FINAL REPORT

Pilot study for future storage and transport of carcases using hypobaric storage of meat

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1.0 EXECUTIVE SUMMARY

Technology for storing perishable products in a hypobaric environment has now progressed to a point where the residual atmosphere and humidity in the container can be managed effectively and this provides many opportunities for storage and transport of perishable food commodities including meat.

In some of our export markets, particularly for lamb and sheep meat, fresh or chilled product needs to pass through wet markets in carcase form. The use of reefers constructed using hypobaric technology could enable processed lamb and sheep carcases to be processed in Australia for export as chilled carcases. On arrival at the overseas port the reefers could be transported to wet markets where the chilled carcases would be released. This would be of benefit to the meat processing sector in Australia and be complementary to the existing live export market.

Research to ascertain whether the hypobaric technology can be successfully implemented for transport of meat is an essential first step. This study was undertaken to verify whether or not meat (lamb) can be safely maintained in a hypobaric (vacuum) environment for 35 days without appreciable loss of weight or spoilage.

The NSW DPI currently has 6 hypobaric chamber research units at their horticultural research facility at Ourimbah on the NSW Central Coast. Two of the units located in a chiller capable of being held at around 0 degrees Celsius were made available for this project.

Lamb loins were held in a hypobaric environment using air as the residual atmosphere in one chamber and carbon dioxide in the other chamber for 5 weeks. Temperature was set at 0 degrees C. The relative humidity in the chambers was maintained at above 95% to minimise weight loss. To maintain humidity levels the chambers were set up to provide a steady flow of air or CO2 through a humidifier and into the chamber. The flow rate effectively replaced the residual atmosphere volume every 2 hours.

The endpoints measured were weight loss, colour, oxidative stability, microbiological quality and shear force as a measure of tenderness. Samples of lamb loins were vacuum packed and held in the chiller containing the chambers as controls. For endpoints other than shear force the study included 4 separate replications with 8 loins in each of the treatment groups and 8 loins as controls. For shear force measurement 8 loins were included in each of replicates 2 – 4 (this was not part of the original project, but was deemed important and so added). The results were aggregated for statistical analysis.

For the first replicate the internal chamber pressure was set at 30 Torr (mm Hg). At this pressure mold growth was observed on the surface of loins in the air treatment group. The pressure was lowered and the mean pressure for the chambers in runs 2, 3 and 4 was between 5.3 and 5.5 Torr. No evidence of mold was observed on the loins in these runs.

In summary based on the findings for the endpoint data it was concluded that:

- The ultimate pH, lipid oxidation and protein oxidation findings after storage were equivalent to that observed with traditional wet aging using a vacuum pack,
- The weight loss during storage was between 6 and 7% which is higher than that observed with wet aging (2-3%),
- Retail colour consistent with consumer expectations breached after 2 days display following removal from storage compared with 2.5 days for the vacuum packed controls,

- In terms of pathogenic bacteria the microbiological quality of meat stored with low pressure air as the residual atmosphere in the chamber was equivalent to traditional wet aging in a vacuum pack,
- In terms of spoilage bacteria meat stored with low pressure air or CO2 as the residual atmosphere in the chamber revealed higher levels than traditional wet aging in a vacuum pack,
- The microbiological quality of meat stored with low pressure CO2 as the residual atmosphere in the chamber revealed some unexpected findings for the presence of pathogenic bacteria, and
- In terms of tenderness as measured by shear force the ageing of the lamb in the hypobaric chambers was equivalent to that observed using traditional wet aging in a vacuum pack.

In conclusion this pilot study has effectively provided proof of concept for the potential use of hypobaric containers for transport and storage of sheep meat. Development of this concept further will open up opportunities for transport of whole chilled sheep carcases directly into wet markets in countries around the world, but particularly into the Middle East. An additional benefit will be the ageing of the meat during transit.

Recommendations for further research include:

- Further investigation of the microbial quality issues and where required, methods to minimise bacterial growth;
- Evaluation of the organoleptic aspects of various lamb and wether meat cuts (as they are currently exported live) after treatment;
- Determination of acceptable ranges for the pressure and humidity chamber parameters;
- Studying the effect of shorter and longer storage timeframes and determining an upper limit of storage time for acceptability of product from a food safety and meat quality perspective;
- Investigate a suitable reefer design for hypobaric storage and transport of chilled whole sheep carcases, including the engineering aspects required to reliably manage the temperature, humidity and pressure.

It is recommended that the focus of the next stage of research be on the first two areas listed above in the first instance. It is also recommended that the use of hypobaric storage and transport of beef be considered for research in the future.

2.0 INTRODUCTION

Hypobaric storage and transport of meat and other food products was attempted in the 1980s and at that time was reportedly unreliable. The US company that was producing the hypobaric containers ceased to do so and the use of the technology was on hold until interest in its use was revived in the last 4 or 5 years. The vacuum technology for storing perishable products in a hypobaric environment has now progressed to a point where the residual atmosphere and humidity in the container can be managed effectively.

The technology has been shown to be very successful for the storage of cut flowers and is now used commercially for this purpose in North America. Flowers have been stored this way for 30 days and were perfect on release with the normal expected shelf life after they were released.

This project was developed in recognition of the expectation in some of our export markets, particularly for lamb and sheep meat that fresh or chilled product needs to pass through wet markets in those countries in carcase form. Successful implementation of this technology could enable processed lamb and sheep carcases to be processed in Australia and sent to markets such as the Middle East by ship as chilled product and then transported to and released directly into wet markets overseas as chilled carcases. As such it has the potential to benefit the processing sector in Australia and be complementary to the existing live export market.

This pilot study was designed to ascertain the potential for the use of sophisticated hypobaric container technology for storage and transport of meat as chilled carcases (sheep and lamb). The intention of the study was to verify that meat (lamb) can be safely maintained in a hypobaric (vacuum) environment for 35 days without appreciable loss of weight or spoilage. A review of the literature revealed that there was no credibly researched information available regarding weight loss and spoilage of meat held under hypobaric conditons.

The NSW DPI is currently conducting research to establish the parameters for use of this technology for treatment of fruit for insect pests. There are 6 hypobaric chamber research units at their research facility at Ourimbah on the NSW Central Coast. Two of the units are located together in a chiller capable of being held at around 0 degrees Celsius and were made available for this project.

The project utilised the 2 vacuum research vessels to hold meat (lamb) in a vacuum environment and at -1 to 0 deg C for 5 weeks. The objective was to maintain the humidity in the residual atmosphere at above 95% to minimise purge or weight loss. The endpoints to be measured were colour, oxidative stability, weight loss and microbiological quality. In the course of the project shear force was included as a measure of meat tenderness.

