# final report

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# Lactic Acid application to extend shelf life of long aged chilled lamb racks

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# Executive Summary

This project has been developed following feedback from a customer who reported seemingly sporadic, unpredictable spikes in microbiological counts on long aged vacuum packaged lamb product. A comprehensive review of post-slaughter processes was initiated, including chilling practices, cold chain integrity, and sanitation and hygiene procedures. Though no causative factors could be identified. However, previous work identified that application of 5% lactic acid into bags prior to vacuum packing could reduce microbial counts on products. Consequently, a trial was set up to:

1. Develop and assess a commercially adaptable, reliable system of delivering lactic acid application to barrier bag directly before product packaging using an automatic bag loader, and
2. Increase shelf-life of lamb racks by applying a 5% lactic acid solution and improve the quality of lamb racks at the end of the stated shelf life.

To achieve these objectives an automatic taped bag loader was modified by mounting a spray nozzle into the unit which allowed a lactic acid solution to be sprayed into inflated bags immediately prior to placing the lamb racks inside the inflated bag. The footprint of the bag loading machine was unchanged, and testing of the unit indicated that the time to pack racks into bags was similar for when the spray was turned off or on. The machine operated reliably throughout the trial and was consequently considered to have commercial potential.

Cap-off 50mm frenched lamb racks, each containing 8 ribs, were packed using the machine. Eighty racks were placed into bags that had been sprayed with 5% lactic acid (treated) and 80 racks were packed without lactic acid (control) – racks were similar in weight and pH. Initial total viable counts were 2,600 cfu/g on average.

Racks were then stored for up to 105 days at an average temperature of -1.51°C. Five racks per treatment group were removed from storage at weekly intervals and tested for Total Viable Counts (TVC), Lactic acid bacteria (LAB), Enterobacteriaceae and *Clostridium perfringens*. *C. perfringens* was not detected in any samples, and Enterobacteriaceae were detected in 2 treated and 10 control samples. Microbial growth was limited in both treatment groups during the first 40 days due to cold storage temperatures (-2.0°C on average), and LAB were largely absent during this period. Similar growth was subsequently observed in both treatment groups, reaching levels of around $10^7$ cfu/g by 90-100 days. The lack of organoleptic assessments makes it impossible to ascertain the quality, and hence acceptability, of racks at the end of the trial (105 days). However, using inputs from this trial and temperatures from a shipment, predictive models indicate that a shelf-life of 85 days is achievable without lactic acid, provided good temperature control is maintained.

It is recommended that future shelf-life trials include sensory assessment to ascertain quality and acceptability of the product, which may be achievable by undertaking microbiological testing in-house, rather than outsourcing. In addition, shelf-life specifications, especially organoleptic, should be negotiated with the customer, as microbiological shelf-life indicators are of limited use in establishing end of shelf-life, especially under good temperature control.

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# 1 Background

This project has been developed following feedback from a customer who reported seemingly sporadic, unpredictable spikes in microbiological counts on long aged chilled vacuum packaged lamb product. Amongst the range of products tested the lamb racks received by this customer returned elevated microbiological results the most frequently. In an effort to understand the factors that may have contributed to the elevated microbiological results, the company who initiated this project conducted a comprehensive review of post-slaughter processes including chilling practices, cold chain integrity, and sanitation and hygiene procedures. This review provided valuable insight and developments in how the products sent to this customer are produced and improvements were implemented in the production of these products. However the unexplainable elevated microbiological results provided by the customer continued to be reported despite improving the processing practices. This indicated that factors outside of the control of the company during processing may affect the microbiological load of products towards the end of their shelf life.

During a visit to the customer, company representatives identified that the customer was opening the packaged racks and treating these with lactic acid before repackaging and sending the products for retail. The customer indicated they would support the development of a system where lactic acid was applied to the racks at packaging in Australia, prior to sea freighting the product to the customer. This could remove the need for the customer to open and treat the product on arrival.

The use of chemical intervention to decrease microbiological contamination is common place in the beef industry. However, the research is closely centred on using a chemical control process to decrease contamination at, or directly following slaughter, on primal cuts prior to boning, or for meat to be used for manufacturing.

Research involving sheep meat appears to be largely limited to using chemical interventions (such as lactic acid) to improve the shelf life of fresh sheep meat or to decontaminate the whole carcase following slaughter. There is no information on the effect on shelf life that chemical intervention can have on long aged chilled lamb products (40 days or more).

