Final Report

Extending the shelf life of vacuum-packed sheep meats

Project code: V.MFS.0452

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Executive summary

The overall aim of this project was to understand further the microbiology of vacuum-packed (VP) lamb spoilage with a view to enable the development of an innovative and practical strategy for shelf-life extension. The proposed strategy/intervention and its underpinning science will facilitate its acceptance and adoption by Australian lamb processors to reliably produce VP lamb with a longer shelf-life than 90 days in cold chains (at approximately -0.5°C). This would benefit the industry by being able to assure and maintain customer/consumer trust in product quality, while increasing its profitability through a reduction in wastage and markdowns.

This project was conducted from January 2021 to January 2024. The project expanded upon previous MLA-funded projects (G.MFS.0.289 and V.MFS.0402), and specifically investigated the key factors (*e.g.,* meat pH, glucose, lactic acid, the presence of bone marrow, and fat) that may affect shelf-life and underpin the differences between VP lamb and beef, and also between VP bone-in and boneless products. In this report, the key achievements during the course of the project are summarised and described below.

Results from this project have confirmed that the application of glucose and lactic acid can extend the shelf-life of VP lamb. These highlight its potential to be developed and evaluated further as a practical and cost-effective approach for the Australian red meat industry for shelf-life extension of VP lamb. The project has also provided fundamental knowledge that future research can build upon to create further opportunities for the development of targeted approaches to increase the shelf-life of lamb to be similar to that of beef. Specifically, it was found that meat pH is not the key factor contributing to the differences in shelf-life of VP beef and lamb. These differences were, however, driven by the availability of substrates in each meat type and the subsequent development of spoilage communities. The results also showed that fat content, but not bone marrow, played an important role in the shelf-life of VP red meat.

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1. Publications (preparation, submitted or published)

1.1 Manuscripts in preparation

- Rood, L., Bowman, J. P., Ross, T., Nichols, D., D'Agnese, E., Corkrey, R., and Kocharunchitt, C. Effects of additional glucose as a surface treatment on the microbial community, shelf-life and associated volatilome of vacuum-packed lamb.
- Rood, L., Bowman, J. P., Ross, T., Pagnon, J., Yang, S., Toomik, E, Nichols, D., and Kocharunchitt, C. Determination of meat pH as a key factor contributing to the different shelf-life between vacuum packed lamb and beef.
- Toomik, E., Rood, L, Bowman, J. P., and Kocharunchitt, C., Effects of different fat contents on the shelf-life of vacuum-packed beef and lamb.

1.2 Submitted manuscripts

Not applicable

1.3 Published manuscripts

- Rood, L., Bowman, J. P., Ross, T., Corkrey, R., Pagnon, J., Yang, S., and Kocharunchitt, C. (2022) The effects of glucose on microbial spoilage of vacuum-packed lamb, *Meat Science*, 188, 108781.
- Rood, L., Bowman, J. P., Ross, T., Corkrey, R., Pagnon, J., Kaur, M., and Kocharunchitt, C. (2022) Spoilage potential of bacterial species from chilled vacuum-packed lamb, *Food Microbiology*, 107, 104093.
- Toomik, E., Rood, L, Bowman, J. P., and Kocharunchitt, C., Microbial spoilage mechanisms of vacuum-packed lamb meat: A review, *International Journal of Food Microbiology* 387, 110056.

2. Report and theses

- Rood, L. (2021). Understanding and minimising microbiological spoilage of Australian chilled vacuum-packed lamb. PhD thesis, University of Tasmania. Hobart. Australia.
- Kocharunchitt, C., Bowman, J., and Ross, T. (2021). Extending the shelf life of vacuum-packed sheep meats: Milestone 2 report (V.MFS.0452). Meat and Livestock Australia, North Sydney, Australia.
- Rood, L., Bowman, J., Ross, T., and Kocharunchitt, C. (2022). Extending the shelf life of vacuum-packed sheep meats: Milestone 3 report (V.MFS.0452). Meat and Livestock Australia, North Sydney, Australia.
- Rood, L., Bowman, J., Ross, T., and Kocharunchitt, C. (2022). Extending the shelf life of vacuum-packed sheep meats: Milestone 4 report (V.MFS.0452). Meat and Livestock Australia, North Sydney, Australia.

- Rood, L., Bowman, J., Ross, T., and Kocharunchitt, C. (2023). Extending the shelf life of vacuum-packed sheep meats: Milestone 5 report (V.MFS.0452). Meat and Livestock Australia, North Sydney, Australia.

- Rood, L., Bowman, J., Ross, T., and Kocharunchitt, C. (2023). Extending the shelf life of vacuum-packed sheep meats: Milestone 6 report (V.MFS.0452). Meat and Livestock Australia, North Sydney, Australia.

3. Where we were in 2021

Australia is the world's largest exporter of sheep meat, exporting $11.3 billion worth of sheepmeat (in 2021) to more than 80 countries with the majority being chilled, vacuum-packed (VP) lamb (MLA, 2022). Although Australian VP lamb has a superior shelf-life in export markets, challenges still exist for meat processors/exporters, typically due to non-optimal temperature and/or delays during shipment. This is particularly relevant for export to distant markets, which require sufficient shelf-life to allow flexibility to distribute and sell product in their intended retail markets. Therefore, being able to produce VP lamb with a longer shelf-life is critical for the success of the industry (*i.e.,* maintaining and/or increasing market access, while assuring consumer trust in product quality).

The shelf-life of VP lamb meat at -0.5°C is much shorter than that of VP beef meat (~12 *versus* 26 weeks) (Kaur et al., 2021). This is thought to be mainly due to their differences in pH. Specifically, lamb has a higher pH (pH 5.6-6.8) compared to beef (pH 5.5-5.7), which has been attributed to the higher fat content distributed throughout the muscle tissue of lamb cuts (Gill and Penney, 1985). The higher pH of lamb may result in more favourable conditions for growth of spoilage organisms compared to beef. Indeed, previous MLA-funded projects (G.MFS.0.289 and V.MFS.0402) confirmed the faster rates of total viable count (TVC) increase on VP lamb than on VP beef across all storage temperatures (0°C to 8°C). It was also evident that both VP beef and lamb had similar members within their microbial communities after a period of their storage. These included lactic acid bacteria (LAB, *e.g., Carnobacterium* spp., *Leuconostoc* spp.), species of psychrotrophic Enterobacteriaceae, *Brochothrix* spp., and *Clostridium* spp. etc. However, the ability of these individual species to grow and potentially cause spoilage was systematically different between meat types and thought to be driven by differences in their pH (Kaur et al., 2017; 2021). These results provide the basis of the microbiology of VP lamb spoilage and further highlight the potential for manipulation of meat pH for shelf-life extension of VP lamb.