Air and Carbon dioxide atmospheres were used to make up the residual gas in the chamber to establish if there was any variation in terms of colour, oxidative stability and microbiological quality of the meat due to the different residual atmosphere. Four sets of samples subjected to each atmosphere were used to establish that the results are repeatable (replicates). Samples of lamb loins were also held vacuum packed (chilled) outside the chambers as controls.

It was intended that this pilot, if successful, would lead to further research to look at the organoleptic qualities of the meat subjected to this treatment and to determine the optimum parameters for maintaining meat in a hypobaric chamber. In the long term assuming this method of storage and/or transport can be used there is an opportunity for lamb/sheep carcases or beef quarters to go directly into ‘wet markets’ overseas as chilled product.

3.0 PROJECT OBJECTIVES

The project objectives were:

1. Investigation of the potential to extend the shelf life of lamb using a hypobaric chamber, and
2. Examination of the potential of hypobaric chambers for increasing the flexibility of supplying lamb for export wet markets.

4.0 METHODOLOGY

4.1 Study Overview

The study comprised two components. The first or primary component was designed to address the program of research as approved by AMPC. This primary study component included 4 separate replications (or runs) for the treatment groups and controls. Details of study design are outlined in 4.3.1.

After commencement of first run the opportunity to include additional loins in the remaining runs for the purposes of shear force testing was identified. This secondary component was undertaken and the details of the study design are outlined in 4.3.2.

4.2 Equipment and Facilities

Two hypobaric research chambers acquired by NSW DPI for horticultural research purposes were made available for this pilot study. The chambers were located in a blast chiller capable of being operated at a relatively constant temperature. Each chamber consists of thick metal walls with an opening at the front capable of forming an air tight seal with a door. The chamber has a thick metal door with a window in the centre. A silicon seal located a few centimetres from the inside edge of the door is held against the chamber opening and the airtight seal required to maintain the hypobaric conditions is achieved by fixing the door to the chamber using specially designed bolts.

Each chamber is designed to control the level of humidity and the rate of exchange (refreshing) of the residual atmosphere in the chamber. This is achieved by use of an inlet valve to control the flow rate of gas entering the chamber and the placement of a water based humidifier in the gas inlet line to control the relative humidity of the residual atmosphere in the chamber. Inlet lines can be open to the air or connected to the required source of gas for the residual atmosphere.

The pressure in the chamber is reduced using a rotary vane vacuum pump and maintenance of the pressure of the residual atmosphere is achieved by sensors in the chamber which activate a control valve in the line between the chamber and the vacuum pump.

All sensors and valves provide input to a computer program that enables the operator to set the level of vacuum, humidity and rate of refreshing of the residual atmosphere. When operational the chambers are continually monitored electronically for vacuum, temperature and humidity and the records stored electronically.

Prior to commencement of the experiment a racking system for placement of lamb loins in the chambers was designed and built. The racking system comprised an aluminium frame and food grade

stainless steel racks for placement of the meat samples.

Approximately 1 hour before each run the internal surfaces of the chambers, the chamber door, probes inside the chamber and all surfaces of the racks were sanitised using a sanitiser commercially available for food preparation areas.

The internal appearance of a chamber with the racking system in place is shown in Figure 4.2.1 and the external appearance of the chambers with the doors in place is shown in Figure 4.2.2.

Figure 4.2.1 Chamber and racking system

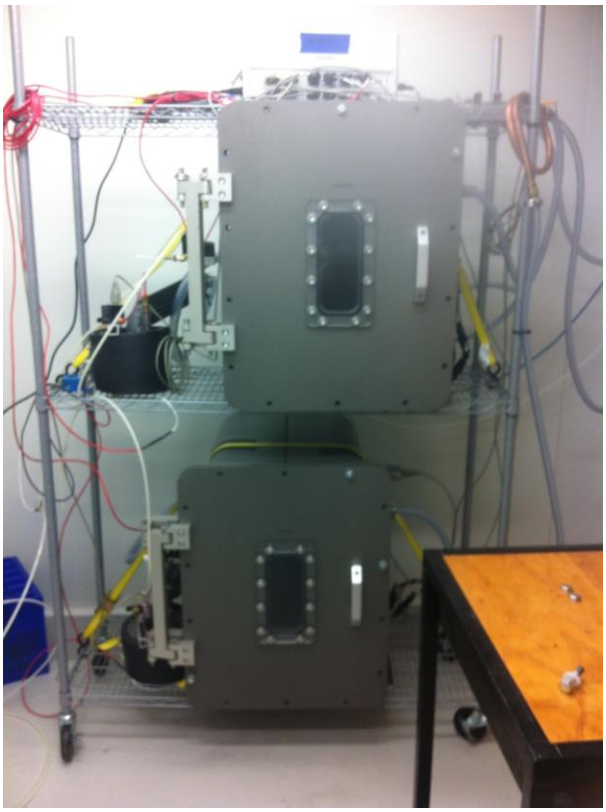

Figure 4.2.2 Chambers with doors in place

4.2 Study Design

4.2.1 Primary Study

To provide confidence in the repeatability of the experiments four separate replications (runs) were conducted using a sample size of 8 lamb loins per treatment group (air and carbon dioxide as the residual atmosphere in the chamber) and 8 loins in a single control group for each run.

The left and right loins from 12 carcases (24 loins) were boned out and collected 4 times over a 6 month period (total of 96 loins from 48 carcases; Figure 4.2.3). Once boned out (with the subcutaneous fat still attached), loins were uniquely identified and sections excised for 0 week microbiology and oxidative stability tests (TBARS). The loins were allocated into treatment groups.

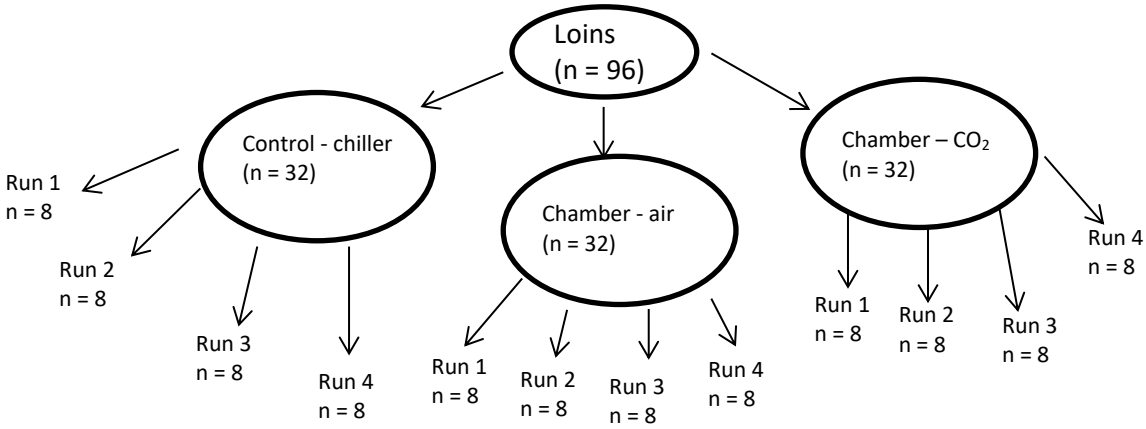

Figure 4.2.3. Flow chart representing the number of samples per treatment group and number from each treatment group for each run.

