Impact of extended shelf-life of chilled beef into overseas markets

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TABLE OF CONTENTS

Table of Contents 2

1.0 EXCECUTIVE SUMMARY 4

2.0 INTRODUCTION 6

3.0 PROJECT OBJECTIVES 6

4.0 METHODOLOGY 7

Experimental design 7

Selection of animals 8

Packaging and distribution 8

Microbiology 9

Meat biochemistry 9

Pack sensory assessments 10

Total volatile basic nitrogen (TVB-N) 10

Fatty acid analysis and TBARS 10

Measurement of non-volatile changes in VPCB 11

Measurement of volatile changes in VPCB 11

Statistical analysis 11

5.0 PROJECT OUTCOMES 12

Carcass attributes 12

Initial microbiology 12

Initial biochemistry 13

Timelines, shipping and storage 13

Temperature log histories 14

Temperature deviation from ideal 17

Aerobic plate counts in VPCB 18

Lactic acid bacteria growth in VPCB 19

VPCB shelf-life prediction using the University of Tasmania model 19

Sensory assessment of VPCB 20

Changes in pH over time 26

Relationship between temperature deviation parameters, pH and microbiological data 26

Relationship between pack sensory scores and temperature deviation 27

Chilled beef storage colour changes 29

Total volatile basic nitrogen 29

Changes in meat lipids during storage and TBARS 30

Changes in non-volatile compounds.....31
Changes in volatile compounds in raw and grilled VPCB.....32
Rapid volatile profiling by PTR-MS.....35
Collaboration with SDAU 36
7.0 CONCLUSIONS & RECOMMENDATIONS 37
9.0 APPENDICES 38
9.1 Appendix 1 _ Sensory Assessment Ballot sheet – English version 38
9.2 Appendix 1 _ Sensory Assessment Ballot sheet – Chinese version 41
10.0 BIBLIOGRAPHY 43

1.0 EXCECUTIVE SUMMARY

The temperature control of the current cold supply chain for Australian vacuum packaged chilled beef (VPCB) exported to China, was tested over an extended storage period; up to 140-days. The temperature of VPCB was monitored from the processing plant in Queensland, during refrigerated shipping to Shanghai (China) and within the Chinese cold supply chain. Extended storage VPCB control samples, held under near ideal conditions (-1 ^{o}C) at CSIRO (Brisbane, Australia), were also extensively biochemically characterised.

Beef *longissimus lumborum* (striploins) were obtained from a commercial beef processor, currently exporting meat to China. Meat Standards Australia (MSA) graded carcasses (n = 40) were within microbiology and biochemistry specification for export. Striploins were vacuum packaged as per standard plant protocol and allocated into cartons and shipped to one of four destinations:

1) Australia (CSIRO) under near ideal conditions (control, -1 ^{o}C)
2) China: Shandong Agricultural University (SDAU, Shandong, Tai'an)
3) China: Huadong distribution centre (greater Shanghai area)
4) China: Fuzheng distribution centre (greater Shanghai area)

Temperature profiles during transport of each carton was recorded from meat processor to final destination, using two different temperature loggers (i-buttons and LogTags). During the 16-day shipping journey to Shanghai, the temperature of VPCB was maintained at ~ 0^{o}C. On arrival in Shanghai International Port (SIP), samples were held and inspected by Chinese Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) officials, for an extended period (41-days) before release. During that time the average temperature of boxes increased to more than 3-4^{o}C for a 7-day period. The extended holding period and temperature excursion at the SIP should be considered "atypical". The Chinese cold-supply chain integrity was generally good at the distribution centres and during road transportation within China. The main temperature deviations occurred during customs handling at the SIP.

VPCB samples held at distribution centres in China were sent to SDAU for testing at subsequent time points; 84-days (12 weeks), 98-days (14 weeks), 120-days (17 weeks) and 140-days (20 weeks). The microbiological, biochemical and sensory assessment tests were also conducted on the VPCB samples stored at CSIRO on the same days. After 84-days (12 weeks), microbiological, biochemical and sensory assessment was conducted on the VPCB. Despite low initial counts, the aerobic plate counts (APC) and lactic acid bacteria (LAB) counts were somewhat higher than expected in all VPCB samples, compared to previous studies (Small, 2011). Although a decline in some of the pack sensory parameters was measured, there was no evidence of an abrupt change in the odour of the raw meat indicative of spoilage. The pack sensory properties of the VPCB before and after opening and blooming were higher in the CSIRO control samples, which had optimal temperature control. In general, the APC and LAB counts were weakly correlated with the pack sensory scores, whereas the temperature deviations of VPCB samples were more strongly correlated with the sensory changes. The findings confirmed that APC and LAB counts alone are not sufficient for shelf-life prediction.

Independent modelling of the storage data using the University of Tasmania (UTas) prediction model demonstrated that all of the CSIRO VPCB would easily attain more than 140-days shelf-life. Despite temperature deviations, most of the Chinese VPCB samples were predicted to attain 140-days shelf-life, and all were predicted to achieve 120-days.

Total volatile basic nitrogen (TVB-N), is a measure of meat freshness/spoilage recognised by Chinese authorities and was also conducted on the samples at each time point. The TVB-N limit for fresh and frozen beef in China is 15 mg/100g. Most of the Chinese VPCB samples had a TVB-N level slightly higher than 15 mg/100g, indicating they may not be considered compliant according to the current Chinese criteria. There was, however, no indication of organoleptic degradation or spoilage in any of the Chinese VPCB product from sensory pack assessments and other measures. The sensory assessments suggested that the limit of 15 mg/100 g is not an appropriate TVB-N cut-off for VPCB**.** Other groups have previously shown that a higher limit, e.g.; 20 mg/ 100 g is a more appropriate TVB-N limit for beef, in which case all of the Chinese VPCB samples in this study would be considered compliant. Unlike microbiological counts, the TVB-N clearly increased with time in VPCB, suggesting that this biochemical parameter could be a useful addition to shelf-life prediction models.

For VPCB samples stored under near ideal conditions, quantitative changes in lipids (measured as fatty acid methyl esters) indicated a slight reduction in some saturated and unsaturated lipids between day zero and 84-days perhaps due to unknown microbiological processes. Non-volatile metabolite analysis indicated, large increases in free amino acids and carnosine, indicative of proteolysis still occurring until around day-120, which then slowed. The increased proteolysis could be due to the microbiological populations present and could positively impact on sensory attributes, such as tenderness and flavour intensity. Key non-amino acid meat metabolites such as creatine and carnitine underwent smaller changes over time. Nucleotide metabolites, such as inosine monophosphate, also showed a progressive decline over the storage period. These metabolites are of interest for understanding the development of sensory attributes of the meat.

In raw and grilled VPCB, there were small increases in some volatiles over time, but none were indicative of spoilage. A sharp increase in the concentration of ethanol between 120 and 140-days indicated that this volatile may be a useful indicator of extended shelf-life.

Overall, the data supported justification for extension of shelf-life limit to 140-days for Australian VPCB with the caveat that extremely good temperature control needs to be maintained at all times throughout the entire cold supply chain.

2.0 INTRODUCTION

Australia exports more than 18,000 metric tonnes of VPCB, the bulk of it (7,884 tonnes) destined for Japan, but China is rapidly becoming the next major East Asian export market for Australian VPCB (http://www.agriculture.gov.au/export/controlled-goods/meat/statistics). China does not have an 'official' shelf-life limit for Australian VPCB. Other countries such as Japan, have a limit of only 77-days or 11 weeks (Huynh, 2016). An extension of the shelf-life of VPCB beyond 11 weeks would allow greater flexibility to exporters and beef suppliers, especially in overseas markets, including China.

End of shelf-life of VPCB has traditionally been defined by a microbiological limit; e.g. when aerobic plate counts (APC) or lactic acid bacteria (LAB) counts, exceed ~10^6 log. It is widely thought that arbitrary microbiological limits are simplistic measures of shelf-life, because VPCB can have higher microbial counts and display little evidence of spoilage and retain good organoleptic properties (anecdotal evidence from processors and observed in this study). The odour of the raw meat after pack opening – "confinement odour" – may be used as an initial indicator of beef freshness. The character of the pack odour may change in odour strength and character over a given storage period, however this may not necessarily be strongly correlated with bacterial growth or the eating quality. Additional objective biochemical markers of VPCB shelf-life would be useful to incorporate in prediction models and also to better understand the biochemical changes that occur during extended vacuum storage.

In addition, if the storage temperature of VPCB is very well controlled at –1.0°C; a much longer shelf-life is achievable, with eating quality increasing up to 140-days (20 weeks) (Hughes, McPhail, Kearney, Clarke, & Warner, 2015; Small, 2011). In a controlled environment, this is optimal, but for export markets, achieving this can be more problematic. Variations in temperature are difficult to maintain throughout the supply chain, especially when other international shipping companies and customs have their own procedures to follow. Using the Australian-Chinese supply chain as an example, this study aimed to test the temperatures that VPCB is exposed to during the transportation process, from Australian processor to Chinese customer. This will provide Australian beef processors with an unbiased perspective of the temperature fluctuations that occur to VPCB whilst creating an opportunity for all parties involved in the process to achieve a favourable product for the Chinese consumer.

3.0 PROJECT OBJECTIVES

- Determine typical temperature fluctuations which occur in vacuum packaged chilled beef during international shipping and within the cold supply chain in China.
- Demonstrate that a longer shelf-life for exported chilled beef can be supported, aiming for 120-days (17 weeks) to 140-days (20 weeks).
- Strengthen international collaboration between Australian and Chinese red meat researchers
- Provide Australian beef processors with evidence based recommendations for achieving longer shelf-life of chilled beef for international export markets, such as China.