Among all spoilage organisms, LAB (including *Carnobacterium* spp., a dominant species on VP meat) have been considered as both a protector and spoiler for meat under anaerobic conditions. This is because they have a growth advantage at cold storage temperatures under anaerobic conditions, and preferentially utilise available glucose (in the meat) to produce organic acids, such as lactic acid. This metabolism generally does not result in spoilage of VP meat, but instead prolongs the shelf-life by impeding the growth of other spoilage organisms through the accumulation of organic acids, potentially reducing meat pH, along with the production of other antimicrobials (*e.g*., bacteriocins) (Mills et al., 2014). Indeed, the growth of *Enterobacteriaceae* and *Brochothrix thermosphacta* is inhibited by pH <5.8, while *Shewanella putrefaciens* does not grow at pH <6.0 (Kaur et al., 2021). However, when available glucose becomes limiting/exhausted, LAB and other spoilage organisms begin to metabolise other carbon sources, such as amino acids, producing compounds with unpleasant odours (*e.g.,* amines, dimethyl sulphide). Such compounds cause the quality of VP meat to deteriorate as characterised by off-odours and discolouration (Mills et al., 2014). This information,

taken together, lead to the hypothesis that the addition of available glucose to VP lamb or further acidification of meat surface (*e.g.,* by lactic acid or equivalent) may extend its shelf-life.

A preliminary study to evaluate the potential effects of additional glucose on shelf-life of VP lamb showed very promising results (Rood et al., 2022). Both VP bone-in and boneless lamb shoulders stored at 4°C had a longer shelf life when treated with glucose (up to 0.4 g/kg) by up to 76% (compared to the control). This shelf-life extension might have occurred through a reduction in meat pH (due to elevated levels of lactic acid and other organic acids produced by LAB), and/or an increased level of glucose available for LAB and other spoilage organism (*i.e.,* prolonging the time before other carbon sources are metabolised). The results of that study, therefore, highlight the potential for the proposed application to be applied or exploited for shelf-life extension of VP lamb.

From the above, the ultimate aim of this project was to investigate potential applications that can be used for manipulation of the microbial spoilage of VP lamb for shelf-life extension. Specifically, the project investigated the key factors (*e.g.,* meat pH, glucose, lactic acid, the presence of bone marrow, and fat content) that may underpin the differences in shelf-life of VP lamb and beef, and also between VP bone-in and boneless products. The new knowledge gained will aid the development of practical approaches for the Australian red meat industry to effectively and reliably extend the shelf-life of VP lamb. The following objectives were given to achieve the stated aim:

- to assess and compare the microbial spoilage of VP lamb and beef cuts that have similar and different pH during cold storage;
- to investigate the roles of other key factors (*i.e.*, fat content and bone marrow) in the shelf-life of VP red meat; and
- to evaluate the effects of glucose and lactic acid application on the shelf-life of VP lamb, including its eating quality.

4. Where we are in 2024

A series of studies were conducted addressing both basic and applied aspects to achieve the proposed objectives of this project. Detailed descriptions of those studies, and results and interpretations obtained, are available in their corresponding milestone reports (*see* Section 2), publications (*see* Section 1.3), and Appendices. Key observations and outputs include:

4.1 Outputs

- Laboratory trials confirmed that meat pH was not the key factor contributing to the differences in the shelf-life of VP beef and lamb (Milestone Reports 3 and 5).

- It was evident that the observed shelf-life of each meat type (*i.e.*, beef and lamb) was similar regardless of its pH, and that beef cuts consistently had a longer shelf-life than lamb cuts (by approximately 25%). This was despite that the rates of TVC increase were dependent upon meat pH rather than meat type. The results suggested that the growth rate of TVC as affected by meat pH did not contribute to the faster rate of quality loss of VP lamb (Milestone Reports 3 and 5)

- Subsequent analysis using 16s rRNA gene amplicon sequencing revealed that the microbial community composition of VP red meat was mainly driven by meat type rather than its pH.

This suggests that different substrate availability in beef and lamb play an important role in the development of spoilage communities. Specifically, the analysis revealed differences in relative abundance of key spoilage organisms (*i.e., Carnobacterium divergens*, *Carnobacterium maltaromaticum, Lactococcus* spp., and *Pseudomonas* spp.) between VP beef and lamb, which may contribute to differences in their shelf-life (Milestone Report 5).

- Fat content (≥20% *w/w*) was found to have a negative effect on the shelf-life of VP beef and lamb. VP beef and lamb mince containing 20% and 50% fat had a shorter shelf-life (based on odour quality) by up to approximately 25% compared to the control (approx. <5 % fat) when stored at 2°C. This was accompanied by the observations that VP red meat with a higher fat content had faster growth rates of LAB and higher maximum population densities of *Enterobacteriaceae* without changing its pH. The results suggest that fat may facilitate the growth of LAB and *Enterobacteriaceae* due to differences in nutrient composition (Milestone Report 5).

- The presence of bone marrow on VP lamb boneless products did not have a negative effect on the shelf-life. Preliminary shelf-life trials using boneless samples with and without addition of bone marrow (*i.e.,* 0.01 g/cm^2; to simulate the bone marrow content of bone-in products) showed no differences in the shelf-life. A similar observation was also made for pH, growth rates of LAB, and glucose, protein, lipid, and moisture content. However, the rates of TVC increase were faster on meat with bone marrow than those on the control (Milestone Report 4).