Samples from the left and right side of three carcases were randomly assigned to treatment groups so that the left and right sides of one carcase were assigned to air and CO_2 treatment groups, another left and right loin from the second carcase were allocated to air and control and loins from a third carcase were allocated to CO_2 and control treatment groups (Fig 4.2.4). This was repeated 4 times within each run.

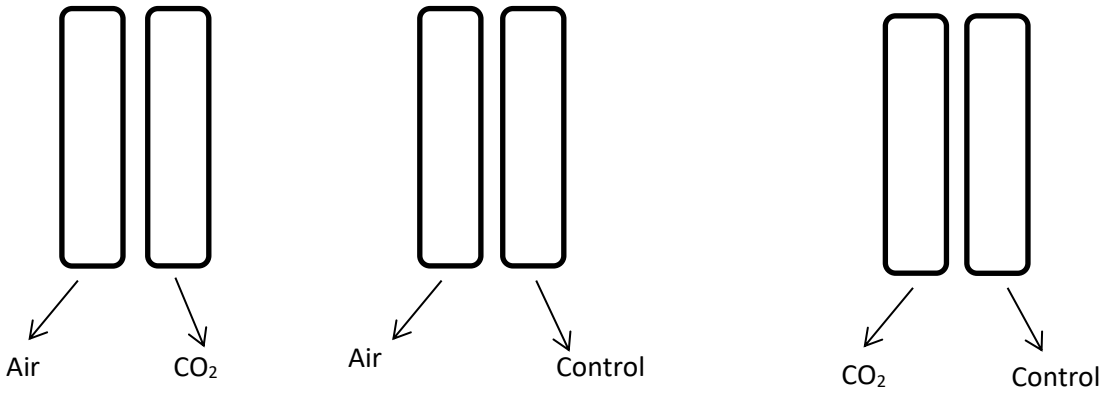

Figure 4.2.4. Allocation of left and right loins from 3 carcases into treatment groups.

The full sampling procedure is summarised in Table 4.2.1 and Figure 4.2.5. Loins allocated to treatment groups were weighed prior to placement into the vacuum chambers and loins assigned to the control group were weighed prior to being vacuum packed and held in the chiller adjacent to the chambers at 0 to -1°C.

After 5 weeks ageing each loin in the treatment and control groups was individually removed from the chambers and weighed. Loins in the control group were removed from the vacuum pack and any purge remaining on the surface removed prior to weighing. After each loin was weighed a 90 gm section for the 5 week microbiology testing was excised, individually identified, vacuum packed and placed in a polystyrene container two thirds full of dry ice for transport of all the samples to the laboratory. The remaining section of each loin was individually identified, packed and placed in a transportable refrigeration unit for transfer to the NSW DPI Cowra Meat Laboratory.

Once transported back to the Cowra Meat Lab sections were taken for further analysis for oxidative stability, lipid oxidation (TBARS), ultimate pH (pHu) and retail colour stability. The location of the sections taken from each loin is shown in Figure 4.2.5.

Sections taken for retail colour stability were kept under retail conditions in a chiller and measured each day using the HunterLab colorimeter over the course of 3 days before being sampled again for TBARS (5 g) and protein carbonyl content (5 g).

Table 4.2.1. Summary of measured meat quality traits, measuring time and the amount of muscle required.

Trait	**Time Measured**	**Amount Required (g)**
Microbiological Load	24 h	90 – 100
	5 weeks	90 – 100
Retail Colour	5 weeks	3 cm thick slice
TBARS	5 weeks + 3 days (from Retail Colour)	5
Ultimate pH	5 weeks	1 - 2
Carbonyl Content	5 weeks + 3 days (from Retail Colour)	5
Purge	24 h & 5 weeks	

Figure 4.2.5. Diagram of the sampling of treatment loins.

Loins in the treatment groups were placed on the upper rack in each chamber. A label uniquely identifying each loin was placed on the rack beside the loin and fixed in position using a cable tie as shown in Figure 4.2.6. Placement of loins on the rack is shown in Figure 4.2.7.

Figure 4.2.6. Placement of loins on the rack

Figure 4.2.7.Rack placement in chamber

4.2.1 Shear force study

The shear force study was conducted in conjunction with runs 2, 3 and 4 of the primary study. The loins used for this study were additional to the loins used for the primary study. As was the case with the primary study the sample size comprised 8 loins per treatment group (air and carbon dioxide as the residual atmosphere in the chamber) and 8 loins in a single control group for each run. Allocation of loins to treatment groups or the control group was done the same way as for the primary study.

For each loin a sample was collected immediately prior to placement in the hypobaric chambers or vacuum packing of the control group (ie at 1 day post mortem) and then at 35 days after treatment. All shear force blocks were held frozen until subsequent analysis at the NSW DPI laboratory at Cowra.

Loins in the treatment groups were placed on the lower rack in the chamber. A label uniquely identifying each loin was placed on the rack beside the loin and fixed in position using a cable tie.

4.3 Analysis of Samples

4.3.1 Purge

Purge results for each individual loin sample was the measured weight loss over the 5 weeks expressed as a percentage of the weight at day 0. The weight of each individual loin after sectioning to remove the day 0 samples required for analysis was recorded as was the weight of the loin after the 5 week experimental period. Any excess fluid present on the surface of each loin was removed prior to recording the 5 week weight.

4.3.2 Ultimate PH

The ultimate pH of the *m. longissimus thoracis et lumborum* (pHuLL) was measured after 35 days of ageing. Approximately 1 g of tissue was removed from each still frozen sample, and then homogenised at 19,000 rpm for 2 bursts of 15 s (Ystral homogeniser: Series X10/25, Ystral, Germany) in 50 mL Falcon tubes containing 6 mL of buffer solution (Dransfield, Etherington & Taylor, 1992). The samples were placed in a water bath at 20°C, and the pH measured using a pH meter (smartCHEM-

CP, TPS Pty Ltd, Brisbane, Australia) with a polypropylene spear-type gel electrode (Ionode IJ 44) calibrated using two pH buffers (pH 4.01 and pH 6.86). Duplicate pH measures were recorded and a third was taken if 2 readings differed by more than ±0.03.