4.0 METHODOLOGY

Experimental design

Australian VPCB was collected from an existing exporter and allocated to four different destinations and stored for four different storage times (84, 98, 120 and 140-days). Constant temperature monitoring was conducted using i-buttons and LogTags throughout the whole duration of storage. VPCB was held at CSIRO under near ideal conditions (- 1 °C) and the remaining samples were shipped to China and transported via three different cold supply routes within China (**Figure 1**). An earlier time point was planned (50-days), but delays in Shanghai International Port (SIP) precluded this. After sample collection (day 0, 16th August, 2016) the positive control VPCB samples were transported to CSIRO, Brisbane. Remaining samples were then shipped to China (via Brisbane Port) in two separate shipping containers (departed, day 20, 5th September). Container-1 (containing SDAU and Chinese distributor-1 or Huadong samples) had some delays, but arrived at the destination on day 77 or 1st of November. Container-2 (containing distributor-2 or Fuzheng samples) had no delays and arrived at destination on day 45 or 30th September. Both distributors are located within the greater Shanghai area, approximately 800 km from SDAU in Tai'an in Shandong province (**Figure** 2). After storage at the distribution centres (Huadong and Fuzheng) designated boxes of VPCB were then shipped back to SDAU by refrigerated truck prior to each storage time point; 84, 98, 120 and 140-days, for chemical, microbiological and sensory testing. The same tests were conducted on the CSIRO samples at the same time. In addition, extensive biochemical testing was carried out on the CSIRO VPCB samples.

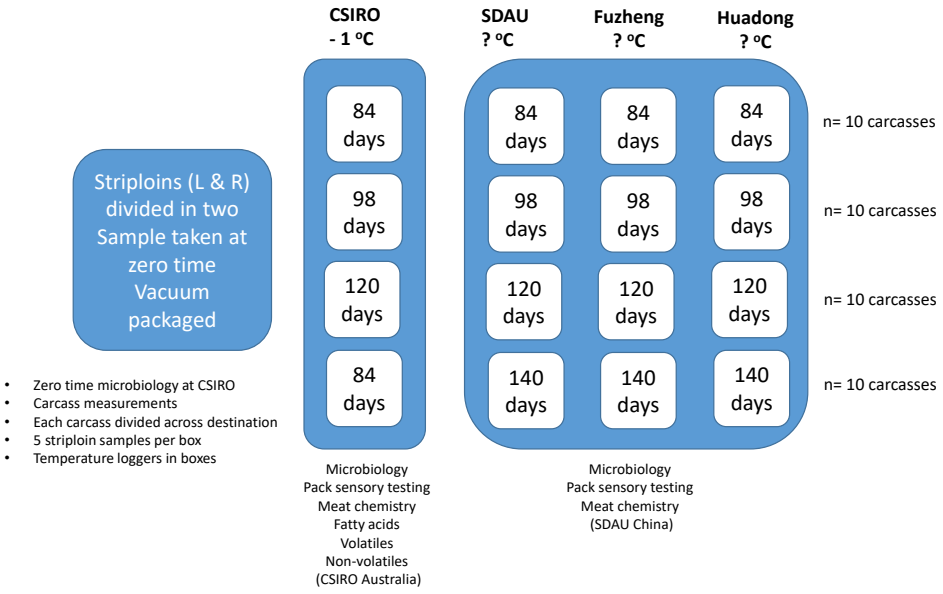


Figure 1: Experimental design. Striploins from each carcass were distributed across four destinations. Due to delays in shipment by Chinese Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) officials, the first time point was modified to 84-days.

Figure 2: Location of the Chinese university (SDAU) in Shandong province and the two distribution centres (Huadong and Fuzheng) located in the greater Shanghai area.

Selection of animals

Pasture-fed Santa Brahman cross steers, approximately 42 months old, from the same group of animals were selected. Animals had less than 76 % highest tropical breed content (TBC) and were hormone growth promotant (HGP) free. Animals were transported to the meat processor >24 h prior to slaughter, where they were provided with shade and water and were not exposed to any adverse conditions or mishandling. Steers were stunned using captive bolt, hind leg shackled and exsanguinated by thoracic stick. No immobilisation or electrical stimulation was used. The hide puller was used at 40 Hz for 7 seconds and a hot water decontamination unit was used at 82°C for 19 seconds. Carcasses were Achilles hung and held between 0 to 6 °C for >18 h prior to chiller assessment. Carcasses were quartered between the 10th and 11th rib and were graded according to AUS-MEAT (2014) assessment criteria for meat colour (MC), pH, rib fat, eye muscle area (EMA), Meat Standards Australia marbling score (MSA-MB), ossification and hump height. Dentition scores and the hot carcass weight (HCWT) was also collected.

Packaging and distribution

Striploins (*longissimus lumborum)* were halved and were placed in a Cryovac high shrink barrier vacuum bag (low density polyethylene, polyvinyl acetate and polyvinyldichloride, with maximum oxygen transmission rate of 30 cc/m^2/24 h/ @23°C) of the type normally utilised by the plant (see illustrations in **Figure 3,** a, b and c). All samples were processed as a group through the standard evacuation and shrinking process (80 - 86°C). Samples were packed into cartons according to the time of storage with five half striploins placed in each carton. A small cross section of the striploin was taken from each carcass for zero time measurements and transported to the CSIRO laboratory in insulated containers with freezer blocks. The remaining samples for the four time points destined for transportation, were allocated to a carton and had 2 different temperature data loggers inserted; i-button and a LogTag (**Figure 3**). Each carton was chilled according to the standard plant procedures and then either transported to CSIRO via commercial refrigerated road transport several days later or transported to China via a standard sea-freight shipping container. On arrival at the laboratory, the day zero samples were immediately placed in a chiller operating at 5°C and processed for initial microbiology and biochemistry. The eight cartons allocated for long term storage were placed at -1 °C until the storage time point was processed at time points consistent with product in China.

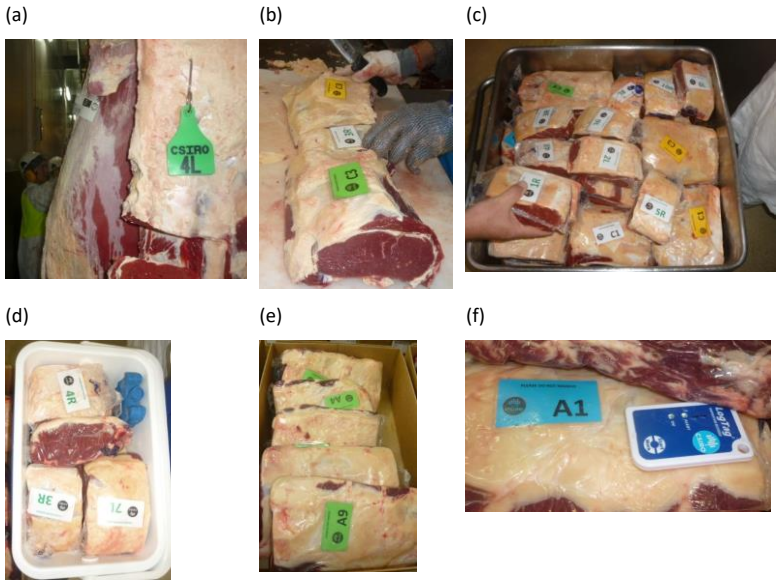


Figure 3: Summary of collection procedure: (a) MSA graded carcasses identified in the chiller, (b) striploins cut in half and randomly allocated to destinations (centre white piece for time zero microbiology assessment), (c) all samples vacuum packed, (d) time zero microbiology samples transported immediately back to CSIRO for testing, (e) all other samples packed in cartons, with 5 half striploins/ per carton and (f) every carton containing 2 temperature loggers (i-button and LogTag).

Microbiology

Initial microbial contamination on the surface of striploins was determined for each of the 40 sampled animals as follows. Four × 10 cm^2 surface slices comprised of two subcutaneous fat and two lean portions of meat were excised from each striploin. Surface slices from the same striploin were combined with 100 mL of 0.85% saline in sterile bags and stomached for 1 minute. An aliquot of each sample was decimally diluted in 0.85% saline and plated onto Petrifilm Aerobic Count (AC) Plates (3M Microbiology, Minnesota, USA) for determining Aerobic Plate counts (APC) and *E. coli* / Coliform (EC) Count Plates (3M) for determining *E. coli* / Coliform counts. Parallel dilutions were also prepared in de Man, Rogosa, Sharpe (MRS) broth (Oxoid, Basingstoke, UK) and plated onto AC Plates for enumerating lactic acid bacteria (LAB). AC Plates were incubated at 25°C ± 1°C for 96 ± 3 h; EC Plates were incubated at 35°C ± 1°C for 24 ± 2 h and LAB Plates were incubated anaerobically at 25°C ± 1°C for 120 ± 3 h. Samples with counts of zero (no colony forming units or CFU detected at the lowest dilution tested) were arbitrarily assigned a value of half the limit of detection (i.e. 0.1 $\log_{10}$ CFU/cm^2 for APC and EC and 1.10 $\log_{10}$ CFU/cm^2 for LAB).