- Laboratory trials confirmed that surface treatments of lactic acid (up to 2.5% *w/v*) can achieve a longer shelf-life of VP lamb at 2°C by up to >40 % (>16 days) when compared to the control. This was despite no detectable effects on the meat surface pH and sensory qualities (odour and colour) (Milestone Report 5).

- A series of in-plant trials confirmed that addition of glucose to lamb primals (0.04 g/kg after 5% glucose treatment) before vacuum packaging can achieve a longer shelf-life without compromising their eating quality. The effects were also found to be similar regardless of the cut types and the presence of bone. Specifically, the shelf-life of VP lamb (bone-in shoulder, bone-in foreshank, and boneless shoulder) with elevated glucose levels were 24 - >29% longer compared to the control during storage at low temperatures (-0.67 and 2.28°C) (Milestone Reports 5, 6 and Appendix A).

- Studies to provide an insight into the potential mechanisms underpinning the shelf-life extension by glucose treatment suggested that glucose extends the shelf-life of VP lamb through complex mechanisms. These complex mechanisms cannot be solely explained by measuring residual glucose and lactic acid content alone. It was found that the glucose levels in meat did not correlate to the point at which meat loses its quality shelf-life. There was also no detectable difference in lactic acid levels (ranging between 8 – 12 g/kg) between treatments. These, taken together, suggest that glucose depletion is not directly related to the onset of spoilage under anaerobic conditions and that lactic acid did not play a major role in shelf-life extension by glucose treatment (Appendix B).

4.2 Outcomes

The outputs of this project have significantly advanced our scientific understanding of the microbial spoilage of VP beef and lamb. The pH of meat was ruled out as a key factor contributing to the shelf-life differences between VP beef and lamb, but rather due to differences in substrate availability of

each meat type and the subsequent development of spoilage communities. Furthermore, the effects of other key factors (*i.e.*, fat content and bone marrow) on the shelf-life of VP red meat were established. This has provided fundamental knowledge that future research can build upon to create opportunities for the development of targeted approaches to increase the shelf-life of lamb to be similar to that of beef.

This project also provided scientific evidence to support that glucose and lactic acid application can successfully extend the shelf-life of VP lamb. This knowledge can be used by the industry to develop innovative practical approaches for shelf-life extension of VP lamb. Once successfully developed and implemented, it will allow the industry to maintain and/or increase market access, while assuring consumer trust in product quality.

In addition to the above, a key outcome of this project is the increased scientific capabilities in the Australian red meat industry. This has been achieved through training of post-doctoral experts for red meat microbial quality who can support the industry to respond to market challenges and recognise and exploit new innovative opportunities. These experts can also provide other training opportunities for young scientists whose research would be relevant to the Australian red meat industry.

5. Where to from here

As described in Section 4, this project has provided scientific evidence supporting the application of glucose and lactic acid as an effective and reliable approach for the shelf-life extension of VP lamb. The results strongly indicate that VP meat with elevated surface glucose or lactic acid levels can achieved a longer shelf-life. These highlight the potential for the proposed application to be developed further as a practical and cost-effective approach for the Australian red meat industry to extend the shelf-life of VP lamb. To this end, future studies can focus on two different aspects, which are described below.

1. Given that the levels of available glucose in meat are an important factor contributing to the shelf-life extension of VP lamb, studies can be conducted to develop best practices to allow optimal levels of glycogen/glucose in livestock prior to slaughter. This is to ensure that the meat has sufficient residual glucose, allowing the longer shelf-life without the need to implement any intervention technology.
2. Further investigation can be undertaken to develop the application of glucose or lactic acid as an intervention technology. These can involve the use of existing technologies such as the Rinse and Chill technology to deliver glucose to lamb carcases (after slaughter). Alternatively, a novel technology can be developed by using an Automatic Taped Bag Loader (Model number BL19M2-V1, Cryovac) mounted with a spray nozzle or an equivalent system for delivery of a fine mist of glucose or lactic acid solution into the bag immediately before product insertion to facilitate coating during vacuum packaging.

Further to the above, it will be necessary to evaluate the performance of the developed practices or technology (on a commercial scale) including their cost *vs.* benefit as a practical approach for shelf-life extension of VP lamb. This information will be used to facilitate industry adoption, while also supporting negotiation with regulators and/or customers in export countries, *i.e.,* as demonstrating the longer shelf-life of VP lamb.

Apart from the development of a practical approach for shelf-life extension of VP lamb, further research is still required to understand better the microbial spoilage of VP red meat. This will involve assessing changes in the levels of other key substrates apart from glucose (*e.g.,* amino acids, short-chain fatty acids, alkaloids etc.) as spoilage occurs and how these are influenced by changing microbial communities over the course of shelf-life. Such knowledge will provide scientific basis to explain the differences in the shelf-life of VP beef and lamb, while also supporting the underpinning science of the proposed approaches for shelf-life extension of VP meat.

6. References

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7. Appendices

7.1 Appendix A: Evaluate the performance of glucose surface treatments for shelf-life extension of VP lamb in a commercial setting

7.1.1 Background

Both laboratory and industrial trials to date have demonstrated that vacuum-packed (VP) lamb with elevated glucose levels achieved a longer quality shelf-life. It was evident that surface treating (0.01 ml/cm^2) VP lamb primals with 5% glucose treatment (0.4 g/kg) extended the shelf-life (based on odour assessment) by up to >76% relative to the control at different storage temperatures (Rood et al., 2022, 2023a; 2023b). The observed shelf-life extension could be due to changes in the microbial spoilage community through elevated levels of organic acids as a result of glucose fermentation by lactic acid bacteria (LAB) (Rood et al., 2022; Shelef, 1977). Furthermore, an increased level of available glucose for the microbial community, may prolong the time before other carbon sources (*e.g.,* amino acids) are metabolised, which typically produce compounds with unpleasant odours (*e.g.,* amines, dimethyl sulphide). These ultimately delay the process of spoilage (Kumudavally et al. 2010; Lambropoulou et al., 1996; Newton and Gill, 1978; Nychas et al., 1988). The results of these trials, therefore, indicate the potential for development of a practical approach that aims to increase or maintain high glucose levels in meat for shelf-life extension.