During 2013, a small scale product trial was undertaken using multiple products that were sent overseas to the customer providing the microbiological feedback. These products included short-loin pairs, boneless legs, hand frenched racks and water frenched racks. These products were used in the small trial to determine the best concentration of lactic acid solution needed to see a result on long aged chilled vacuum packaged lamb products. The products in this trial were treated with 1%, 3% and 5% lactic acid solution. There were also control products where no treatment was given. There appeared to be little difference in the overall microbiological load of the product tested with the 1% and 3% lactic acid solution. The 5% dilution produced a noticeable reduction in Total Viable Counts (TVC) for hand racked product at 82 days. This has provided the basic structure for extending the previous trial work with this PIP in developing a commercially viable method with reliable application of a known quantity of lactic acid solution.

One direct method of delivering an automated commercial application of a lactic acid solution at the point of packaging is being developed as part of this project. This method is the delivery of a fine mist of lactic acid solution through a spray nozzle system mounted to an automatic bag loading machine. The mist coats the inside of the packaging and is transferred onto the surface of the meat once it has been placed inside the bag. If this method is proven to be effective at delivering the lactic acid solution to coat the meat products, it may result in an antimicrobial effect. This may aid in decreasing the elevated microbiological results reported by the customer, thus improving the quality of the product they receive and the shelf life of the products and result in commercial gains.

# 2 Projective Objectives

The objectives for this project were as follows:

1. Increase shelf-life of lamb racks treated with 5% lactic acid Solution.
2. Develop a commercially adaptable, reliable system of delivering lactic acid application through applying to barrier bag directly before product packaging.
3. Develop a commercially adaptable, reliable system of delivering lactic acid application through automatic bag loaders.
4. Improve quality of product at the end of a product’s stated shelf life.

# 3 Methodology

## 3.1 Trial Design

The trial consisted of 160 frenched lamb racks with cap removed and a frenched portion of 50mm, each containing 8 ribs. The racks were divided into two treatment groups, 80 control and 80 treated with 5% Lactic Acid solution. All 160 racks were collected from lambs slaughtered on 24$^{th}$ August 2015. The carcases were chilled overnight and the racks recovered and packaged on 25$^{th}$ August 2015.

Immediately prior to packing, racks were weighed and a pH measurement taken (TPS Aqua pH/Temperature Meter with an Ionode IJ44C combination pH Electrode).

## 3.2 Product Packing and Treatment Application

The machine used in this project was an Automatic Taped Bag Loader, Model number BL19M2-V1, supplied by Cryovac. There is no human contact with the inside surface of the bag during packaging. The bag loader machine works by using a jet of air to inflate the bag, allowing a person to feed the cut of meat into the bag, peal the loaded bag off the tape and away from the machine and the machine loads the next bag and inflates it. The packaged meat is then vacuumed and sealed.

The bag loader machine used in this project was modified by mounting a spray nozzle onto it that pulsed a fine mist of lactic acid solution into the bag immediately before product insertion, and thus coating the product during vacuum packing.

The lactic acid was purchased as an 88% solution (L(+) Lactic Acid 88% - 25kg from Redox Pty Ltd) and diluted to 5% prior to application, using 56.8 ml of lactic acid solutions and 943.2 ml of purified water.

The commercial viability of the automatic bag loader machine applying the lactic acid solution was assessed by evaluating if the bag loader machine can sustain throughput that matches production. To assess this, the bag loader machine was used without the spray nozzle activated, this emulates normal production conditions. The machine was used again with the spray nozzle system activated to deliver the lactic acid solution to the treatment group of racks. The time taken to package the racks was measured for both treatment groups by using a stop watch to measure the time taken to load 10 consecutive bags. This was repeated three separate times, using treated and control racks to assess if there was a significant impact on time taken to package the racks when using the spray nozzle system.

## 3.3 Product Storage

Both the control and the lactic acid treatment group of racks were treated the same during storage throughout the project. The individually vacuum packaged racks were stored in a lid and base style carton with 10 racks per carton. A data logger (Model TC, Thermocron) was used to monitor refrigeration temperature at 20-minute intervals throughout the trial. The logger was placed in the thermal centre of a carton of product. The control racks and the treated racks were stored under refrigerated conditions in the same refrigerator.

Five control racks and five treated racks were removed from storage at weekly intervals ranging from Day 0 (production date; immediately after packing) to Day 105 (15 weeks post production). The samples were packed into eskys with freezer blocks and transported overnight to the laboratory.

A summary of the dates at which samples were removed from storage and were transported to the laboratory, and the microbiological testing dates is shown in Table 1.

