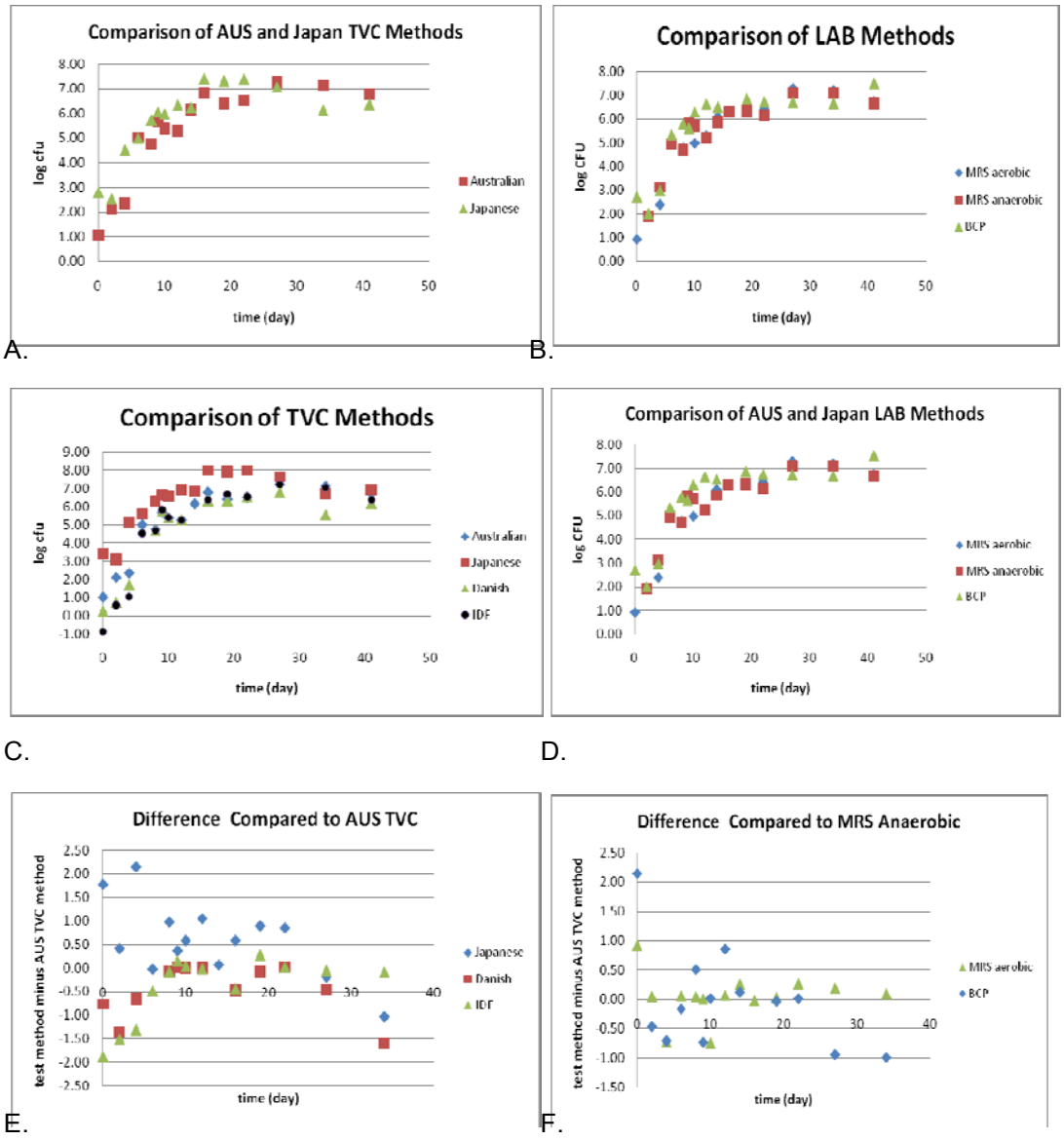
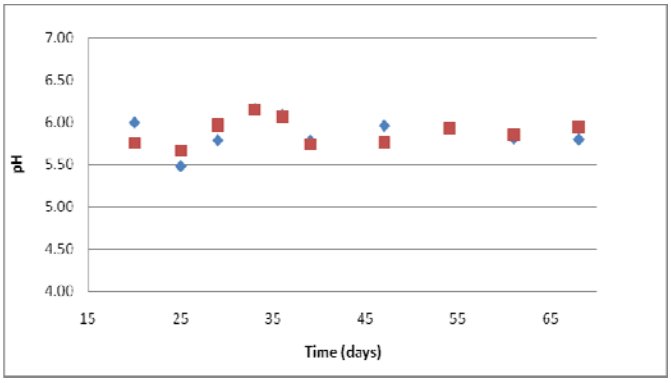


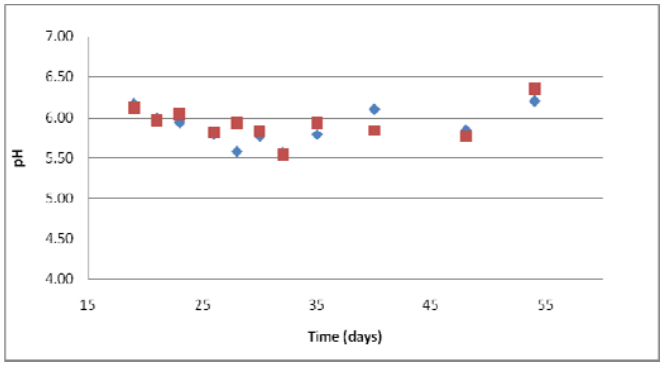
Figure 48. Comparison of TVC (A, C, E) and LAB (B, D, F) counts at 7°C among all methods (A, B) and Australian versus adjusted Japanese method (C, D). In addition, the difference in $\log_{10}$ counts among all TVC methods was compared to the Australian method (E) and LAB counts compared to the Australian MRS anaerobic method (F).