4.3.3 TBARS Content

TBARS content determination was adapted from Hopkins, Clayton, Lamb, van de Ven, Refshauge, Kerr, Bailes, Lewandowski & Ponnampalam (2014) with a 50.0 mg sample added to 500.0 µL RIPA buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals, Michigan, USA) and homogenised using micro-tube pestles. Supernatant was then analysed as per the OXltek TBARS assay kit technical bulletin (Zeptometrix 2016) and absorbance read at 532 nm on a bench top spectrophotometer. Results were expressed as mg malondialdehyde (MDA) per kg fresh meat.

4.3.4 Retail Colour

Following their prescribed ageing period, a cutting guide was used to section the LL samples to a uniform 3 cm thickness with the myofibrils perpendicular on the measured surface. These sections were then individually placed on black foam trays and overwrapped with PVC food film wrap (15 µm) and permitted to bloom for 45 min before colorimetric analysis. Colorimetric measurements were taken over four display time intervals (0, 24, 48, and 72 h) during which all samples were displayed under simulated retail lighting (mean: 851 lx) and refrigeration (mean: 1.6 °C). A HunterLab spectrophotometer (Miniscan Model 45/0-L: Reston, VA, USA) with a 25 mm aperture was calibrated as per manufacturer guidelines (X = 80.4, Y = 85.3, Z = 91.5). This was set to illuminant D-65 and viewing angle 10°. At each reading, measurements were replicated after rotating the spectrophotometer 90° in the horizontal plane. The oxymyoglobin/metmyoglobin ratio (R630/580) was estimated by dividing the captured light reflectance at wavelength 630 nm, by that at wavelength 580 nm (AMSA, 2012).

4.3.5 Shear Force Measurement

Samples to determine the effect of storage in hypobaric chamber on shear force were collected at 1 day post mortem, prior to treatment in hypobaric chambers and at 35 days after treatment from each loin. This was undertaken for 3 replications after it became apparent there was space in the chambers for additional loins and it would be useful to establish any effects on this important trait. All shear force blocks were held frozen until analysis. Determination of shear force values was conducted using shear force blocks (mean weight = 66 g, ± s. d. = 2.55 g) cooked at 71°C for 35mins and analysed using a Lloyd texture analyser with a vee-blade as described by Hopkins, Toohey, Kerr & van de Ven (2010) on 6 replicates per shear force block. Where the co-efficient of variation exceeded 24% for the 6 replications, the median of the values was reported rather than the average of the 6 repetitions (Hopkins, Kerr, Kerr & van de Ven, 2012). Shear force blocks were weighed before and after cooking to determine cooking loss (calculated as a percentage of weight lost during cooking).

4.3.6 Protein oxidation

Protein oxidation was determined by measuring the stable carbonyl groups, to this end approximately 25.0 mg of sample was homogenised in 200 µl of RIPA buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals, Michigan, USA) using a micro-pestle. These were centrifuged at 5 600 rpm and the supernatant was analysed using the Protein Carbonyl Assay Kit (no. MAK094,

Sigma-Aldrich Pty. Ltd., Missouri, USA) technical bulletin (Sigma-Aldrich, 2015) using a micro-plate reader (FLUOstar OPTIMA™, BMG, Labtechnologies, Victoria, AUS). Absorbance was measured at 375 nm. The same supernatant was also analysed using the Bicinchoninic Acid Kit for Protein Determination (no. BCA1, Sigma-Aldrich Pty. Ltd., Missouri, USA) technical bulletin (Sigma-Aldrich, 2015) protocol and measured using the same microplate reader set to measure absorbance at 540 nm to determine the sample protein content. Carbonyl content was then calculated from these two measures and expressed as nmole/ mg protein.

4.3.7 Microbial Load

Microbial loads were determined at the start and end of each ageing period by taking a 90 g sample aseptically, which was diluted in 90 mL peptone salt solution (0.1%) for 30-60 seconds for microbial loading before being measured as total viable count (TVC), of Lactic Acid Bacteria (LAB), *Enterobacteriaceae* (ENT), *Brocothrix thermospacta* (*B. thermospacta*), Escherichia coli (E. coli), listeria and salmonella. For LAB, once samples were diluted, they were plated at 0.1 mg on MRS agar and incubated in an anaerobe jar with the addition of Campygen for 72 ± 2 hours at 30 ± 1 °C prior to counting. Counts of ENT were conducted using 1 mL of each dilution pour plated onto VRBG agar and overlaid with the VRBG agar prior to being incubated for 21 ± 3 hours at 36 ± 2 °C and counted. B. thermospacta was measured by plating diluted samples on an STAA spread plate and incubated for 48 ± 4 hours at 22-25 °C. For E. coli, 1 mL was inoculated onto a Petrifilm count plate and incubated for 24-48 hours at 37 ± 1 °C. All TVC are given as colony forming units (CFU) per g of meat.

The presence of Listeria was detected using an Enzyme-Linked Immunofluorescent Assay (ELFA) VIDAS LIS Assay screening Method (AOAC method 999.06). If detected, the presence of Listeria was confirmed by completing the Australian Standard method 24.1 (AS 50.13.24.1). Similarly, the presence of Salmonella was also tested using an ELFA VIDAS assay method (Salmonella - bioMerieux VIDAS Salmonella Assay (AFNOR (BIO 12/16-09/05)) and confirmed using the Australian Standard method AS 5013.10-2009. Both Listeria and Salmonella were recorded as either detected or not detected for each sample. All microbial analysis was undertaken at a commercial laboratory.

4.4 Statistical Analysis

Statistical analysis to determine whether there was a significant difference in mean traits measured between treatment groups was conducted using REML mixed models in (Genstat ed 18). Run (replication), chamber and the side of the carcase the loin was taken from where used as random effects and were combined to determine whether there was any interaction between these terms. Colour traits measured during display were analysed using a model with treatment as a fixed effect and time of display as a fixed effect with random terms as previously described. Microbiology variables were analysed depending on the data type by one of three methods including comparison of 95% confidence intervals for proportions, generalised linear models with poisson errors and log link function for counts and generalised linear models with binomial errors and logit link function for proportions.

5.0 PROJECT OUTCOMES

5.1 Hypobaric Chamber Operational Parameters

Overall the operation of the chambers ran very smoothly with the exception of one incident resulting in a short period of a few hours when the vacuum pump was not operational during run 4. The incident related to a power failure to the vacuum pump caused by an electrical maintenance operative needing to cut the power to that circuit for a short period due to a maintenance requirement.