*Table 1: Summary of sampling and testing dates.*

| Week | Sent to lab | Date tested | | Week | Sent to lab | Date tested |
|---|---|---|---|---|---|---|
| **0** | 25/08/2015 | 26/08/2015 | | **8** | 20/10/2015 | 21/10/2015 |
| **1** | 1/09/2015 | 2/09/2015 | | **9** | 27/10/2015 | 28/10/2015 |
| **2** | 8/09/2015 | 9/09/2015 | | **10** | 3/11/2015 | 4/11/2015 |
| **3** | 15/09/2015 | 16/09/2015 | | **11** | 10/11/2015 | 11/11/2015 |
| **4** | 22/09/2015 | 23/09/2015 | | **12** | 17/11/2015 | 19/11/2015 |
| **5** | 29/09/2015 | 30/09/2015 | | **13** | 24/11/2015 | 25/11/2015 |
| **6** | 6/10/2015 | 7/10/2015 | | **14** | 1/12/2015 | 2/12/2015 |
| **7** | 13/10/2015 | 14/10/2015 | | **15** | 8/12/2015 | 9/12/2015 |

## 3.4 Microbiological Testing

On arrival at the NATA accredited laboratory the product temperature was measured. Bags were opened aseptically and a 10g excision sample removed and diluted in Buffered Peptone Water. An aliquot was removed, according to the test method and organisms tested. The following microbiological tests were undertaken:

- Total Viable Count (TVC) (AS 5013.1): Incubated at 30°C+/-1°C for 72hrs +/-3hrs under aerobic conditions.
- Lactic Acid Bacteria (LAB) (APHA 2001): Incubated at 30°C+/-1°C for 72hrs +/-3hrs under microaerophilic conditions.
- Enterobacteriaceae (AS 5013.8-2004): Incubated at 37°C+/-1°C for 24hrs +/-2hrs under aerobic conditions.
- Clostridium perfringens (AS/NZ 4276.17.1:2000): Incubated at 37°C+/-1°C for 18-24hrs under anaerobic conditions.

### 3.5 Organoleptic assessments

As sensory expertise was not available at the microbiological laboratory, it was not possible to undertake organoleptic assessment of the racks after storage. Consequently, product spoilage was assessed using the MLA/UTAS meat spoilage predictor.

### 3.6 Data analysis

Differences in average product weight and pH were assessed using a Welch's two-sample t-test (Moore and McCabe, 1998).

Microbial counts – TVC and LAB – were log transformed and plotted over time and visually assessed.

# 4 Results

### 4.1 Product Packing and Treatment Application

Video footage, taken of the bag loader with and without the spray nozzle system in operation indicates that the loading of bags onto the machine and the packing of the product occurred without delay to production.

Additionally, the time taken to package ten racks was recorded at three separate occasions and these are shown in Table 2. From these times it can be seen that loading/packing racks into untreated bags (control) was marginally faster than loading/packing racks into bags sprayed with lactic acid. This difference was not considered to be of practical importance.

*Table 2: Time taken to pack racks, with and without lactic acid spray into the bag*

| Treatment Group | Time (seconds) | Number of Racks | Time/rack (seconds) | Average (seconds) |
|---|---|---|---|---|
| **Lactic Acid** | 37.6 | 10 | 3.76 | 4.2 |
| **Lactic Acid** | 43.2 | 10 | 4.32 | |
| **Lactic Acid** | 45.2 | 10 | 4.52 | |
| **Control** | 36.0 | 10 | 3.60 | 3.9 |
| **Control** | 36.0 | 10 | 3.60 | |
| **Control** | 44.3 | 10 | 4.43 | |

Control racks weighed an average of 638.5g (SD = 45.2g) and treated racks weighed an average of 650.1g (SD = 46.8g). The difference in average weight was not statistically significant (P-value = 0.11), nor of practical importance, i.e., control and treated racks were comparable.

### 4.2 pH

Box plots of the pH levels of the control and the treated lamb racks immediately prior to packing are shown in Figure 1. The average pH of control racks was 5.86 and of that treated racks was 5.75, and the difference was statistically significant (P-value < 0.001). The practical importance of this difference is questionable, though clearly some control racks were high in pH (> 6), which may limit shelf-life.