Meat biochemistry

After initial microbiology samples were collected, a 25 mm steak was cut and allowed to bloom for 60 minutes at 10 °C prior to colour measurement. Meanwhile, a secondary 25 mm steak was cut for pH analysis. The pH was measured using a TPS Model WP80 pH meter fitted with an Ionode IJ44 combination pH electrode (TPS Pty Ltd, Springwood, Qld, Australia) after calibration at 10°C. After blooming, the colour was measured using a Minolta CR-400 (Minolta Pty Ltd, Japan, light source D65, observer angle 2°, light projection tube CR-A33d, with φ22mm disc).

Three determinations of lightness and redness (L* and a* respectively) were made. The same microbiology and meat biochemistry protocols were used in China at SDAU and at CSIRO to ensure the data was directly comparable. CSIRO staff were present for the first time point in SDAU, China to ensure consistency in measurements.

Pack sensory assessments

A standardised questionnaire (English and Chinese versions – see appendices) was developed to measure the sensory differences between packs and time points using a 100 mm line scale. The 100 mm scale is a validated instrument to measure sensory responses and is used in Meat Standards Australia untrained consumer testing. Before assessment, a briefing session was held to clarify the method of assessment and to clarify the attributes. Unopened packs were removed from refrigerated storage and placed on a large table at SDAU (China) and CSIRO (Brisbane) on the same day. Assessments were performed by 10 Chinese postgraduate students or 10 CSIRO staff members available at each time point. Packs were assessed one at a time by each person in a randomised order using a paper ballot. Assessors were asked first to visually inspect the unopened packs and; (Q1) rate the vacuum integrity on scale of 1 to 4, (Q2) rate freshness of appearance, (Q3) visually rate the amount of weep or liquid in the pack and (Q4) rate the overall liking of the appearance in the pack. After assessing the unopened packs, the bags were opened to assess the "confinement odour". A small cut was made in the packaging and then immediately re-sealed with a bulldog-clip. Assessors were asked to remove the bulldog clip, sniff the headspace and quickly rate the intensity of various attributes; (Q5) overall odour intensity, (Q6) odour freshness, (Q7) rotten/sulphur odour, (Q8) fruity/rotten odour and (Q9) cheesy/fermented odour. Bulldog clips were used to reseal the bags between sniffs.

The VPCB was then fully opened and allowed to bloom for 60 min and the assessors were asked to return and rate the post-bloom samples; (Q10) overall liking of post-bloom appearance, (Q11) post-bloom freshness appearance, and (Q12) post-bloom freshness odour. The last question (Q12) can be considered a measure of any persistent decrease in organoleptic quality that remains after the confinement odour has dissipated and is hence probably the most important indicator of the overall raw meat freshness/ acceptability. The average sensory scores were calculated for each pack at each time point and used in further statistical calculations. Six packs from the original design (SDAU) went missing and a further four packs were removed due to a loss of vacuum integrity. Hence only 148 packs were used in the overall statistical analyses.

Total volatile basic nitrogen (TVB-N)

TVB-N was measured in accordance to method specified in **GB 5009.228-2016**, in the National Standard of the People's Republic of China - GB 2707-2016 - "Hygienic Standard for Fresh (Frozen) Meat of Livestock". Briefly, 100 g of raw meat (free of subcutaneous fat) was minced and 10 g was weighed into 100 mL Milli-Q water and left with intermittent shaking for 30 m. The solution was filtered and 5mL of filtrate was added to a round bottom flask.

Immediately prior to distillation, a 10 mL volume of magnesium oxide solution (10 g/L) was added to the sample and the sample was steam distilled for 5 minutes. The distillate was condensed into a receiving flask containing boric acid (20 g/L) with indicator. The solution was back-titrated with 0.01 M hydrochloric acid solution and the total volatile basic nitrogen was calculated in mg/100g meat.

Fatty acid analysis and TBARS

Fatty acids were extracted from methanolic raw meat slurries (200 mg) and directly converted to fatty acid methyl esters according to a modified method (Lepage & Roy, 1986).

Briefly, the meat slurry was suspended in methanol (3 mL) in a Pyrex glass tube and tridecanoic acid was added as an internal standard (IS, 2 µg). Acetyl chloride was added to the sample drop-wise (200 µL X 2) in a fume hood (caution exothermic reaction, potential for explosion!) and hexane was added (1 mL) and the contents vortexed. Samples were sealed and incubated at 70 °C (3 h), cooled, and washed with potassium carbonate solution (6%). The hexane layer was dried with sodium sulphate and a 1 µL volume was injected onto the gas chromatograph (240 °C, 1:20 split) (Shimdazu QP-2010-Plus GC–MS,). Separation was achieved on a Zebron-Wax Plus (Phenomenex) column (0.25 mm, 30 m. 0.5 µm). The amount of each lipid in the meat was determined using the integrated total ion current (TIC) for each peak compared to the IS and experimentally determined response factors (Dodds, McCoy, Rea, & Kennish, 2005). Thiobarbituric-reactive substances (TBARS) was determined according to previously reported methods (Hughes, McPhail, Kearney, Clarke, & Warner, 2015).

Measurement of non-volatile changes in VPCB

Selected non-volatile compounds were measured by LC-MS/MS (TSQ-Quantiva, Thermo Scientific, Australia) equipped with a heated- electrospray ionisation source. Raw underivatised meat extracts (500 mg) were homogenised in 70 % methanol solution. Stable isotope internal standards were spiked into samples (50 µg/mL). ^{15}N-glutamine was used to quantify free amino acids, carnosine, carnitine and creatine, adenosine monosphosphate-$^{15}N_5$ for quantification of ribonucleotides, nucleotides and nucleosides and succinic acid-d_4 for quantitation of organic acids. Reference compounds were used for optimisation and calibration of the MS detector and determination of unique MRM-transitions. Response factors were determined against the labelled internal standards. Separation was achieved on an Intrada amino acid column (150 cmx 3 mm, 35°C, 200µL/min) using a solvent gradient; acetonitrile/formic acid (0.1%) (A) and 100 mM ammonium formate (B). Initial 14% B (0-3min) then 14% to 100% B (3 -10min), 100% B (10-12min) and 100% to 14%B (12-15min).

Measurement of volatile changes in VPCB

Volatiles were extracted at 37 °C for 40 m above raw and grilled VPCB slurries at zero time and after storage for the CSIRO samples only (n=50 samples raw and n=50 samples grilled). Volatiles were concentrated using solid phase microextraction (Carboxen-polydimethylsiloxane-divinylbenzene- 23 gauge, 2 cm fibres) in an autosampler and analysed by gas chromatography mass spectrometry (GC-MS). Volatiles were desorbed at 250 °C (splitless, 5 min) and separated on Zebron Wax-Plus column (0.25 mm, 30 m, 0.5µm).

Statistical analysis

Data analysis for summary statistics and adjusted means was performed using GenStat 16th edition (VSN-International) using the standard REML procedure. Destination (CSIRO, SDAU, Huadong, and Fuzheng) and time point (zero time, 84-days, 98-days, 120-days and 140-days) were used as fixed effects and MSA-MB was coded as a fixed covariate to adjust for differences in the amount of intramuscular fat. HCWT and carcass number were coded as random variables. The total number of hours of exposure to temperatures above -1 °C— was calculated for each carton and also used as a fixed variable for use statistical models. For the analysis of the sensory data, initial APC and LAB counts were also coded as covariates, to ascertain whether they had a significant effect. Significant effects were shown as p values ($p < 0.05$). Correlations and linear regression modelling of data was performed using the standard procedures in GenStat. The two-sided test for significance was calculated for the correlation coefficient (r) and shown as a p value.

5.0 PROJECT OUTCOMES

Carcass attributes

Attributes of the 40 carcasses used in the study are summarised in **Table 1.** All carcasses were MSA compliant for pH, meat colour and rib fat depth. Most animals had a dentition score of 4, with one having 8 teeth. The hump height indicated the fairly high tropical breed content, which is to be expected in these cross-bred steers. In terms of fat, the majority of carcasses had a whitish colour and moderate fat depths (rib and P8) and marbling scores (MSA-MB) showed a fair range in values. Overall, the carcasses were fairly consistent in terms of attributes, so the treatments were randomised over all the storage time points.

Table 1: Mean initial (zero time) carcass attributes from 40 different carcasses.

Carcass attribute	Mean	Median	Minimum	Maximum	Std dev
Dentition	4	4	1	8	2
Ossification	150	160	120	190	20
HCWT (kg)	330	324	279	404	29
Hump height (mm)	114	115	80	165	19
EMA (cm^2)	62	62	44	97	10.1
MSA marbling	310	300	160	530	90
P8 Fat depth (mm)	12	12	5	20	3.9
Rib fat depth (mm)	9	8	3	20	4.3
Fat colour	2	2	1	4	1
Meat colour	3	3	1C	3	1
Grader pH	5.59	5.61	5.40	5.68	0.07

Initial microbiology

The mean and range of counts obtained for APC and LAB are listed in **Table 2**. The initial microbiological counts for the beef samples were consistent with values obtained in previous CSIRO shelf-life projects (Small, 2011). Hence, stored under ideal conditions the VPCB samples were predicted to achieve an extended shelf-life and were considered suitable.

Table 2: Initial (week 0) bacterial counts (mean and range) on beef Longissimus lumborum (striploin) muscles from 40 different carcasses.

Target	Mean (log_{10} CFU/cm^2)	Range (log_{10} CFU/cm^2)
Aerobic Plate Count (APC)	**2.25**	**1.44 – 3.01**
Lactic Acid Bacteria (LAB)	**2.05**	**1.10 – 3.01**
E. coli	**0.1***	**N/A**
Coliforms	**0.1***	**N/A**

**E. coli* and coliforms were not detected in any of the 40 samples tested. These samples were arbitrarily assigned a value of half the limit of detection.