This short period of 2 hours 50 minutes where vacuum was not maintained would have had negligible impact on the results for the loins in run 4. To this end the results for the chamber pressure for that period have been treated as outliers and have been excluded from the data set when determining the mean and standard deviation presented here.

5.1.1 Humidity

The objective of the management of the relative humidity in the residual atmosphere in the chamber was to keep it as high as possible and consistently above 95% to reduce the loss of moisture from the loins. Table 5.1.1 summarises the operational recordings for humidity for each chamber.

Table 5.1.1. Mean and Minumum Relative Humidity for each run.

Run	Relative Humidity in Chamber (%)			
	Air		CO2	
	Mean	Minimum	Mean	Minimum
1	99.6	98.5	98.9	97.7
2	99.7	96.2	98.3	95.6
3	99.67	96.1	99.4	96.3
4	98.37	93.9	97.8	95.5

5.1.2 Chamber Pressure

The mean and standard deviation for the internal operational pressure in each of the chambers for the 4 runs is provided in Table 5.1.2.

Table 5.1.2. Mean and Standard Deviation for the chamber pressure readings.

Run	Chamber Pressure (Torr)			
	Air		CO2	
	Mean	SD	Mean	SD
1	30.00	0.12	29.97	0.29
2	5.37	0.32	5.43	0.27
3	5.42	0.24	5.45	0.22
4	5.45	0.23	5.43	0.25

5.1.3 Temperature

Temperature probes were subjected to a calibration trial using the ice water method and the temperature of the ice water confirmed at 0 degrees Celsius using an officially calibrated thermometer. For each probe the temperature readings were adjusted by value for the temperature

recorded by the probe in the calibration trial.

For each run the mean and standard deviation for the adjusted temperatures for the chamber and loin probes are provided in table 5.1.3.

Table 5.1.3. Mean and Standard Deviation for the temperature readings of the chamber and loin probes for each run.

Run	Probe Location	Temperature (Deg C)			
		Air		CO2	
		Mean	SD	Mean	SD
1	Chamber	-0.30	0.432	-0.30	0.542
	Loin	-0.42	0.223	-0.24	0.243
2	Chamber	-0.41	0.409	-0.46	0.534
	Loin	-0.61	0.203	-0.51	0.219
3	Chamber	-0.30	0.397	-0.34	0.515
	Loin	-0.56	0.250	-0.38	0.216
4	Chamber	0.22	0.361	0.18	0.483
	Loin	-0.09	0.251	0.09	0.198

5.2 Sample Results

5.2.1 Purge loss, Lipid Oxidation, Ultimate pH and Carbonyl content

The means (± s. e.) for each trait measured across the treatments and control groups are given in Table 5.2.1 with the least significant differences of means.

Table 5.2.1. The predicted means for each trait measured across the treatment and control groups with the least significant differences (l. s. d.) of means and the standard error (s. e.) of the mean.

Trait	Mean			s. e.	L. S. D.	*P value*
	Control	Air	CO_2			
Purge Loss (%)	**3.0a**	**6.8b**	**6.4b**	**0.91**	**2.29**	**0.01**
Lipid Oxidation (MDA mg/kg)	**1.08**	**1.09**	**1.18**	**0.15**	**0.40**	ns
pHu	**5.68**	**5.69**	**5.69**	**0.02**	**0.07**	**ns**
Carbonyl Content (nmole/ mg protein)	**4.18**	**4.42**	**3.98**	**0.45**	**0.64**	**ns**

Means with different letters are significantly different at P = 0.05.

There was a significant difference between purge for loins treated in hypobaric containers compared with loins stored under control conditions (Table 5.2.1: P = 0.013) with greater fluid losses in loins held in the chambers. However, there was no difference in ultimate pH, lipid oxidation or protein oxidation.

5.2.2 Retail Colour

The means (± s. e.) for each retail colour trait measured across the treatments and control groups are given in Table 5.2.2 along with the least significant differences of means.

Table 5.2.2. The predicted means for each retail colour trait measured across the treatment and control groups including the least significant differences (l. s. d.) of means and the standard error (s. e.) of the mean.

Trait		Mean			s. e.	L. S. D.	*P value*
		Control	Air	CO_2			
L*	5 weeks	36.27a	34.99b	35.54ab	0.68	1.00	0.05
	5 weeks + 1 day	36.01	34.98	35.24	0.87	1.23	ns
	5 weeks + 2 days	35.79	34.56	34.94	1.22	1.18	ns
	5 weeks + 3 days	35.61	34.29	34.76	1.11	1.18	ns
a*	5 weeks	19.06	18.81	18.98	0.21	0.46	ns
	5 weeks + 1 day	19.01	17.81	18.22	0.83	1.17	ns
	5 weeks + 2 days	16.59a	14.71b	15.33b	0.69	0.99	0.01
	5 weeks + 3 days	14.18a	11.65b	13.27a	0.46	1.35	0.01
b*	5 weeks	17.08	16.61	16.76	0.16	0.38	ns
	5 weeks + 1 day	17.71	16.76	17.71	0.58	0.51	0.01
	5 weeks + 2 days	16.67	15.76	16.00	0.42	0.64	0.03
	5 weeks + 3 days	15.66	14.47	15.24	0.67	0.67	0.01
630/580 ratio nm	5 weeks	5.86	5.94	5.95	0.16	0.34	ns
	5 weeks + 1 day	4.70	4.42	4.50	0.3	0.42	ns
	5 weeks + 2 days	3.65 a	3.14 b	3.28 ab	0.24	0.39	0.05
	5 weeks + 3 days	2.88 a	2.26 b	2.65 ab	0.15	0.41	0.03

Means with different letters are significantly different at P = 0.05.

The decline in L* values and b* measured was not significant between treatments or over time on retail display. The loins in treatment and control groups also did not fall below the L* value threshold of 34 for consumer acceptability during retail display.

There was a significant difference in the decline in the a* values over the retail display period with both treatment groups having significantly lower values ($P < 0.001$) than the control group for

consumer acceptance on day 2 with the air treatment having fallen below the a* threshold (14.8) at the day 2 reading.

Treatment also resulted in a significant in difference in the 630/580 nm ratio after 5 weeks storage and 48 h (P =0.046) and 72 h under retail display (P =0.029). The loins from the hypobaric treatment groups fell below the R630/580 threshold for consumer acceptance (3.3) at the 2 day time point for retail display compared to the control which did not fall below this threshold until the 3 day time point.