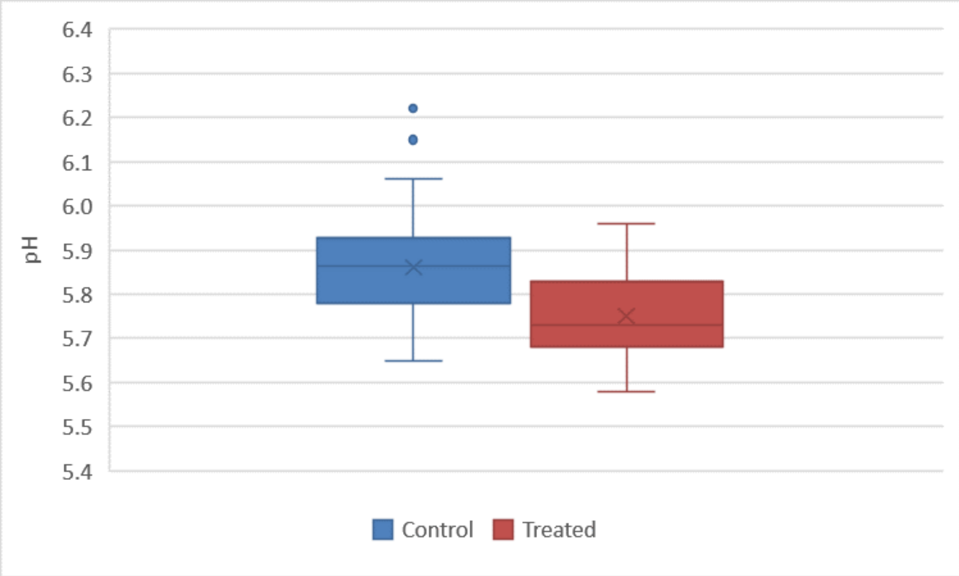


*Figure 1: Box plots of pH of control and treated racks, immediately prior to packing.*

### 4.3 Storage temperature

On completion of the storage phase, the data logger was removed, the records downloaded onto a PC and the temperature trace is shown in Figure 2. Within the first 12 to 24 hours the temperature had dropped to 2.1°C and 0.01°C, respectively. The average temperature throughout the trial was -1.51°C, though clearly it was colder during the first 40 days (-2.0°C; excluding the initial 24 hours).

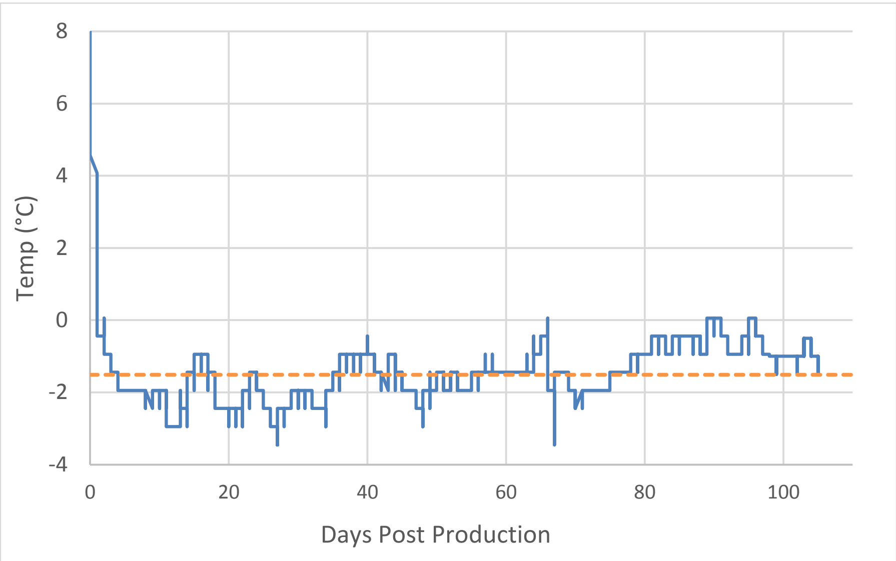


*Figure 2: Temperature trace of product throughout the storage trial; average temperature throughout the trial is shown as a dashed line.*

## 4.4 Microbiological results

*Clostridium perfringens* was not detected in any of the samples. Enterobacteriaceae were detected ten times in control racks and twice in treated racks. In control racks the counts ranged from 10 cfu/g (8 days post production – DPP) to 3,600 cfu/g (105 DPP), while treated racks ranged from 30 (64 DPP) to 260 (86 DPP).

Scatter plots of TVC and LAB are shown in Figure 3 for control racks and in Figure 4 for treated racks. From Figure 3 it can be seen that TVC in control racks appears to show a group of unusually high TVC counts around 40 days (highlighted). The reason for this pattern is unknown, though it may be possible that they relate to unobserved confounding factors that were not taken into account in the trial design. Except for these data points, there appears to be a long lag phase, before growth is observed around 50-60 days. Treated racks also show a long lag phase for TVC, with no growth occurring until about 60 days, at which time rapid growth becomes evident (with large variability noted in TVC counts between different racks).

With respect to LAB, these are noticeably absent during the initial shelf-life period – about 40 days for control racks and 60-80 days for treated racks. Lactic acid bacteria grow under anaerobic conditions and generally become dominant after about 40 days of storage (depending on temperature). The almost complete lack of LAB growth, together with the limited growth of TVC during the first 40 days is likely related to the cold storage temperatures during the initial stages of the trial.