To facilitate the development of that practical approach, we conducted a further in-plant trial to specifically characterise the effects of glucose application (at 5% *w/v*) on the shelf-life and eating quality (*i.e.,* taste profiles) of VP lamb boneless shoulder when stored at 2°C (*see* Rood et al., 2003b). While the shelf-life trial was still ongoing (at the time of preparing that report), we reported that no systematic differences in taste qualities were perceived by a 'consumer' panel between meat with and without glucose treatment. This indicates that the treatment had no noticeable effect on eating qualities. In this section, the complete results for the effects on glucose application on the shelf-life (based on both microbiological and organoleptic assessments) are described.

7.1.2 Approach

All methods were carried out as described in section 4.1.2 of the Milestone 6 report (Rood et al., 2023b).

7.1.3 Key Results and Discussion

Effects of glucose on VP lamb shelf-life and pH

- Table A1 describes the observed shelf-lives (based on odour quality) of VP lamb boneless shoulder previously treated with or without glucose (5% *w/v*) during storage at 2.28°C. The shelf-life data for VP lamb primals treated with 5% glucose obtained from earlier trials (Rood et al., 2022; 2023a; 2023b) was also included for comparison.

Table A1. Summary of the shelf-life data for VP lamb primals previously treated with glucose solutions followed by storage at different temperatures.

Storage temperature	Cut	Glucose treatment % *w/v*	Observed shelf-life[1] (days)	Shelf-life difference[2] (days)	Shelf-life extension[2] (%)	Source
2.28	Boneless shoulder	Control (0%)	55	-	-	Current trial
		5%	49	-	None	
	Bone-in foreshank	Control (0%)	25	-	-	Milestone 6 Rood et al., 2023b (industrial setting)
		5%	31	6	24%	
-0.67	Bone-in shoulder	Control (0%)	90	-	-	Milestone 5 Rood et al., 2023a (industrial setting)
		5%	> 116	>26	> 29%	
4.0	Boneless shoulder	Control (0%)	26	-	-	Rood et al., 2022 (laboratory setting)
		5%	35	9	35%	
	Bone-in shoulder	Control (0%)	17	-	-	
		5%	> 30	>13	>76%	

[1.] The observed shelf-life was determined as the time taken for the average odour score to reach a score of ≤ 4
[2.] Shelf-life extension was determined relative to the observed shelf-life of the control of the respective trial

- It was evident that VP boneless shoulder previously treated with 5% glucose solution had a shorter shelf-life relative to the control by approximately 10% (~6 days) (Table A1).
- These results were unexpected and do not agree with the observations from previous laboratory and industrial trials (Rood et al., 2022; 2023a; 2023b). In those trials, various cuts of VP lamb previously treated with glucose at the same concentration (5% *w/v*) achieved a

much longer shelf-life compared to the control when stored at different temperatures (Table A1).

- A possible explanation for the lack of shelf-life extension of VP boneless shoulder observed in this study might be due to compromised vacuum/packaging integrity. Compromised vacuum integrity typically results in a shorter shelf-life. This is due to higher levels of residual oxygen within the pack, which can facilitate the growth of higher potential spoilers such as *Pseudomonas* spp. (Kaur et al. 2017). Indeed, it was found that the vacuum integrity of meat previously treated with glucose consistently scored lower compared to that of the control after 33 days of storage onwards (Figure A1). The scores for the vacuum integrity also fell below the unacceptable threshold (≤4) earlier for treated meat than that of the control (after ~48 vs ~54 days; Figure A1). This corresponds well with the time when the treated meat was rated as commercially unacceptable (*i.e.,* after 49 days of storage based on odour). The basis of the apparent loss of vacuum integrity of meat with elevated glucose remains to be elucidated. However, our previous studies to date demonstrated that the vacuum integrity of various lamb primals was maintained throughout the shelf-life duration at different storage temperatures (Rood et al., 2022; 2023a; 2023b).

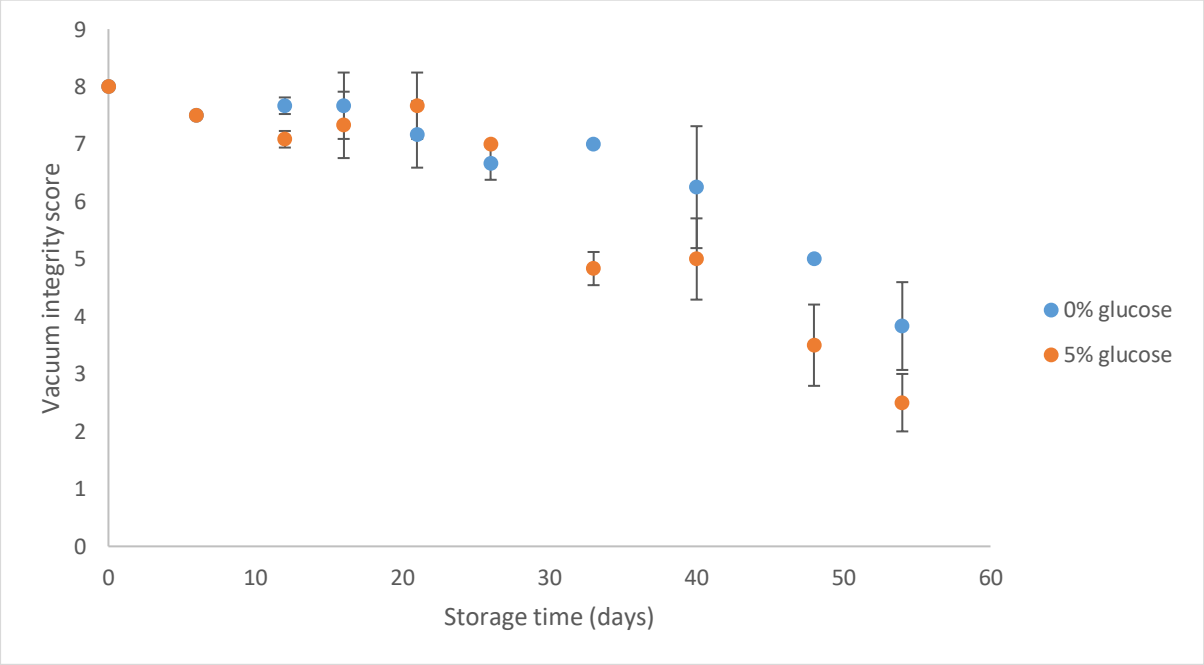

Figure A1. Average (± standard deviation) vacuum integrity score of VP boneless shoulder during storage at 2.28˚C after surface treated with different glucose concentrations.