5.2.3 Microbiology

At the conclusion of Run 1 which was operated at an internal chamber pressure of 30 Torr there was a visual presence of mould colonies on the fat covered surface of some of the loins in the air treatment group. In addition there was an odour indicative of spoilage present when the air chamber for Run 1 was opened. The type of mould present was visually consistent with the surface mould that may be present with dry aging of meat and aging of cheeses. The type of mould involved was not explored further and the decision was made to use a significantly lower pressure (5 to 7 Torr) for the remaining 3 runs. No evidence of mould or odour was noted for the remaining 3 runs.

The *B. thermosphacta* counts for loins in the Air and CO2 hypobaric chamber treatments (100% and 94% respectively) were significantly higher (at the 95% confidence interval) when compared to the Control group held in the chillers (69%). The results are shown in Table 5.2.3.

Table 5.2.3. Brochothrix thermosphacta measured 5 weeks after slaughter. Proportion of loins (n=32) in each group (± standard errors) and 95% confidence intervals.

B.thermosphacta count (cfu/g)	Treatment		
	Air	CO2	Ctrl
Low count category < 300 000	0/32=0	2/32=0.06 ±0.04 95% CI=[0, 0.14]	10/32=0.31 ±0.08 95% CI=[0.15, 0.47]
High count category > 300 000	32/32=1	30/32=0.94 ±0.04 95% CI=[0.86, 1]	22/32=0.69 ±0.08 95% CI=[0.53, 0.85]

All loins had less than < 10 cfu/g of *E. coli* at 24 hours post slaughter, however by 5 weeks post mortem, 1 loin treated in the CO_2 hypobaric chamber had 10 cfu/g of *E. coli* and 1 loin had 150 cfu/g of *E. coli*. All other loins had < 10cfu/g at 5 weeks post mortem.

The average count of *Enterobacteriaceae* was significantly higher for the CO_2 treatment compared to the Air and Control (df =2, deviance ratio=60.30; P<0.001) (Table 5.2.4).

Table 5.2.4. The effect of storage treatment on *Enterobacteriaceae* at 5 weeks post mortem. Predicted means and standard errors on the log_e scale and back transformed means in brackets.

Treatment		
Air	CO2	Ctrl
4.975 ± 0.511 b (2298)	6.435 ± 0.493 a (9897)	5.270 ± 0.505 b (3086)

Means with different letters are significantly different at p=0.05. Average pairwise l.s.d.= 0.424.

There was no significant difference between the treatments for mean LAB (df = 2, deviance ratio = 2.91; P = 0.131) or Listeria (deviance ratio = 0.67, P = 0.512) and Salmonella was not detected at either 24 h post mortem or after 5 weeks ageing.

5.2.4 Shear Force

The results for shear force measurement are provided in Table 5.2.5.

Analysis of shear force values demonstrated that ageing was significant ($F_{1,6}$=213.07; P<0.001), as average shear force values at day 1 (67.9 N) were higher than the average shear force values after 35 days storage (27.4 N). However, there was no effect of treatment in the hypobaric chambers and no interaction between storage and treatment in the hypobaric chambers (treatment $F_{2,4}$=3.31; P=0.142, interaction $F_{2,6}$=0.32; P=0.736).

Table 5.2.5. Effect of hypobaric treatment and storage time on shear force.

	Time Post Mortem (days)	
Treatment	Day 1	Day 35
Air	71.77	28.42
CO2	67.98	27.59
Ctrl	63.95	26.05
mean	67.90 a	27.36 b

Storage time means with different letters are significantly different at P = 0.05; L.S.D.=6.796.

6.0 DISCUSSION

6.1 Chamber Parameters

Effective management of the chamber parameters of temperature, pressure and humidity were expected to be critical to the success of hypobaric storage of meat.

The records show that the temperature was maintained at around 0 degrees Celsius as was intended to minimise the potential for microbial growth of pathogens and other microbes that cause spoilage of meat. The humidity level was maintained as close to saturation as possible and the records show the mean relative humidity was above 97% in the chambers in all runs. The records for mean internal chamber pressure show it was below 6 Torr for runs 2, 3 and 4. A higher pressure (30 Torr) was used in run 1 however this proved to be unsuccessful in terms of control of mould on the surface of the meat.

The importance of temperature management in storage of meat is widely known and as a parameter for chamber operation will not be discussed further here.

6.1.1 Relative Humidity of the Residual Atmosphere

Maintenance of the relative humidity as close as is practical to saturation is important to minimise loss of moisture from the product. The results for purge loss show a reduction in the weight of loins in the treatment groups by between 6 and 7 % compared to 3 percent for the vacuum pack controls.

Cooling at the surface where moisture is being lost is also a consequence of the evaporation of the water. There was no appreciable difference in the temperature of the product and the chamber temperature which indicates that this level of moisture loss over the timeframe of 5 weeks did not affect the temperature of the product. The use of the stainless steel metal rack with a metal frame may have provided a heat sink in contact with the product and assisted in heat transfer.

6.1.2 Chamber Pressure

Run 1 was operated at an internal chamber pressure of 30 Torr. On opening the chamber after 5 weeks there was a visual presence of mould colonies on the fat covered surface of some of the loins and an odour indicative of spoilage in the air treatment group. Given that it was only observed in the air treatment group it was presumed that the level of oxygen in the residual atmosphere was sufficient to enable the mould growth.

Consideration was given to lowering the pressure so that it would be much closer to the expected vapour pressure of water in the chamber at 0 degrees Celsius thereby minimizing the partial pressure of air in the residual atmosphere. At saturation point the vapour pressure of the water at 0 deg C would be 4.6 mm Hg (or 4.6 Torr). Adjustments were made to the equipment to facilitate this and the mean chamber pressure for runs 2, 3 and 4 was between 5.3 and 5.5 Torr. No evidence of mould or odour was noted in either treatment chamber for the remaining 3 runs.

Based on the outcome for Runs 2, 3 and 4 an internal chamber operating pressure of 5.5 Torr was proven successful in maintaining the visual appearance of the meat. It is possible that higher operating pressures could be used and this would require further experiments to establish a

threshold maximum internal pressure suitable for future use.

6.2 Sample Results

6.2.1 Purge loss, Lipid Oxidation, Ultimate pH and Carbonyl content

The findings show that there was no difference observed in ultimate pH, lipid oxidation or protein oxidation between the treatment groups and the controls. From a meat quality perspective this shows that breakdown products from fat and protein degradation are not impacting on the quality of meat stored under the hypobaric conditions used in this pilot study.

There was a significant difference between purge for loins treated in hypobaric containers compared with loins stored under control conditions (Table 5.2.1: P = 0.013) with greater fluid losses in loins in the treatment groups. The results for purge loss show a reduction in the weight of loins in the treatment groups by between 6 and 7 % compared to 3 percent for the vacuum pack controls.