With exception of the high TVC counts around 40 days in the control group, the TVC levels for both groups look similar, as do LAB levels after about 70-80 days.

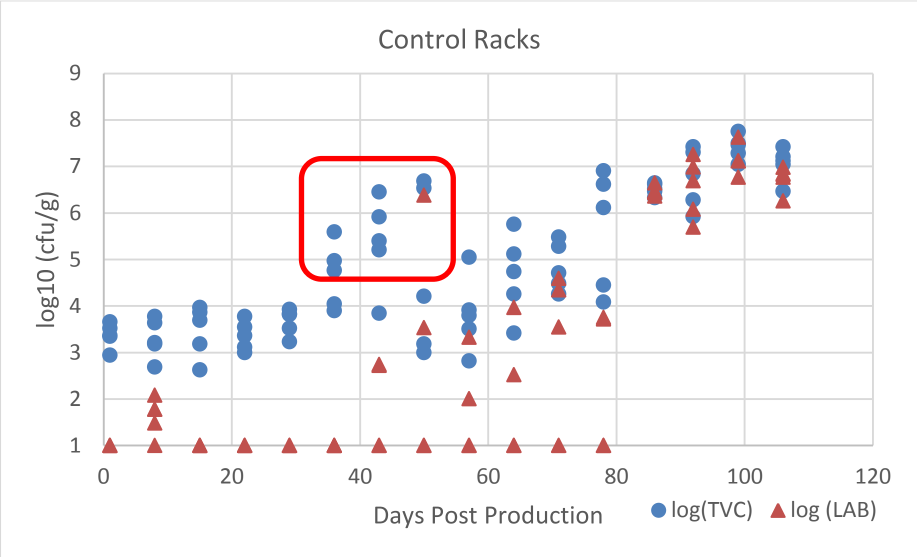


*Figure 3: Scatter plot of log(TVC/g) and log(LAB/g) on control racks over time; points at '1' indicate samples with counts below the limit of detection.*

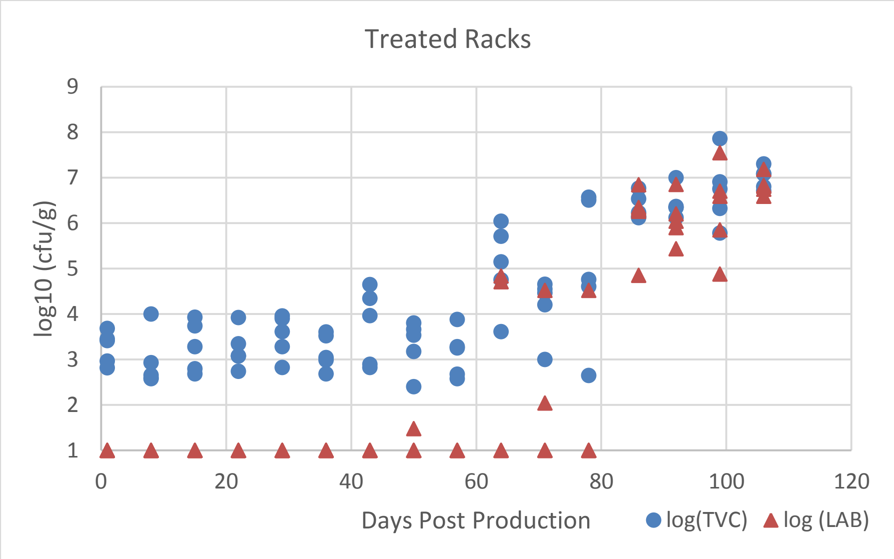


*Figure 4: Scatter plot of log(TVC/g) and log(LAB/g) on treated racks over time; points at '1' indicate samples with counts below the limit of detection.*

## 4.5 Predicted shelf-life using the MLA / UTAS meat spoilage predictor

As noted above, organoleptic assessment of lamb racks was not possible. Therefore, the MLA / UTAS meat spoilage predictor was used to predict the shelf-life of control racks – no prediction is possible for treated racks as the model has not been validated for this situation.

Since the model accepts a maximum of 2,400 temperature points, every forth temperature record was extracted from the logger trace (i.e. temperatures every 80 minutes), and inserted into the model. A starting microbial level of 2,600 cfu/cm$^2$, reflecting the average TVC count one day after packing[1], was entered into the model – the graphical output is shown in Figure 5. In addition, the model indicates a remaining shelf-life of 33 days at the average temperature of -1.51°C, or 17 days at the preferred storage temperature of -0.5°C.