Surface pH dynamics of VP lamb with elevated glucose

- Figure A2 shows the representative change in lean and fat surface pH of VP boneless shoulder throughout storage at 2.28˚C.

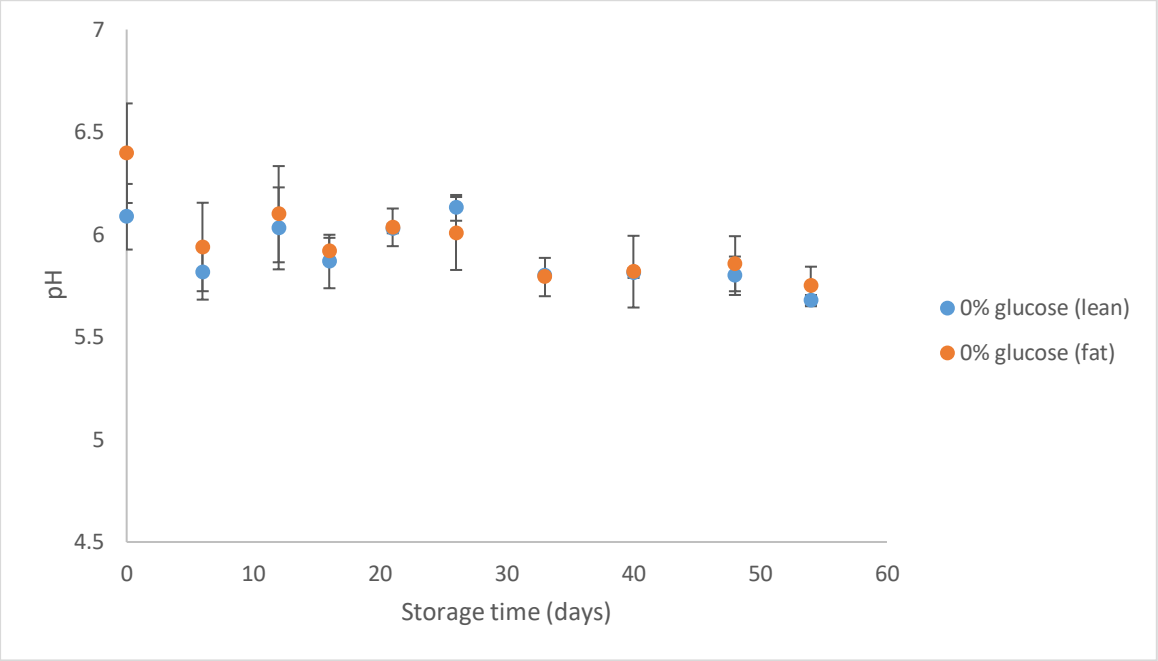

Figure A2. Representative changes of the average (± standard deviation) lean and fat surface pH of VP boneless shoulder throughout storage at 2.28˚C.

- In all cases, the pH of lean and fat surfaces appeared to be similar throughout storage, which is likely due to the higher intramuscular fat content distributed throughout the lean muscle tissue of shoulder (Figure A2).
- There were also no systematic differences in meat pH between the control and glucose treatment throughout storage (data not shown). These results are consistent with Rood et al. (2023a; 2023b) where VP lamb primals treated with up to 5% glucose had a similar pH to the control throughout storage at -0.67 and 2.28˚C. By contrast, it was evident in a laboratory setting that boneless shoulder had a significantly lower pH (up to 0.4 units) when previously treated with up to 10% glucose compared to the control (Rood et al., 2022). This could have been due to the accumulation of organic acids via fermentation of glucose by LAB (Leisner et al., 2007; Shelef, 1977). However, as discussed in Rood et al. (2023b), the apparent inconsistency in the pH results between trials could be because the addition of 5% glucose treatments might only slightly increase organic acids, which may not be consistently detectable, especially due to the natural pH variability of meat (Bendall, 1978).

Microbial growth on VP lamb with elevated glucose

- Figure A3 illustrates the typical growth of bacterial indicators (TVC, LAB and *Enterobacteriace*ae) on VP boneless shoulder treated with and without glucose (5% *w/v*) during storage at 2.28˚C. In all cases, all bacterial indicators increased in their numbers before they reached their maximum population density.