Initial biochemistry

As shown in **Table** 3, across the 40 carcasses, the initial colorimetric and pH values were fairly similar, with no incidence of high pH or dark meat colour (confirming grader observations) and consequently ruled out any problems that are known to occur with storing high pH meat for long periods of time.

Table 3: Initial (week 0) colorimetric (L and a*) and pH values on beef Longissimus lumborum (striploin) muscles from 40 different carcasses, where each carcass was allocated to a specific storage time point.*

	Lightness (L*)	Redness (a*)	pH
Minimum	28.6	19.0	5.41
Maximum	35.0	25.7	5.59
Mean for all samples	32.0	21.8	5.48
Standard deviation	1.58	1.76	0.04

Timelines, shipping and storage

The distribution and extended storage was executed as planned, however there were uncontrollable delays in clearance of VPCB by AQSIQ officials at Shanghai International Port (SIP) (http://www.portshanghai.com.cn/en/) and the timeline of main events are summarised in **Table 4**. Container-1 (SDAU and Huadong destinations) was held at SIP for an extended period of time; 41-days, due to problems with the labelling of cartons. This delay postponed the first experimental time point from 50 to 84-days. Furthermore, during the delay carton temperature deviations were found to be fairly high ~ 4 °C compared to controls, for more than 7 days, which likely accelerated the spoilage processes, thus shortening the shelf-life of the VPCB. The industry stakeholder indicated these temperature deviations were not considered typical.

Table 4: Timeline of events for the shipping and distribution of VPCB within China

Day	Date 2016	Event
0	August 16	Samples collected and packaged
15	August 31	Loading date (from Queensland plant)
20	September 5	Depart Brisbane in shipping container
36	September 21	Huadong and SDAU samples (container-1) arrived in Shanghai (16-days transit). AQSIQ officials inspected samples on September 27th and held the product for an extended period (41-days) before release. Some cartons were opened and some samples were confiscated. No explanation given.
38	September 23	Fuzheng samples (container 2): Arrived in Zhangjiagang (different terminal within Shanghai port - (18-days transit)
45	September 30	Fuzheng samples (container 2): Collected and cleared by AQSIQ officials and transported to Fuzheng distributor until sent to SDAU at various time points
77	November 1	Huadong and SDAU samples (container 1). Samples released from customs (held for 41-days at customs).
84	First time point	Microbiology and sensory evaluation performed in China (SDAU) and Australia (CSIRO)
98	Second time point	Microbiology and sensory evaluation performed in China (SDAU) and Australia (CSIRO)
120	Third time point	Microbiology and sensory evaluation performed in China (SDAU) and Australia (CSIRO)
140	Fourth time point	Microbiology and sensory evaluation performed in China (SDAU) and Australia (CSIRO)

Temperature log histories

The temperature history data for each of the 32 cartons of VPCB are shown below. As expected, i-button and LogTag temperature profiles were very similar for each carton. In **Figure 4**, CSIRO samples were held at ~3.0 °C for around 7 days (during storage at the meat processor) and then reached ~ 4 °C during road transport to Brisbane. Temperatures were maintained at ~- 0.5 to -1.0 °C for the duration of storage at CSIRO. The initial elevated temperature may be considered a deviation from ideal, but overall they were optimal for shelf-life extension of VPCB (between -1.0 and -0.5 °C).

The temperature histories of cartons from container 2 (Fuzheng distribution centre) in Shanghai are shown **Figure 5.** Prior to shipping, the samples were held at ~ 2 °C and temperatures were maintained close to 0 °C during the 16-day shipping journey. The container was opened on day-45 at SIP and cleared, but one i-button was lost in transit (84-day time point). At SIP the average temperature increased to ~5 °C for a number of days, before slowly returning to ~ 0 °C by day-66. The delays and elevated temperature at SIP were not typical for VPCB (personal communication from industry stakeholder). Once at the Fuzheng distribution centre, samples were generally maintained at ~-1 °C.

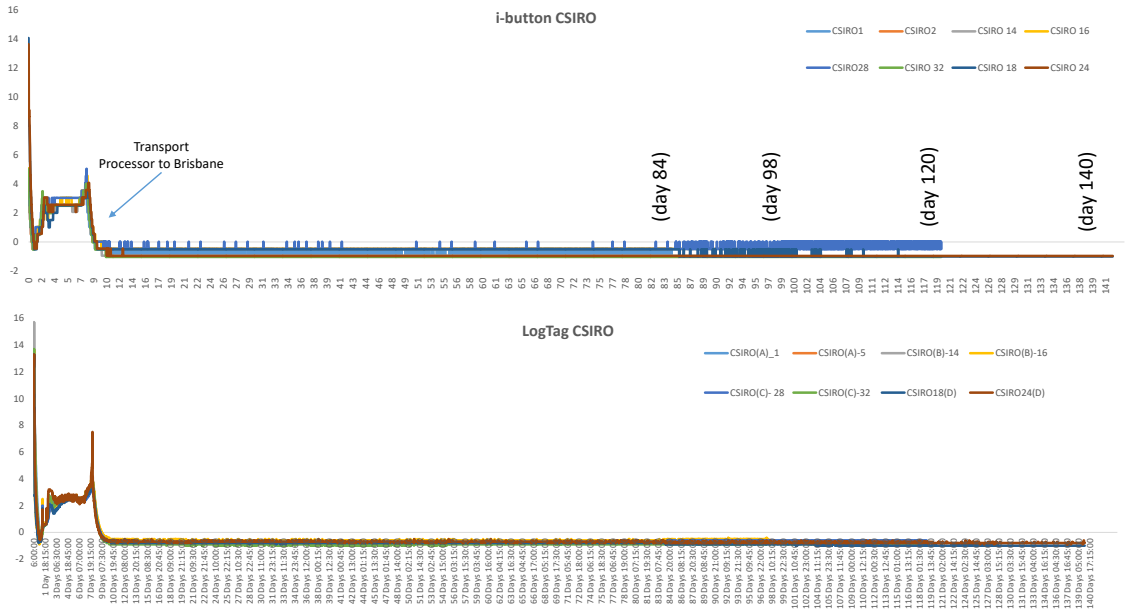


Figure 4: i-button (top) and LogTag (bottom) temperature logger histories for CSIRO samples. After being held at ~ 3 °C for a number of days during transport samples were maintained between -0.5 and – 1 °C at CSIRO.

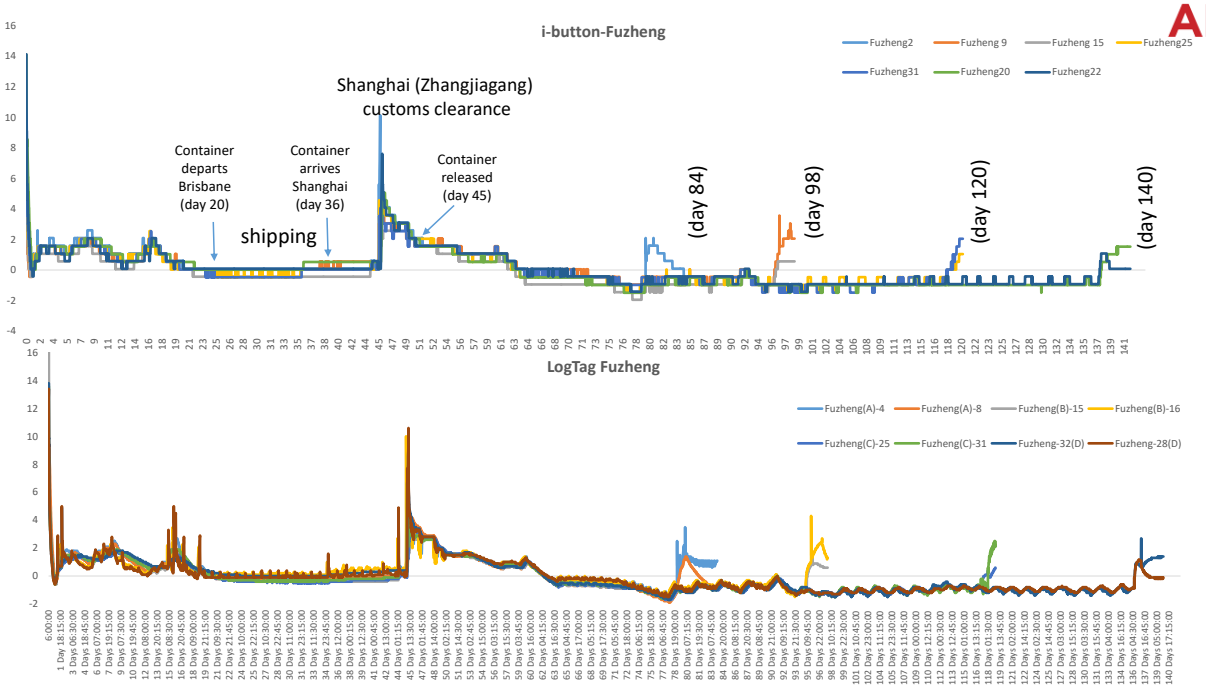


Figure 5: i-button (top) and LogTag (bottom) temperature logger histories for Fuzheng samples. Note that one of the i-buttons went missing (84-day sample)