In addition, data from a temperature logger that was included in an overseas shipment was available for entering into the model. The model output is shown in Figure 6, again assuming starting TVC levels of 2,600 cfu/cm$^2$. The temperature trace indicates that temperatures were well controlled during this shipment, with an average temperature of -1.41°C, which is similar to the temperatures during the storage trial (except for a small spike during the last 24 hours, possibly related to product receival and unpacking). The model predicts that during the 40 days of transport, microbial levels would have increased by 2.8 log cfu/cm$^2$, or about 630-fold, with 92 days of shelf-life remaining if product was stored at -1.41°C. Even at the preferred storage temperature of -0.5°C a remaining shelf-life of 60 days is predicted.

[1] Assuming a 1-1 relationship between weight and surface area.

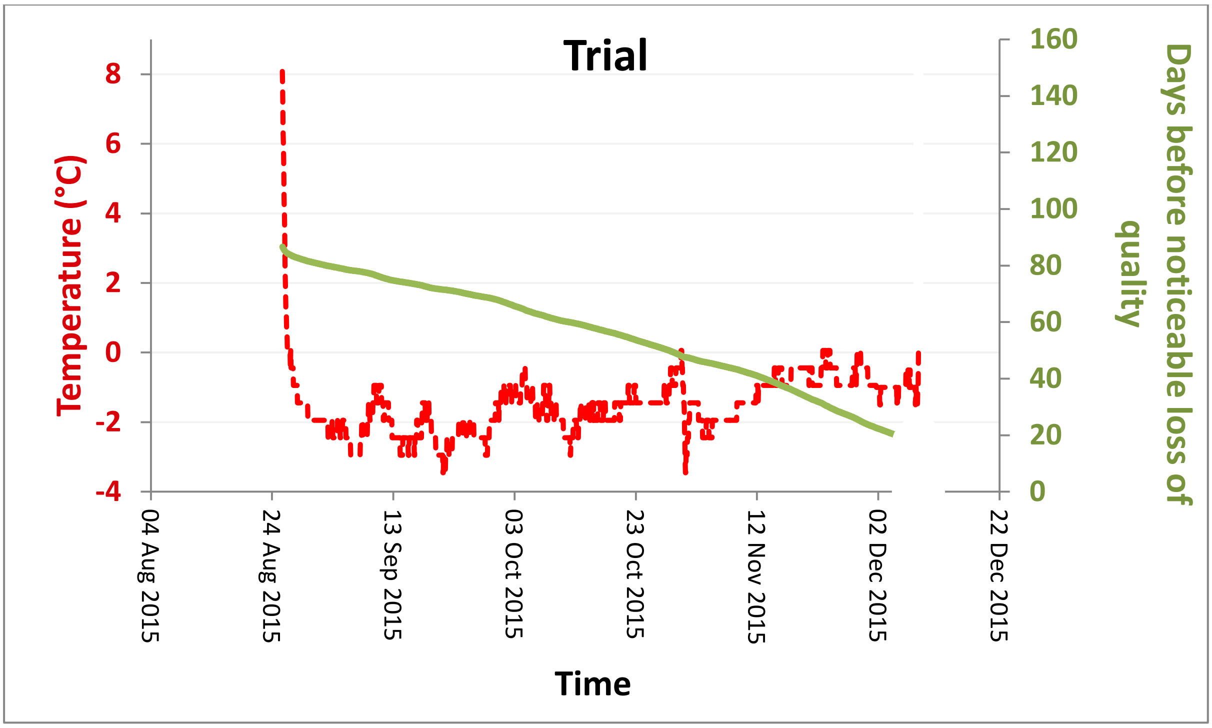


*Figure 5: Temperature trace and expected days until noticeable loss of quality for the shelf-life trial (MLA/UTAS meat spoilage predictor).*