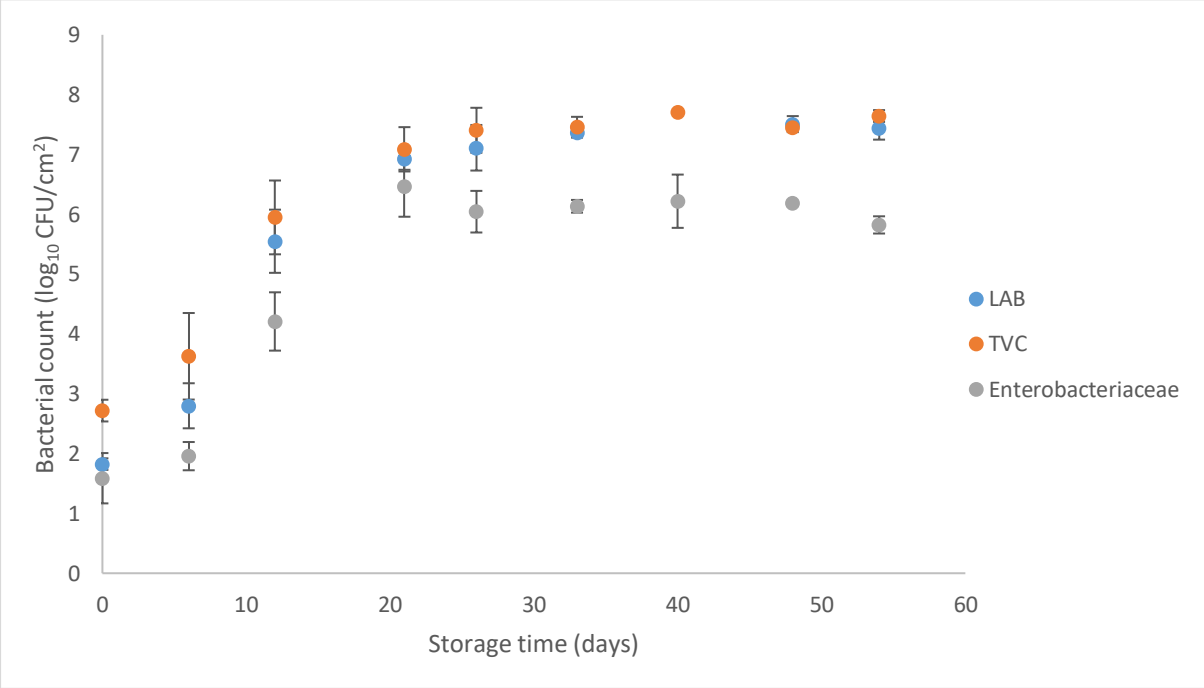

Figure A3. Representative bacterial counts (± standard deviation) on VP boneless shoulder throughout storage at 2.28˚C.

- The initial counts and growth kinetics of TVC, LAB and *Enterobacteriaceae* on VP boneless shoulder with and without glucose treatment (5% *w/v*) during storage at 2.28˚C are summarised in Table A2.

Table A2. Summary of the microbial growth kinetics for VP boneless shoulder after surface treated with different glucose concentrations stored at 2.28˚C.

Growth kinetics		Treatment	
		Control (0%)	Glucose (5%)
Total Viable Count (TVC)	**Initial population[a]**	2.90 (±0.27)	2.70 (±0.23)
	Growth rate[b]	0.69 (±0.10)	0.64 (±0.08)
	Maximum population[a]	7.34 (±0.12)	7.47 (±0.10)
Lactic acid bacteria (LAB)	**Initial population[a]**	1.29 (±0.21)	1.85 (±0.22)
	Growth rate[b]	0.63 (±0.06)	0.62 (±0.04)
	Maximum population[a]	7.30 (±0.11)	7.40 (±0.12)
Enterobacteriaceae	**Initial population[a]**	1.56 (±0.28)	1.55 (±0.19)
	Growth rate[b]	0.75 (±0.04)	0.73 (±0.19)
	Maximum population[a]	6.29 (±0.12)	6.09 (±0.08)

a. Initial population: $\log_{10}$ CFU/cm^2
b. Growth rate: $\log_{10}$ CFU/cm^2/day

- The initial TVC ranged from 2.70 – 2.90 $\log_{10}$ CFU/cm^2, which was higher compared to that of LAB and *Enterobacteriaceae,* both of which ranged between 1.29 – 1.85 $\log_{10}$ CFU/cm^2 (Table A2). The initial counts for each bacterial indicator were similar between treatments.
- There were no differences in the growth rate of all bacterial indicators (TVC, LAB and *Enterobacteriaceae*) between the control and glucose-treated meat. This agrees well with the observations of Rood et al., (2022; 2023b) showing no differences in growth kinetics (*i.e.,* growth rate and maximum population) on meat previously treated with up to 5% glucose solution.
- *Enterobacteriaceae* had a lower maximum population density compared to TVC and LAB. This is in accordance with Rood et al. (2023b) and was expected as these storage conditions (*i.e.,* anaerobic and low storage temperature) favour the growth of lactic acid bacteria, which typically outcompete members of the *Enterobacteriaceae* family, such as *Serratia*, *Hafnia* and *Rahnella* (Doulgeraki et al., 2012).

7.1.4 Conclusions

- The results of this study showed that VP lamb boneless shoulder with elevated glucose (up to 0.04 g/kg after 5% glucose treatment) had a shorter shelf-life compared to the control (by 10% at 2.28°C). These are inconsistent with the observations from previous trials in which VP

lamb primals treated with glucose at the same concentration can achieve a longer shelf-life compared to the control.

- The apparent lack of efficacy of glucose treatment to extend the shelf-life of VP boneless shoulder appeared to be due to compromised packaging integrity.
- Despite the above, there were no differences in pH and the growth kinetics of bacterial indicators between the control and glucose-treated samples.

7.1.5 References

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7.2 Appendix B: Residual glucose and lactic acid content in VP lamb after glucose surface treatments.

7.2.1 Background

Previous shelf-life trials have demonstrated that vacuum-packed (VP) lamb with elevated glucose levels achieved a longer quality shelf-life. Specifically, surface treating (0.01 ml/cm^2) VP lamb primals with 5% glucose treatment (0.4 g/kg) extended the shelf-life (based on odour assessment) by up to >76% relative to the control at different storage temperatures (Rood et al., 2022, 2023a; 2023b). As previously described in Section 7.1.1, the possible mechanisms driving the shelf-life extension could be due to: (i) changes in the microbial spoilage community through elevated levels of organic acids (*e.g.*, lactic acid) as a result of glucose fermentation by lactic acid bacteria (LAB) (Rood et al., 2022; Shelef, 1977); and (ii) an increased level of available glucose (a preferred carbon source) for microorganisms present on VP meat, which prolongs the time before other carbon sources (*e.g.,* amino acids) are metabolised to form compounds with unpleasant odours (*e.g.,* amines, dimethyl sulphide) (Kumudavally et al. 2010; Lambropoulou et al., 1996; Newton and Gill, 1978; Nychas et al., 1988).

To provide an insight into the potential mechanisms outlined above, residual glucose and lactic acid content of VP primals previously treated with and without glucose (at 5% *w/v*) were assessed throughout storage.

7.2.2 Approach

Meat Samples

- Meat samples (bone-in shoulder and foreshank) were obtained from previous shelf-life trials, as described in Rood et al. 2023a and 2023b. Specifically, at each timepoint, meat was aseptically sliced (~1 cm thick) from the surface of VP lamb primals (previously treated with and without glucose solution, 5% *w/v*) at multiple locations until 10 g was taken and stored at -80°C. Upon completion of the shelf-life trials, these samples were used to determine glucose and lactic acid concentrations using ultra performance liquid chromatography (UPLC) (see details below).