An increase in moisture loss in the treatment groups was not unexpected. As outlined in 6.1 under hypobaric conditions the maintenance of high humidity in the residual atmosphere is critical to minimising weight loss through moisture evaporation.

The rate of moisture loss is a function of the surface area and the relative humidity. The level of moisture loss observed in this pilot would be expected to be less for whole carcases held under similar conditions as the surface area to volume ratio would be much lower than the loins used in this experiment .

In the report on Dry Aged Lamb Proof of Concept Stage 2 prepared for Meat and Livestock Australia (Project V.RMH.0045) the moisture losses reported after 39 days of dry aging were in the order of 14 to 15%. Considering these findings in the context of the potential for use of hypobaric storage as a means of transporting or storing meat the impact of the weight loss (6 to 7%) on organoleptic properties of the meat would be expected to be negligible.

6.2.2 Retail Colour

Retail colour measurements provide an insight to consumer acceptability of meat under retail display conditions. The decline in L* values measured was not significant between treatments or over time on retail display and the loins measured in this study did not fall below the consumer acceptability threshold of 34 during retail display.

The a* values significantly declined over the retail display period ($P < 0.001$) and the decline varied between treatments resulting in the loins treated in hypobaric chambers falling below the a* threshold (14.8) for consumer acceptance earlier than the vacuum packed control group. The rate of decline is shown graphically in Figure 6.2.1. It can be seen that the air treatment group reached the threshold by day 2, the CO2 treatment group reached the threshold during day 2 and the control group reaches the threshold by day 3.

There was also a significant in difference observed in the 630/580 nm ratio after 5 weeks storage and 48 h (P =0.046) and 72 h under retail display (P =0.029). This is shown graphically in Figure 6.2.2. It can be seen that the loins from both the hypobaric treatment groups fell below the R630/580

threshold for consumer acceptance (3.3) by the day 2 time point for retail display compared to the control group which did not fall below this threshold until approximately 2.5 days on retail display.

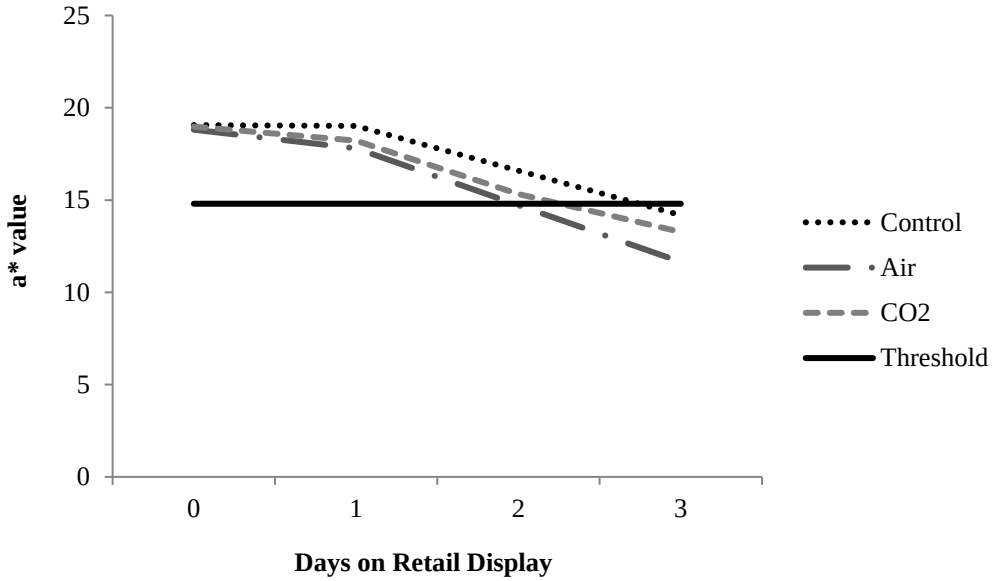

Figure 6.2.1 The decline a* values measured during retail display for loins treated in hypobaric containers held under pressure with Air and CO2 compared to the loins held under chilled storage conditions only.

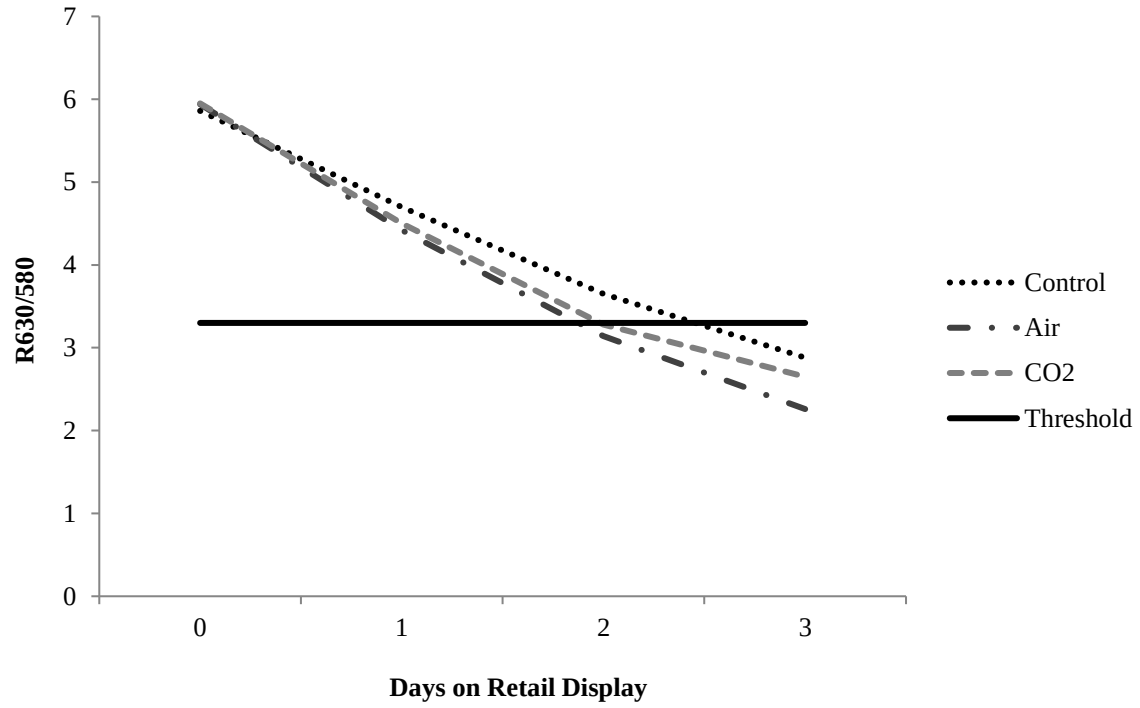

Figure 6.2.2. The decline in the ratio between 630 and 580 wavelengths measured during retail display for loins treated in hypobaric containers held under pressure with Air and CO_2 compared to the loins held under chilled storage conditions only.