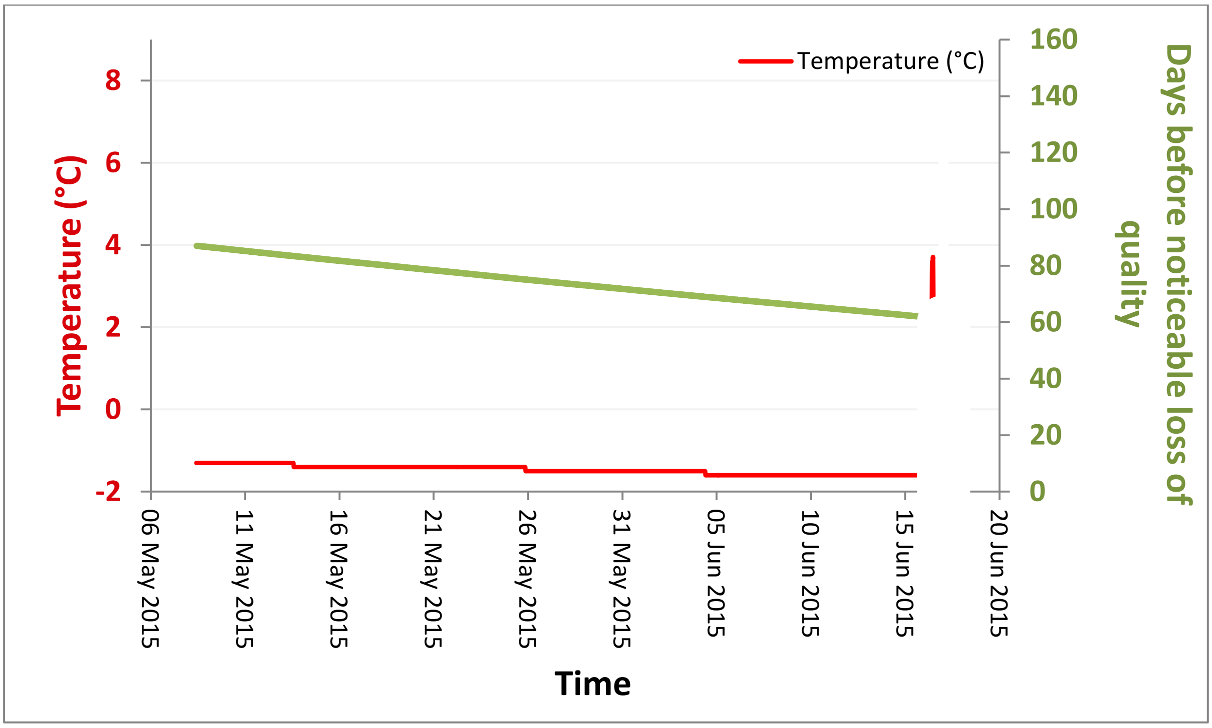


*Figure 6: Temperature trace and expected days until noticeable loss of quality for an overseas shipment (MLA/UTAS meat spoilage predictor).*

# 5 Discussion

## 5.1 Develop a commercially adaptable, reliable system of delivering lactic acid

The method of using a spray nozzle system mounted on an automatic bag loading machine to spray lactic acid solution into a vacuum bag prior to placing a lamb rack in the bag has been shown to be a potentially commercially adaptable system. The spray nozzle system is mounted below the sensor on the bag loading machine that controls the tension of the tape. As a bag is removed the sensor is triggered to apply tension to the tape, which moves the bags on the tape, the sensor then registers that it is covered by a new bag. As the new bag is moved passed the sensor a timer in the spray nozzle system is triggered, which was set to wait 3 seconds to allow for inflation of the bag and for the packer operator to acquire the rack of lamb. Following the 3 second time lapse, the spray nozzle sprays a fine mist of the lactic acid solution into the bag, the operator places the rack into the bag and removes the bag from the tape. The packaged product is then vacuum sealed.

The process of placing the rack into the bag did not change for the operator between the control group and the lactic acid treatment group and the time to undertake this operation was comparable between the two treatment groups.

Problems were encountered when the taped bags had been stored folded in the carton and the bags had a crease present which resulted in the bag not inflating immediately. When this occurred the operator was required to open the bag up, wait for the spray application of the lactic acid solution then place the rack in the bag. If the operator did not open the bag within 3 seconds, then the lactic acid solution would be sprayed and would either miss the semi-inflated bag or would partially be applied inside the semi-inflated bag. This is not a major issue as a manual override button on the operating console can be activated to prompt the machine to spray another quantity of liquid. However, the spray may contaminate surfaces surrounding the machine when not contained inside the intended vacuum bag.

The spray nozzle system can be removed and the bag loader used as normal, i.e. prior to the installation of the spray nozzle system. The spray nozzle is able to be fitted to the bag loader machine without affecting the machine’s integrity or commercial operating speed potential. The system has the capability for different spray nozzles to be installed to change the mist size and range of the lactic acid sprayed into the bag. The delay time of the spraying can also be changed, as can the quantity delivered. All these adaptable factors will help to refine the system to fit into different production requirements. This system appears to be a reliable method of delivering lactic acid application through automatic bag loaders to keep up with commercial speeds of rack production.

## 5.2 Increase shelf-life and improve quality at end of shelf-life

Frenched lamb racks used for this trial were comparable in weight, and all racks – treated and untreated – were stored for 105 days at the same refrigeration temperatures.