The pH of vacuum-packaged lamb shoulders was approximately 6.0 throughout the storage period at -1, 2 and 7°C (Fig. 49).

A



B



C

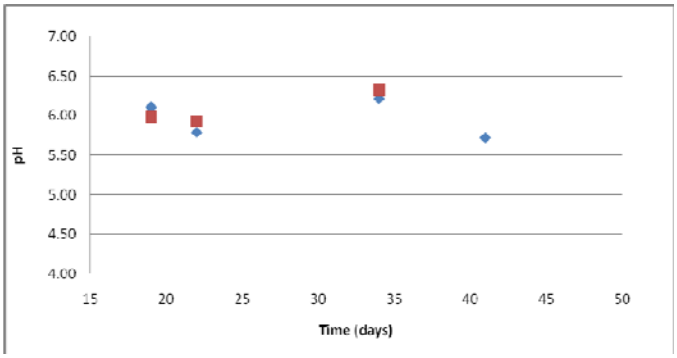


Fig. 49. pH of lamb stored at -1 (A), 2 (B), and 7°C (C). Measurements were made on two separate pieces of lamb (blue=replicate 1; red=replicate 2).

Validation of beef model in vacuum-packaged lamb shoulders

The kinetic parameters for TVC and LAB counts on lamb shoulders (described above) were translated into secondary models and growth rates compared to the beef model prediction (Table 5). Growth rates on lamb were approximately twice that predicted for beef.

Table 5. Growth rates of bacteria on lamb shoulders measured by TVC and MRS methods. Values in bold are beef primal model prediction.

	Storage temperature (°C)		
Test Method	-1	2	7
TVC	0.005 **0.003**	0.010 **0.005**	0.020 **0.012**
MRS-aer	0.005	0.011	0.021
MRS-anaer	0.006 **0.003**	0.012 **0.005**	0.023 **0.012**

5 References

ABARE (2007). Australian Commodities, December Quarter Edited by A. Wright. Canberra.

Borch, E., KantMuermans, M. L. & Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology* **33**, 103-120.

Fleet, G. H. (1999). Microorganisms in food ecosystems. *International Journal of Food Microbiology* **50**, 101-117.

Fontana, C., Cocconcelli, P. S. & Vignolo, G. (2006). Direct molecular approach to monitoring bacterial colonization on vacuum-packaged beef. *Applied and Environmental Microbiology* **72**, 5618-5622.

Jones, R. J. (2004). Observations on the succession dynamics of lactic acid bacteria populations in chill-stored vacuum-packaged beef. *International Journal of Food Microbiology* **90**, 273-282.

Lee, K. T. & Yoon, C. S. (2001). Quality changes and shelf life of imported vacuum-packaged beef chuck during storage at 0 degrees C. *Meat Science* **59**, 71-77.

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

Nissen, H., Sorheim, O. & Dainty, R. (1996). Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. *Food Microbiology* **13**, 183-191.

Nottingham, P. M. (1982). Microbiology of Carcass Meats. In *Meat Microbiology*, pp. 13-65. Edited by M. H. Brown. England: Applied Science Publishers Ltd.

Nychas, G.-J. E. & Skandamis, P. N. (2005). Fresh meat spoilage and modified atmosphere packaging (MAP). In *Improving the safety of fresh meat*, pp. 461-502. Edited by J. N. Sofos. Boca Raton: CRC Press.

Rademaker, J. L. W., Hoolwerf, J. D., Wagendorp, A. A. & te Giffel, M. C. (2006). Assessment of microbial population dynamics during yoghurt and hard cheese fermentation and ripening by DNA population fingerprinting. *International Dairy Journal* **16**, 457-466.

Richardson, K. C. (2001). Preservation of Foods Relying Mainly on Chilling. In *Spoilage of Processed Foods: Causes and Diagnosis*. Edited by F. M. Group. NSW: AIFST.

Sakala, R. M., Hayashidani, H., Kato, Y., Hirata, T., Makino, Y., Fukushima, A., Yamada, T., Kaneuchi, C. & Ogawa, M. (2002). Change in the composition of the microflora, on vacuum-packaged beef during chiller storage. *International Journal of Food Microbiology* **74**, 87-99.

Smith, C. J., Danilowicz, B. S. & Meijer, W. G. (2007). Characterization of the bacterial community associated with the surface and mucus layer of whiting (Merlangius merlangus). *FEMS Microbiology Ecology* **62**, 90-97.