The observations for retail colour may be significant for use of hypobaric storage in situations where the meat will be on display for more than 2 days after exiting the hypobaric container. An important consideration though is that the vacuum pack controls only provided an extra half day before the consumer acceptability threshold was reached.

The impact of these findings for lamb carcases transported in hypobaric containers to overseas wet markets would be negligible as they would be sold within hours of exiting the container and consumed within 2 days.

6.2.3 Microbiological Samples

The results for pathogenic bacteria including the use of the *Enterobacteriaceae* count as an indicator showed that there is minimal health risk associated with the use of hypobaric storage. Salmonella was not detected at either 24 h post mortem or after 5 weeks ageing.

Listeria was present in some loins at 24 h post mortem or after 5 weeks ageing.

All loins had less than < 10 cfu/g of *E. coli* at 24 hours post slaughter and with the exception of two loins from the CO2 test group the loins had < 10cfu/g after 5 weeks. In the CO_2 treatment group 1 loin had 10 cfu/g of *E. coli* and 1 loin had 150 cfu/g of *E. coli*.

The average count of *Enterobacteriaceae* was significantly higher for the CO_2 treatment compared to the Air and Control. The means for the treatment groups were less than 10^4 however at 9897 the mean for the CO2 treatment groups was at the upper end of marginal from a food safety standards perspective.

Given there was only one sample with a high count for E Coli it is unlikely that that particular finding relates to chamber conditions that favoured the growth of E Coli and contamination of the sample is a more likely reason. The high mean count for *Enterobacteriaceae* is however indicative of possible conditions in the CO2 chamber that favour the growth of these organisms. The reasons for this are unclear and warrant further work if CO2 as a residual atmosphere for hypobaric storage is to be pursued further.

The extent of microbial contamination by *Brocothrix thermosphacta* provides an indicator of the potential for spoilage. The level of contamination is assessed as the proportion of samples in a high or low count category. A low count is represented by less than 300,000 cfu per gram whereas a high count category means there is greater than 300,000 cfu/gram. All 32 loins in the Air treatment groups had high counts as did 30 of the 32 loins (94%) in the CO2 treatment groups. This compared to 22 of the 32 loins (69%) in the control group having high counts.

It is unclear why there would be a high proportion of high counts in the treatment groups compared to the control group. The reasons for it and possible management options need to be explored further.

Lactic acid bacteria levels were also considered and the counts for the treatment groups and the controls were not significantly different indicating the growth conditions were similar.

6.2.4 Shear Force

Shear force measurement provides an incation of improvement in tenderness as a consequence of ageing of the meat.

The results demonstrated that the improvement in tenderness through aging in a hypobaric chamber was equivalent to the improvement attributable to aging in a vacuum pack as there was no significant difference in the mean shear force values for the treatment groups and the control group after 35 days. Analysis of shear force values indciated that shear force values at day 1 (67.9 N) were significantly higher than the average shear force values after 35 days storage (27.4 N).

This finding provides confidence that whole sheep carcases could be transported or stored using hypobaric storage methods and be effectively aged while in transit.

7.0 CONCLUSIONS/RECOMMENDATIONS

This pilot study has effectively provided proof of concept for the potential use of hypobaric containers for transport and storage of sheep meat. Further development of this concept and subsequent commercialisation involving the development of hypobaric shipping containers specifically for the transport of chilled sheep carcases will open up opportunities for transport of whole chilled sheep carcases directly into wet markets in countries around the world, but particularly into the Middle East. An additional benefit will be the ageing of the meat during transit.

In this study the combined hypobaric chamber operational parameters for temperature (0 Deg C), humidity (mean above 97%) and pressure (5.3 to 5.5 Torr as in runs 2, 3 and 4) resulted in the successful storage of the lamb loins over a 35 day timeframe.

In relation to product quality under the hypobaric conditions for storage listed above the following conclusions were drawn:

- The ultimate pH, lipid oxidation and protein oxidation findings after storage were equivalent to that observed with traditional wet aging using a vacuum pack,
- The weight loss or purge during storage was between 6 and 7% which is higher than that observed with wet aging (2-3%).
- Retail colour is consistent with consumer expectations for 2 days after removal from storage,
- In terms of pathogenic bacteria the microbiological quality of meat stored with low pressure air as the residual atmosphere in the chamber was equivalent to traditional wet aging in a vacuum pack,
- In terms of spoilage bacteria meat stored with low pressure air or CO2 as the residual atmosphere in the chamber revealed higher levels than traditional wet aging in a vacuum pack,

- The microbiological quality of meat stored with low pressure CO2 as the residual atmosphere in the chamber revealed some unexpected findings for the presence of pathogenic bacteria, and
- In terms of tenderness as measured by shear force the ageing of the lamb in the hypobaric chambers was equivalent to that observed using traditional wet aging in a vacuum pack.

In terms of the value proposition for this concept progressing further the primary use of hypobaric container systems would be the export of chilled sheep carcases and beef quarters to markets currently serviced by the live export trade. The use of such systems is not seen as replacing live exports, but complementing the live export trade through expansion of the total value of the live sheep and sheep meat market to those countries. It would also provide the ability to service markets Australian live exporters are not permitted to send live sheep to due to animal welfare concerns.

In relation to sheep meat further research work required before proceeding to commercialisation includes:

- Further investigation of the microbial quality issues and where required methods to minimise bacterial growth;
- Evaluation of the organoleptic aspects of various lamb and wether meat cuts (as they are currently exported live) after treatment;
- Determination of acceptable ranges for the pressure and humidity chamber parameters;
- Studying the effect of shorter and longer storage timeframes and determining an upper limit of storage time for acceptability of product from a food safety and meat quality perspective;
- Investigate a suitable reefer design for hypobaric storage and transport of chilled whole sheep carcases, including the engineering aspects required to reliably manage the temperature, humidity and pressure; and
- Quantifying the potential market benefits of proceeding with the development and use of hypobaric reefers for transport of sheep carcases.

Similar research could be conducted for hypobaric storage and transport of beef.

It is recommended that the further research for sheep meat referred to above be pursued commencing with the first two areas of research listed.

In relation to similar research for hypobaric storage of beef it is recommended that this should commence once the further research on the organoleptic properties and microbial growth aspects of hypobaric treatment of lamb is completed.

8.0 BIBLIOGRAPHY

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