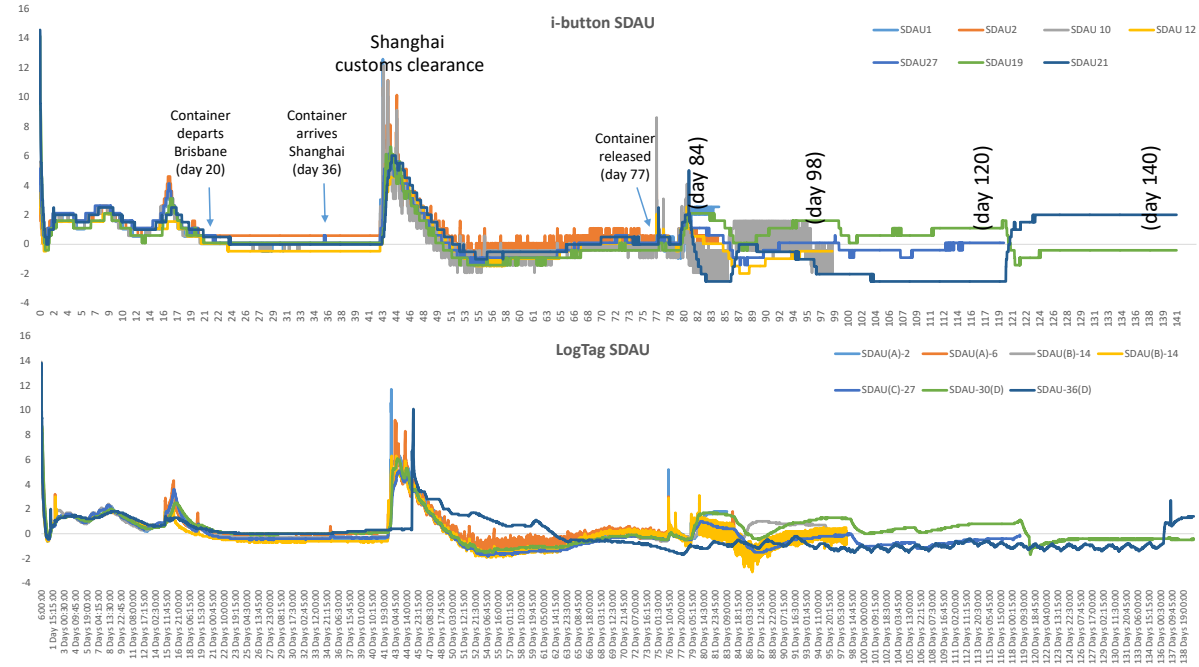


Figure 6: i-button (top) and LogTag (bottom) temperature log histories for SDAU samples. Note that some samples were confiscated and i-buttons and LogTags went missing (98-day sample)

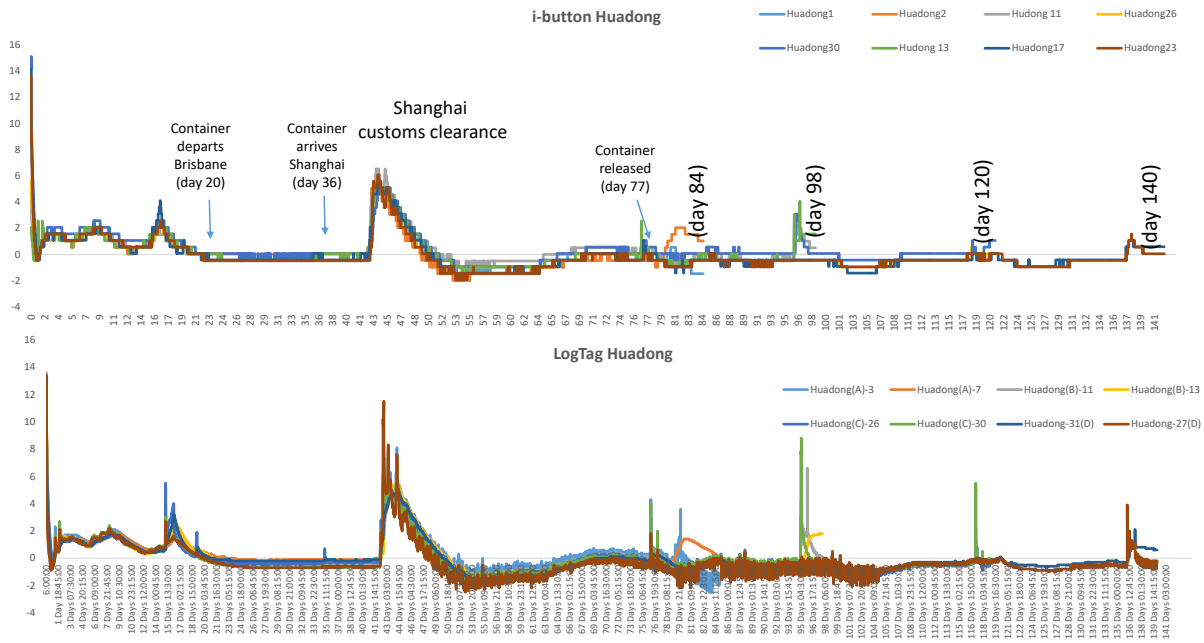


Figure 7: i-button (top) and LogTag (bottom) temperature log histories for Huadong samples.

Figure 6 and **Figure 7** show the temperatures measured in cartons from container 1. There is a larger peak observed during the delay at SIP, indicating higher temperatures were reached in this process. But, similar to container 1, once the distribution centre was reached, temperatures stabilised close to ideal. During road transportation from the distribution centre to SDAU, carton temperatures did increase to ~2 to 4 °C for a number of days, (seen as spikes on the profiles at the various time points) but these were no higher than those observed within Queensland. After transport to SDAU, temperatures were maintained between 2 and – 2 °C, until sampling at various time points.

During the delay period for container 2, a number of cartons were removed and inspected. AQSIQ officials notified us, that four striploin samples from one of the SDAU cartons had to be confiscated altogether, for sampling. All of the Chinese VPCB temperature histories indicated that the largest deviations from ideal storage temperature occurred at SIP during customs clearance. The extended delays at elevated temperatures are not typical and therefore should be considered accordingly.

However, after clearance, both distribution centres showed close to optimal storage temperatures, with Fuzheng maintaining temperatures close to -1 ^{o}C and Huadong close to ~ 0 ^{o}C. The SDAU samples were maintained between -2 and 2 ^{o}C. Road transport trips indicated boxes reached between ~ 2 – 4 ^{o}C for a day or more. Overall the data generally indicates that good cold chain integrity is possible in China, excepting the atypical temperature deviations at the SIP. Because the distribution centres and road transport were aware that the samples were part of a project, extra attention may have been provided to maintain good temperature control. Obviously more experiments would be required to ascertain how variable typical temperature fluctuations are at all stages in the Chinese cold supply chain. The SDAU samples had the largest overall temperature deviations from the ideal during clearance. Some SDAU boxes were opened and sampled by inspectors and quite large temperature deviations ~ 8-10 ^{o}C.

Temperature deviation from ideal

For purposes of statistical analysis, the data were analysed using the fixed effects of destination and time point (REML). Additionally each carton was also considered a unique trip with its own time × temperature history; e.g. 32 unique temperature-time histories, which were used to understand correlations between various parameters. The total temperature deviation, or number of hours × temperature above -1 °C for the entire trip was calculated as an integrated single number for each box; i-button total deviation (i-Bttn-TD). The temperature deviation was higher for the Chinese samples compared to CSIRO (**Figure 8**). Because the main period of temperature deviation occurred during customs clearance at the SIP, the temperature deviation between days 40-65 was calculated (i-Bttn-40-65-TD, **Figure 9**). Similar derived temperature deviation measures of LogTag data were calculated (not discussed in this report). The temperature deviation parameters were used in simple correlations with sensory and microbiological parameters presented in this report. For cartons with missing temperature logger data, the logger information from the corresponding carton from the same time point was used. It should be noted that there may be better methods of extracting a single integrated measure of temperature deviation for use statistical analyses.