Sample preparation

- Frozen samples (50 mg) were added to cold methanol: acetonitrile (1: 1, *v/v*) followed by spiking with 25 µg of $^{3}H_{2}$ labelled L-lactic acid (L113503, Toronto Research Chemicals, Canada) and 18 µg $^{13}C_{6}$ labelled D-Glucose (CIL-CLM-1396-CTM, Cambridge Isotope Laboratories, Inc., USA) to make a final volume of 1000 µl. The meat was homogenised (DLAB D-160, China) with the solvent solution and internal standards on high speed for ~ 1 min. After vortexing, samples were placed on ice and sonicated for 30 min, followed by incubation at −20°C overnight to precipitate proteins. Samples were then centrifuged at 14 000 *x g* for 30 min and supernatant was removed and stored at −20°C until analysis.
- The concentration of labelled standards was selected based on preliminary assessment of the linearity between concentration and peak area (data not shown).

Ultra performance liquid chromatography analysis

- The UPLC instrument was a Waters Acquity H-class UPLC system (Waters Corporation, Milford, MA). Chromatography was performed using an Acquity BEH Amide VanGuard pre-column (5.0 x 2.1 mm, 1.7 µm) and an Aquity BEH Amide column (2.1 × 150 mm × 1.7 µm) (Waters Corporation). The UPLC was operated with a mobile phase consisting of 0.4% (v/v) Ammonium hydroxide (Solvent A) and Acetonitrile (Solvent B). Elution was using a gradient. Initial conditions were 80% B before a gradient to 71.7% B over 2 min, which was held for 2 min. The system was returned to initial conditions at 4.5 min and re-equilibrated for 3 min. The flow rate was 0.35 mL/min and the column was held at 45°C. Injection volume was 1 µL. Typical retention times were: Lactic acid 1.2 min and Glucose 4.3 min.
- The UPLC was coupled to a Waters Xevo TQ triple quadrupole mass spectrometer (Waters Corporation). Analyses were undertaken using multiple reaction monitoring (MRM) in

negative electrospray ionisation mode, with one MRM Transition monitored for the analyte of interest and the Surrogate Standard, respectively. Electrospray ionisation was performed with a capillary voltage of 2.5 kV, and individual cone voltages and collision energies for each MRM transition. The desolvation temperature was 450°C, nebulising gas was nitrogen at 950 L/h and cone gas was nitrogen at 100 L/h. MRM transition dwell times were 120 msec.

Data analysis

- Glucose and lactic acid content (g/kg) were calculated from the peak area ratio relative to the surrogate standard concentration. The concentrations were plotted against time for each product type.

7.2.3 Key Results and Discussion

- Figure B1 illustrates the typical residual glucose content in VP lamb primals with different glucose concentrations (0% and 5% *w/v*) during storage at low temperatures (-0.67˚C to 2.28˚C).

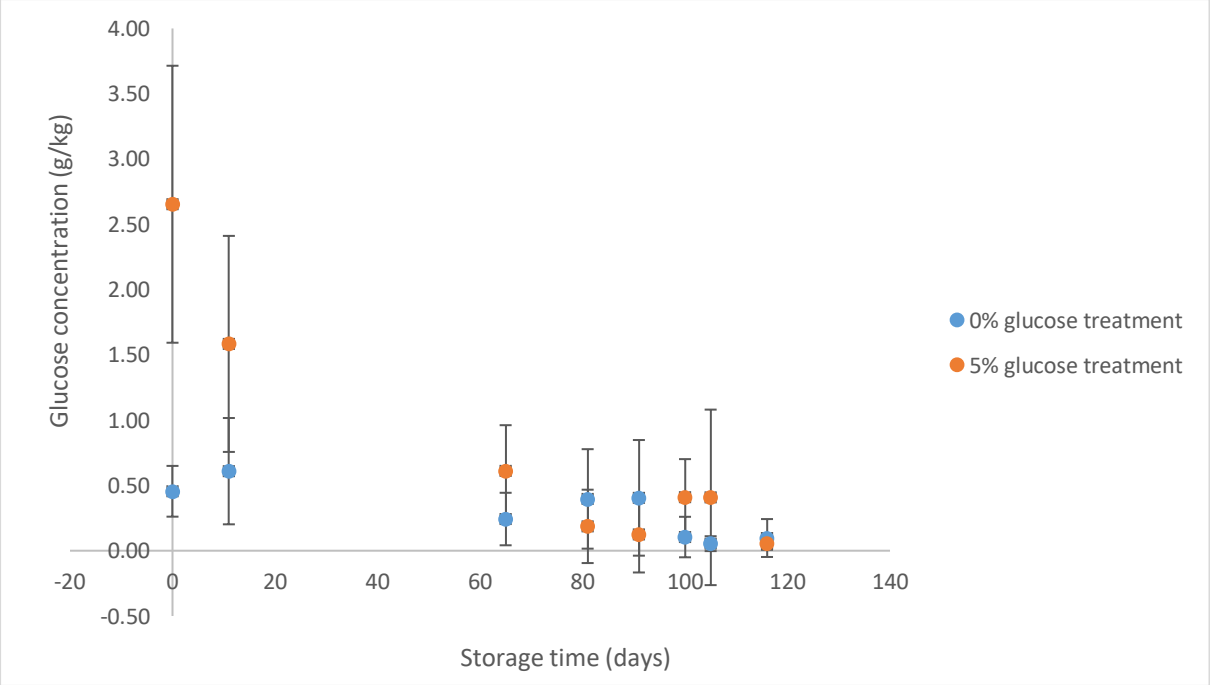

Figure B1: Representative changes in the average (± standard deviation) residual glucose concentration of VP bone-in shoulder treated with different glucose concentrations (0% and 5% *w/v*) during storage at -0.67˚C. The lower detection limit was 0.00045 g/kg.