The pH of control and treated racks was 5.86 and 5.75, respectively, and the 0.11-unit difference, while statistically significant, is unlikely to be of much practical importance. While high pH meat may result in premature spoilage (MLA, 2014), similar pH values have been reported previously for vacuum packed lamb shoulders. In MLA project A.MFS.0238 bone-in and bone-out lamb shoulders had average pH values of 6.07 and 5.87 over 85 days stored at an average temperature of -0.3°C, without organoleptic spoilage being observed.

The average temperature of -2.0°C during the initial 40 days of storage likely resulted in freezing of product, limiting microbial growth in both treatment groups. This temperature is lower than recommended storage temperature of -0.5°C, and temperatures of -2.4°C have been reported to result in a 0.9 decrease in mean log TVC/cm$^2$, which is close to 80% reduction in microbial counts, in vacuum-packed lamb shoulders (MLA, 2014). At the end of the trial, racks from both treatment groups had similar microbial counts of around $10^7$ cfu/g, for both TVC and LAB. While lactic acid bacteria were rarely observed before 40 and 60 days for control and treated racks, this is likely related to the initial cold storage temperatures.

By employing best practice temperature control to products, extended periods of shelf-life can be achieved, especially when initial microbial loads on product is low. However, under vacuum packed conditions microbial levels increase to $10^7$-$10^8$ cfu/cm$^2$ within about 60-80 days (at -0.3°C) without resulting in spoilage (MLA, 2014). This can also be seen from the results of the meat spoilage predictor, which indicates that at the average trial storage temperature of -1.51°C the control racks had 33 days of shelf-life remaining after 105 days, this would be expected to be less when stored at higher temperatures.

Given these results, it is difficult to ascertain to what degree any differences in the microbial levels between control and treated racks were due to the lactic acid treatment or the storage temperature.

Because no organoleptic assessment was undertaken on the stored racks, it is not possible to determine whether racks treated with 5% lactic acid immediately prior to packing differed in sensory attributes from control racks. Nevertheless, the MLA/UTAS meat spoilage predictor indicates that at an average temperature of -1.51°C a shelf-life of 138 days is possible, when the starting microbial levels are 2,600 cfu/cm$^2$.

Similarly, the data from a temperature logger included in product sent overseas via sea-freight indicated good temperature control throughout the 40-day journey, with an average temperature of -1.4°C, and predicted remaining shelf-life of 60 days, provided that product was stored at -0.5°C. At a slightly higher freight temperature of -0.5°C (on average) a shelf-life of 86 days is achievable at the same starting levels. While reducing the starting microbial levels is sometimes desirable, this approach appears to be less effective at extending the shelf-life than good temperature control throughout transport.

# 6 Conclusions/Recommendations

From this project it can be concluded that the automatic bag loading and lactic acid spraying system developed in this project has commercial potential, as the time to pack frenched lamb racks is similar when used with or without the lactic acid spray.

In addition, given the starting microbial levels of 2,600 cfu/$cm^2$ and well controlled freight temperatures of -0.5°C or lower, it appears that the company endorsed shelf-life of 85 days is achievable.

It is recommended that future shelf-life trials include sensory assessment of the product. This may be achievable by foregoing the use of an external laboratory and instead undertaking the necessary microbiological testing for TVC and LAB in-house.

In addition, it is recommended that shelf-life specifications, especially organoleptic, are negotiated with the customer, as microbiological shelf-life indicators, such as aerobic plate counts and lactic acid bacteria, are of limited use in establishing end of shelf-life.

Temperature control during transport is critical for maintaining high quality product with long shelf-life. Consequently, temperature data loggers should continue to be used regularly to monitor supply chains. In cases where temperature abuse has occurred, the MLA/UTAS spoilage predictor model may be useful to evaluate the effect on remaining shelf-life.

# 7 Key Messages

A spray nozzle system has been developed to fit onto an automatic bag loading machine and deliver a measured quantity of lactic acid solution to the inside surface of a vacuum package bag.

Adapting the system to a bag loader machine does not significantly affect the footprint of the machine as the system can be mounted to fit within the confines of the machine with just the liquid pressure pot that contains the lactic acid solution requiring positioning near the machine.

The efficacy of 5% lactic acid application prior to lamb rack packaging for reducing microbial growth during storage is uncertain due to the cold storage temperatures during this trial and the lack of organoleptic assessment.

Nevertheless, under well controlled transport conditions, with a maximum temperature of -0.5°C, a shelf-life of at least 85 days is achievable at the current initial microbial levels.

# 8 Bibliography

MLA, 2014. Shelf life of Australian red meat. Meat & Livestock Australia, North Sydney.

Moore, D.S., McCabe, G.P., 1998. Introduction to the Practice of Statistics. W. H. Freeman and Company.