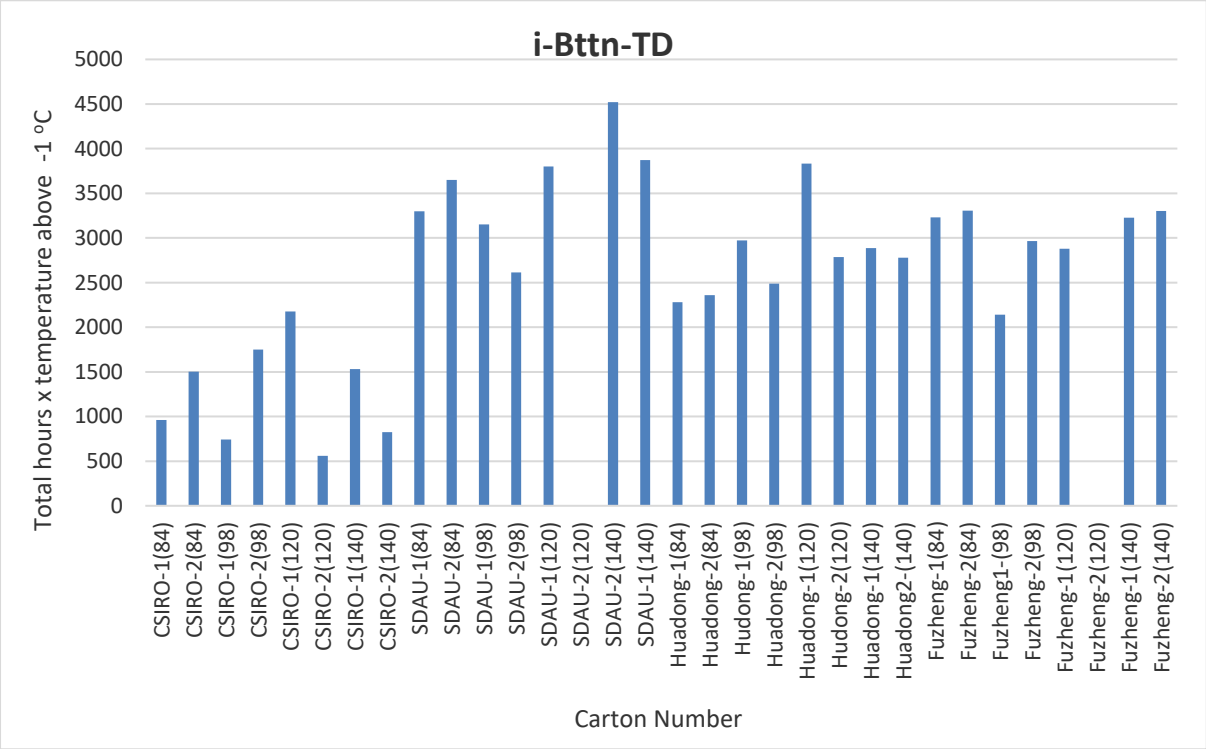


Figure 8: i-button total temperature deviation (hours x temperature above -1°C) for the duration of each of the 32 trips. The integrated number incorporates time and temperature dimensions. Some data are missing – SDAU-2(120) and Fuzheng-2-(120).

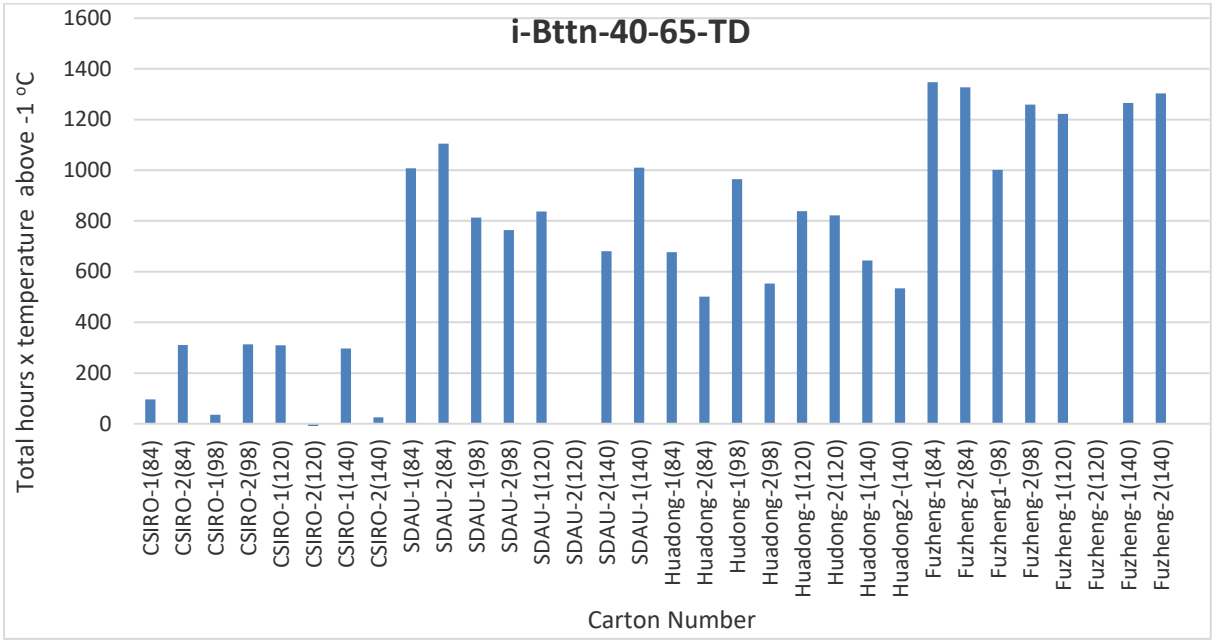


Figure 9: i-button total temperature deviation (hours x temperature above -1°C) from day 40-65 –the time period where the greatest deviation occurred - for each of the 32 trips. The integrated number incorporates time and temperature dimensions. Some data are missing – SDAU-2(120) and Fuzheng-2-(120).

Aerobic plate counts in VPCB

As shown in **Table 5**, the mean APCs on the surface of meat rose from 2.3 $\log_{10}$ cfu/cm^2 at the time of packing to 5.98- log by the first time point (84-days, week 12), which is where counts remained until the final sampling point. This is higher than observed in previous comparable CSIRO studies (A.MFS.0166 and A.MIS1004), in which the microbial load in Australian VPCB increased from 2-3 $\log_{10}$ cfu/cm^2 at the time of packing to around 5 $\log_{10}$ cfu/cm^2 by 8-12 weeks of storage. This is typically followed by a slow rise to around 6 to 7 $\log_{10}$ cfu/cm^2 by week 20. Interestingly, this comparatively high APC count obtained at week 12 was also observed in the CSIRO control samples that were held at close to ideal temperature throughout storage, indicating some difference between these studies. The temperature deviation during the first 7 days of storage of the CSIRO samples during holding at the abattoir (~3 °C above ideal) is the most likely the reason for the elevated APC at week 12, though it is also possible that other factors may have influenced this outcome. Consistent differences in the rate of microflora development in VPCB have been observed between processors previously (CSIRO study A.MFS.0166). While reasons for growth rate variability remain unclear, findings from this and previous studies raise some interesting questions worthy of further investigation. A greater understanding of bacterial growth rates and their association with individual establishments, processing parameters, animal attributes (i.e. what is the impact of breed and geography on microflora), the genetic diversity of microflora on primals at packaging and their corresponding growth potential may be warranted.

Table 5: Mean APC ($Log_{10}CFUcm^2$) for each destination and time point. Differences ($p < 0.05$) for destination×time point comparisons shown by superscripts.

	84-days	98-days	120-days	140-days
CSIRO	5.982[a]	6.042[a]	6.071[a]	6.443[a]
Fuzheng	6.569[b]	6.262[a]	6.771[b]	6.539[a]
Huadong	6.557[b]	6.602[a]	6.446[a]	6.651[b]
SDAU	6.873[b]	7.780[c]	6.331[a]	6.873[b]

Lactic acid bacteria growth in VPCB

Table 6 shows the growth of LAB followed a similar trend to APC, with most samples showing signs of bacterial growth plateau by the first time point (day-84/week 12). As with APC, LAB counts varied considerably between samples and cartons at each time point and in most instances, after week 12 a plateau was observed. Coliforms were not observed in any of the samples except one sample from Fuzheng at 140-days (log 2.63) and three samples from SDAU at 98-days (log 0.699, 3.39 and 3.09). These samples were removed from statistical analysis as there was also evidence of loss of vacuum in packs.

Table 6: Mean LAB ($Log_{10}CFUcm^2$) for each destination and time point. Differences ($p < 0.05$) for destination×time point comparisons shown by superscripts.

Time (days)	84-days	98-days	120-days	140-days
CSIRO	5.89[a]	6.03[a]	6.18[a]	6.46[b]
Fuzheng	6.72[b]	6.21[a]	6.80[c]	6.70[b]
Huadong	6.79[c]	6.55[b]	6.36[b]	6.88[c]
SDAU	7.18[c]	6.57[b]	6.59[b]	6.85[c]

VPCB shelf-life prediction using the University of Tasmania model

The results have been discussed extensively with Dr. Mandeep Kaur and Dr. Tom Ross at the University of Tasmania (UTas). The shelf-life of the VPCB samples in this study were predicted using the UTas shelf-life predictive model for beef (MLA 2017). The model takes into account the initial APC and the time × temperature history of each sample (LogTag information). The predicted shelf-lives of the samples stored for 84, 98, 120 and 140-days are shown in **Table 7**. All of the CSIRO samples, stored under close to ideal conditions, were predicted to easily achieve a shelf-life of 140-days. Even with the atypical delays and temperature deviations experienced at SIP, most of the Chinese samples achieved a shelf-life of 140-days and all of the samples achieved shelf life of 120-days, despite the UTas model being conservative in its approach. VPCB samples stored at CSIRO showed steady trends in some sensory paremeters, including an increase in open odour intensity, post bloom fresh smell, cheesy odour and loss of freshness over time. Overall, sensory-wise CSIRO samples favourably scored better as compared to their Chinese counterparts, which is also reflected in the relatively longer predicted shelf life for these samples. Furthermore, neither of these samples showed abrupt or large negative sensory changes thereby, indicating their acceptability and thus longer shelf-life. Based on the time × temperature profile only the SDAU samples stored for 140-days were predicted not to achieve the expected shelf life of 140-days. This might be because of relatively higher average storage temperature for these particular samples (0.11°C as compared to -0.1 -0.61°C). These samples also had slightly higher TVB-N as compared to Fuzheng and Huadong (Table 13). However, sensory-wise there was not much difference among SDAU, Fuzheng and Huadong samples (**Tables 7-9**).