- In all cases, the concentration of glucose declined over the course of storage. This was expected as glucose is the preferred substrate utilised by microorganisms on VP meat (Nychas et al. 1988) (Figure B1).
- The initial glucose concentration of shoulder treated with 5% solution was higher than expected (by ~1.79 g/kg) based on the amount applied (~0.4 g/kg) (Figure B1). However, the data showed considerable variation between replicates, as indicated by the large error bars. These results suggest that the current application method of glucose may result in uneven distribution of glucose across the surface of the primal. For example, the measured glucose concentration might vary depending on where the subsample was randomly taken from the meat surface, and the distribution of glucose solution over the meat surface when applied.
- As expected, the initial concentration of residual meat glucose for shoulder treated with 5% glucose was higher compared to the control. However, by day 11, and for the remainder of storage, the glucose content of meat with and without additional glucose was similar (Figure B1). These results, taken together with the observed shelf-life differences between the control and glucose-treated meat (90 vs >116 days) (Rood et al., 2023a), indicate that there was no clear correlation between the glucose levels in meat and the point at which meat loses its quality shelf-life. This is in agreement with the study of Rood et al. (2022) reporting that the relationship between glucose depletion is not directly related to the onset of spoilage under anaerobic conditions. To elucidate this further, indicators for protein metabolism (*i.e.*, biogenic amine levels) need to be assessed throughout storage.

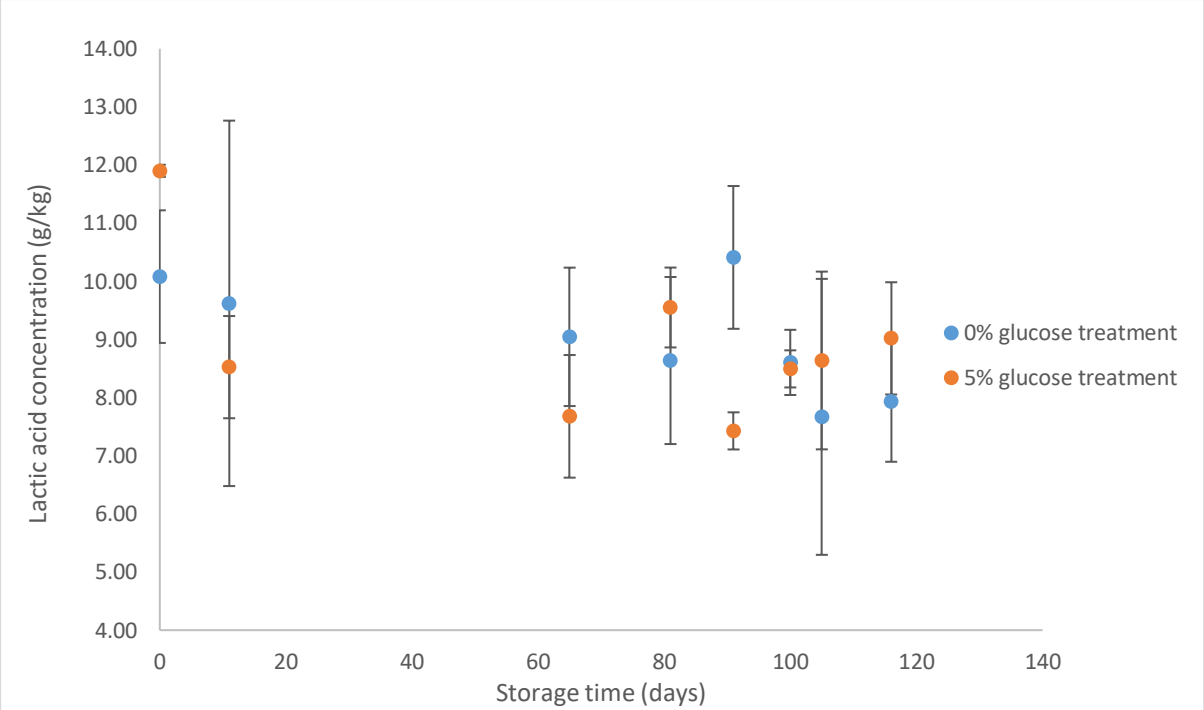

Figure B2: Representative changes in the average (± standard deviation) residual lactic acid concentration of VP bone-in shoulder treated with different glucose concentration (0% and 5% *w/v*) during storage at -0.67˚C. The lower detection limit was 0.00083 g/kg.

- In all cases, the residual concentration of lactic acid appeared to remain unchanged throughout storage, ranging between 8 – 12 g/kg (Figure B2). There was also no detectable difference in lactic acid content between meat with and without added glucose. It, however, should be noted that the initial concentration observed for the 5% treatment was slightly higher compared to all other data points. This was unexpected given that the initial timepoint was completed within ~4 hours after the treatments were applied and vacuum-packed, which is not sufficient time for glucose fermentation by bacteria to occur to influence lactic acid levels of meat.
- The data for lactic acid content was inconsistent with the hypothesis that higher levels of glucose in meat would elevate the levels of organic acids, such as lactic acid, as a result of glucose fermentation by LAB (Rood et al., 2022; Shelef, 1977). However, the data agrees well with the observations of Rood et al. (2022) where a less sensitive enzymatic assay found no change in lactic acid overtime or between the control and treated samples. This suggests that other organic acids (*e.g.,* acetic acid) could have been produced by LAB (Leisner et al., 2007), or the increase in lactic acid is not sufficient at a detectable level to have intensive

lactic acid production (Rood et al., 2022). Further studies involving measurement of total organic acid content should be conducted to support this.

7.2.4 Conclusion

- The results of this study indicated the potential mechanisms underpinning shelf-life extension by glucose treatment are complex and cannot be solely explained by measuring glucose and lactic acid content of meat alone.
- There was no clear relationship between the glucose levels in meat and the point at which meat loses its quality shelf-life. This suggests that the glucose depletion is not directly related to the onset of spoilage under anaerobic conditions.
- It was found that lactic acid did not play a major role in shelf-life extension by glucose treatment. In all cases, there was no detectable difference between control and treatment. This suggests that other organic acids (*e.g.,* acetic acid) might contribute to shelf-life extension.
- Further research (*i.e.,* involving measurement of total organic acid content and key indicators for protein metabolism) is required to specifically determine the potential mechanisms used by glucose for shelf-life extension of meat.

7.2.5 References

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