Table 7: Shelf-life prediction according to the University of Tasmania predictive model. The sample highlighted in orange would not attain the expected 140-day shelf life

	84 days (initial counts = log_{10} 2.546 cfu/cm^2)			
	CSIRO	SDAU	Huadong	Fuzheng
Av. storage temp. (°C)	-0.43	0.42	0.29	0.44
Predicted shelf life (days)	161	109	115	108
	98 days (initial counts = log_{10} 2.270 cfu/cm^2)			
	CSIRO	SDAU	Huadong	Fuzheng
Av. storage temp. (°C)	-0.38	0.14	0.21	0.21
Predicted shelf life (days)	161	126	122	122
	120 days (initial counts = log_{10} 1.972 cfu/cm^2)			
	CSIRO	SDAU	Huadong	Fuzheng
Av. storage temp. (°C)	-0.57	-0.09	-0.07	-0.07
Predicted shelf life (days)	181	143	142	142
	140 days (initial counts = log_{10} 2.219 cfu/cm^2)			
	CSIRO	SDAU	Huadong	Fuzheng
Av. storage temp. (°C)	-0.61	0.11	-0.16	-0.10
Predicted shelf life (days)	182	128	145	141

Sensory assessment of VPCB

Sensory assessment of the VPCB were performed at the same time in China and at CSIRO (**Figure 10** and **Figure 11**) with summaries of sensory attributes during pre-opening (**Table 8**), immediately upon opening **(Table 9**) and post-bloom (**Table 10**) reported. There were significant differences ($p < 0.001$) for destination and time point as well as interactions for all attributes except the amount of weep in pack. The effect of the temperature deviation (i-Butt-40-65-TD) was also highly significant. The MSA-MB, initial APC counts or initial LAB counts did not have a significant effect ($p < 0.05$) on the sensory scores. In general, the CSIRO samples had higher sensory scores for all attributes, compared to the shipped samples and is probably a result of the lower temperatures achieved during the storage period. Between the three Chinese destinations, no obvious differences were observed, with most attributes showing similar variations over similar storage times. The exception of this trend was the post bloom fresh smell (Q12), with Fuzheng showing a consistently higher score compared to other samples stored in the other container, and indicates the longer time spent in SIP had negatively impacted this attribute. Although a standardised sensory assessment method for assessing VPCB was used, there may have been some cross-cultural differences in the use of the rating scale.

For example, the Chinese and other East Asians rate some attributes differently on average compared to Australian (Frank, Watkins, Ball, Krishnamurthy, Piyasiri, Sewell, et al., 2016; Polkinghorne,

Nishimura, Neath, & Watson, 2011; Thompson, Polkinghorne, Hwang, Gee, Cho, Park, et al., 2008). Notwithstanding this possibility, the Chinese and Australian data can be considered internally consistent and comparable across time points.

*Table 8: Adjusted means for pack sensory attributes before opening at different storage times (84, 98, 120 and 140 days) for the four destinations (CSIRO, Fuzheng, Huadong and SDAU) tested for the effects of destination, time and i-button temperature deviation (i-Butt-40-65-TD). Destination×time interaction (D*T). Differences ($p < 0.05$) for destination×time point comparisons shown by superscripts.*

			84-days	98-days	120-days	140-days	LSD	*P* Dest	*p* Time	*p* D*T	*p* i-Butt-40-65-TD
Q1	**Pre Tight Fit**	**CSIRO**	3.1	3.5	3.3	3.5	0.29	<0.001	0.014	<0.001	<0.001
		Fuzheng	3.2	3.0	3.0	2.6					
		Huadong	3.2	2.9	2.6	2.7					
		SDAU	3.1	2.9	2.5	3.0					
Q2	**Pre Fresh Appearance**	**CSIRO**	76.6^{b}	84.9^{a}	79.5^{a}	72.5^{b}	6.4	<0.001	0.005	<0.001	<0.001
		Fuzheng	55.9	46.8^{e}	46.9^{e}	46.9^{e}					
		Huadong	60.0^{c}	52.2^{d}	50.6^{d}	49.8^{d}					
		SDAU	50.3^{d}	53.7^{c}	42.2^{e}	56.0^{c}					
Q3	**Pre Amount Weep**	**CSIRO**	62.7	53.7	62.9	61.9	12.6	0.394	0.78	0.168	0.596
		Fuzheng	52.0	62.2	55.6	60.2					
		Huadong	55.6	58.2	56.2	53.2					
		SDAU	63.9	53.4	70.9	57.5					
Q4	**Pre Like Appearance**	**CSIRO**	71.8^{b}	82.9^{a}	77.6^{a}	72.0^{b}	7.7	<0.001	0.034	<0.001	<0.001
		Fuzheng	53.0^{c}	45.1^{d}	46.3^{d}	41.7^{d}					
		Huadong	55.6^{c}	49.2^{c}	47.5^{d}	46.9^{d}					
		SDAU	47.7^{d}	49.6^{c}	35.3^{e}	53.1^{c}					

When the Chinese packs were analysed separately (no CSIRO data), there were still some differences measured, mainly with time point. Visual assessment of the pack tightness (Q1) generally decreased for the Chinese samples over time and increased slightly for the CSIRO samples after the first time point (84-days). The in-pack fresh appearance (Q2) increased and then decreased for the CSIRO VPCB. Although the Chinese VPCB samples were rated lower than CSIRO samples, the appearance did not sharply decrease over storage, indicative of the consistency in appearance, although the pre-like appearance (Q4) did indicate Chinese consumers preferred samples at 84-days, compared to the later time points, especially for Fuzheng and Huadong samples. The SDAU samples showed an unusually low score at 120-days, which was inconsistent with all other scores across the time points. Overall, the appearance before opening was scored higher with CSIRO samples, but between Chinese destinations, there was no obvious preference, although they did "like" the appearance of the meat more after only 84-days storage. It should be noted that the relationship between the sensory results obtained on the packs and raw meat and the eating quality of the cooked VPCB is not known. The sensory score cut-off for an unacceptable product is also not known.

Figure 10: Students at SDAU assessing the sensory characteristics of the VPCB samples

Figure 11: Staff at CSIRO assessing the sensory characteristics of the VPCB samples

From **Table** 9, over all the time points in the CSIRO samples, the open odour intensity (Q5) increased with an accompanying decline in freshness. The rotten sulphur, fruity and cheesy odours (Q6, Q7 & Q8) all showed a similar trend, generally increasing with time, although high scores were reported at 84-days. These three scores were low compared to those in Chinese samples, and could be a result of either the storage conditions or differences in the scaling used by the panel. In the Chinese samples, the odour intensity and freshness (Q5 & Q6) remained fairly stable over the storage times, but interestingly rotten sulphur, fruity and cheesy odours (Q6, Q7 & Q8) did appear to decline. However, overall these attributes rated quite low in both the CSIRO and Chinese VPCB, not consistent with progressive spoilage.

*Table 9: Adjusted means for pack sensory attributes immediately after opening at different storage times (84, 98, 120 and 140-days) for the four destinations (CSIRO, Fuzheng, Huadong and SDAU) tested for the effects of destination (dest), time and i-button temperature deviation (i-Butt-40-65-TD). Destination×time interaction (D*T). Differences ($p < 0.05$) for destination×time point comparisons shown by superscripts.*

		Time	84-days	98-days	120-days	140-days	LSD	*p* Dest	*p* Time	*p* D*T	*p* i-Butt-40-65-TD
Q5	**Open Odour Intensity**	**CSIRO**	41.7^{b}	48.3^{b}	51.8^{b}	60.2^{a}	7.6	<0.001	0.078	<0.001	<0.001
		Fuzheng	55.5^{a}	40.5^{c}	45.0^{b}	36.6^{c}					
		Huadong	41.4^{c}	44.7^{c}	45.0^{b}	41.0^{c}					
		SDAU	36.7^{d}	47.9^{b}	45.6^{b}	40.8^{c}					
Q6	**Open Freshness**	**CSIRO**	77.8^{a}	81.4^{a}	65.8^{b}	54.8^{c}	5.8	<0.001	<0.001	<0.001	<0.001
		Fuzheng	54.9^{c}	53.8^{c}	51.4^{c}	54.7^{c}					
		Huadong	47.6^{d}	50.8^{c}	51.5^{c}	51.9^{c}					
		SDAU	45.8^{d}	48.0^{d}	49.2^{c}	51.9^{c}					
Q7	**Open Rotten Sulphur**	**CSIRO**	7.2^{a}	1.9^{a}	5.7^{a}	5.2^{a}	5.9	<0.001	<0.001	<0.001	<0.001
		Fuzheng	31.5	23.2^{c}	26.5^{c}	12.5^{b}					
		Huadong	33.7	32.7	24.6^{c}	12.9^{b}					
		SDAU	32.4	31.4	30.1^{c}	14.5^{b}					
Q8	**Open Fruity**	**CSIRO**	7.8^{a}	2.7^{a}	5.1^{a}	7.7^{a}	6.2	<0.001	<0.001	<0.001	<0.001
		Fuzheng	27.0^{d}	19.3^{b}	34.5^{d}	14.3^{b}					
		Huadong	20.2^{c}	26.3^{c}	25.8^{c}	13.1^{b}					
		SDAU	15.8^{b}	25.7^{c}	30.0^{d}	16.7^{b}					
Q9	**Open Cheesy**	**CSIRO**	12.6^{b}	4.4^{a}	9.0^{a}	14.5^{b}	5.8	<0.001	<0.001	<0.001	<0.001
		Fuzheng	45.5^{e}	28.8^{c}	39.2^{d}	18.2^{b}					
		Huadong	34.9^{d}	38.4^{d}	29.8^{c}	17.0^{b}					
		SDAU	37.4^{d}	29.2^{c}	35.5^{d}	20.0^{b}					

Colonisation of undesirable microbial populations, such as psychrotrophic and psychrophilic Clostridium species, have been shown to cause blown vacuum packs and noticeable pungent rotten sulphur odours (Hernández-Macedo, Barancelli, & Contreras-Castillo, 2011; Hernandez-Macedo, Contreras-Castillo, Tsai, Da Cruz, Sarantopoulos, Padula, et al., 2012). No blown packs or corresponding strong sulphur odours were measured in any of the VPCB in the current study.

From **Table 10**, for both CSIRO and Huadong samples, the post-bloom liking, fresh appearance and smell (Q10, Q11 & Q12) all showed a decline over the storage time points, but both Fuzheng and SDAU scores remained fairly constant. It is expected that there would be a decline in these attributes with time, but the consistency in scores for Fuzheng and SDAU is a positive sign that Chinese customers are still liking the appearance and smell of VPCB. Similarly to the before opening scores, CSIRO scores were consistently higher to all of those reported in China. Although, the smell was obviously preferred in the Fuzheng samples compared to other Chinese distributors, and confirms the impact of the storage conditions. It should be emphasised, that these results are preliminary visual and odour tests performed on the raw meat only and no eating quality sensory assessments were conducted on the cooked or grilled meat. It would be interesting to understand the relationship between these sensory pack scores and the eating quality of grilled VCPB, to see if the eating quality can also be improved with optimal temperatures during shipment.

*Table 10: Adjusted means for pack sensory attributes after blooming (30 minutes) at different storage times (84, 98, 120 and 140 days) for the four destinations (CSIRO, Fuzheng, Huadong and SDAU) tested for the effects of destination (dest), time and i-button temperature deviation (i-Butt-40-65-TD). Destination×time interaction (D*T). Differences (p < 0.05) for destination×time point comparisons shown by superscripts.*

		Time	84-days	98-days	120-days	140-days	LSD	*P* Dest	*p* Time	*p* D*T	*p* i-Butt-40-65-TD
Q10	PostBloom Appear Like	CSIRO	81.4^{b}	90.0^{a}	76.4^{b}	72.3^{c}	7.2	<0.001	<0.001	<0.001	<0.001
		Fuzheng	58.2^{d}	58.6^{d}	51.4^{d}	57.3^{d}					
		Huadong	55.6^{d}	50.0^{e}	50.4^{e}	47.6^{e}					
		SDAU	55.1^{d}	51.5^{d}	52.4^{d}	54.4^{d}					
Q11	PostBloomFresh Appear	CSIRO	83.0^{b}	90.9^{a}	74.0^{c}	72.2^{c}	7.0	<0.001	<0.001	<0.001	<0.001
		Fuzheng	57.1^{d}	57.5^{d}	48.7^{e}	58.2^{d}					
		Huadong	57.7^{d}	50.1^{e}	48.2^{e}	46.2^{e}					
		SDAU	54.0^{d}	48.9^{e}	53.1^{d}	53.6^{d}					
Q12	PostBloom Fresh Smell	CSIRO	80.7^{b}	91.6^{a}	73.1^{c}	60.5^{d}	8.6	<0.001	<0.001	<0.001	<0.001
		Fuzheng	54.7^{e}	56.5^{e}	54.0^{e}	55.3^{e}					
		Huadong	46.5^{f}	47.6^{f}	45.5^{f}	38.4^{g}					
		SDAU	48.5^{f}	44.7^{f}	47.4^{f}	47.2^{f}					

The amount of weep in pack (Q3), as assessed visually in unopened packs by the assessors, was strongly positively correlated with the measured amount of weep in the packs (r = 0.69, p < 0.001), clarifying that assessors (whether Australian or Chinese) were able to accurately rate the amount of weep (%) in pack by visual assessment (100 mm line scale) (**Figure 13)**. A clear relationship was modelled by linear regression (p < 0.001); visual assessment of weep (100 mm) = 37.3 + 6.04 × % drip loss (w/w).

The Chinese sensory data did not indicate abrupt negative changes over time. For example, rotten/sulphur or cheesy odour did not increase strongly. The relationships between the various sensory attributes and the APC and LAB microbiology counts for each pack (n=148) are summarised in **Figure 12**. The colour of each square in the correlation matrix is an indication of how strongly two parameters are correlated; dark red - positive, dark blue - negative. Light colours indicate only weak (non-significant) relationships. The matrix allows a quick overview of the strength of the association between two parameters and an overview of the interrelationships between attributes. Many attributes were strongly correlated with each other. For example, reading down the first column, (Q1) tightness of fit (bag tightness) was positively correlated with (Q2) fresh appearance (bright red colour, r = 0.70, p < 0.001) and (Q4) liking of appearance (bright red colour, r = 0.78, p < 0.001) and negatively correlated with (Q3) the amount of weep (dark blue colour, r = -0.53, p < 0.001). In general, the odour intensity of the open bag was not strongly correlated with any other sensory parameters (column 3, mainly light colours). Open freshness odour (Q6) was strongly correlated with most other parameters, e.g. (Q7) a negative association with rotten/sulphur as expected (r = -0.71, p < 0.001), (Q8) fruity (r = -0.61, p < 0.001, (Q9) cheesy/fermented (r = -0.66, p <0.001) and a positive relationship with (Q12) post bloom freshness (r = 0.81, p < 0.001). Relationships between sensory attributes and microbiological counts were generally weaker. Open fresh smell (Q6) was negatively correlated with LAB (r = -0.46, p < 0.001) and TVC (r = -0.42, p < 0.001) and rotten/sulphur odour (Q7) and was positively correlated with LAB (r = 0.35, p < 0.001) and TVC (r = 0.39, p < 0.001).

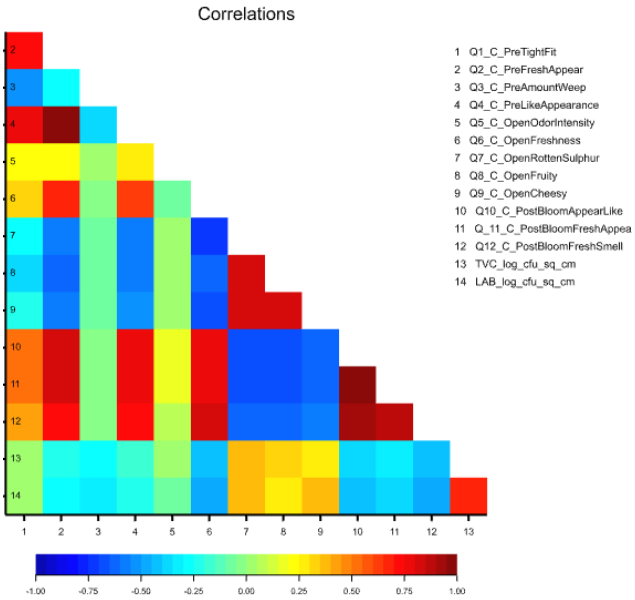


Figure 12: Correlation matrix showing relationships between VPCB sensory attributes and the microbiological counts (n=148). Dark red signifies a strong positive relationship between parameters, dark blue a strong negative relationship. Light colours signify weak and/or insignificant relationships.

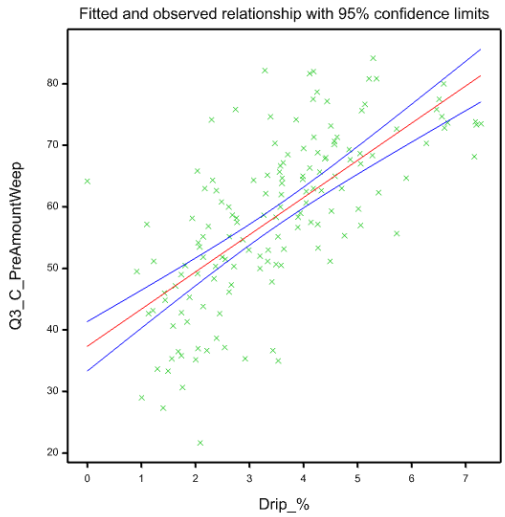


Figure 13: Linear regression model of the relationship between the amount of weep in packs (%) and the visual estimation of amount of weep (rated on a 100 mm line scale, n=148).

Changes in pH over time

As shown in **Table 11,** the pH of the VPCB all remained within a normal expected range for the entirety of the storage experiment. The pH values at the first time point (84-days) were variable, with lower values measured in the Huadong and SDAU samples. These samples were in the same container and had a slightly different temperature profile compared to the Fuzheng samples. The lower pH may have been associated with greater LAB activity. LAB counts were higher in the Huadong and SDAU samples compared to the Fuzheng **(Table 6**). The pH generally increased over time, meaning the samples became slightly more alkaline. This would correspond with generation of basic compounds, such as trimethylamine over time. The pH change may partially explain the TVB-N data discussed in the following sections.

Table 11: Mean pH (n=10) in the VPCB over the storage experiment. Differences ($p < 0.05$) for destination×time point comparisons shown by superscripts.

	84-days	98-days	120-days	140-days
CSIRO	5.62^{c}	5.50^{c}	5.61^{c}	5.65^{e}
Fuzheng	5.68^{d}	5.57^{c}	5.63^{d}	5.77^{e}
Huadong	5.23^{a}	5.71^{e}	5.64^{d}	5.70^{d}
SDAU	5.45^{b}	5.7^{d}	5.72^{e}	5.74^{e}

Relationship between temperature deviation parameters, pH and microbiological data

Correlation coefficients between the temperature deviation parameters, ultimate pH and microbiology data are shown (**Figure 14**). Moderate positive relationships between the temperature deviation parameters and microbiological counts (LAB, TVC) were found. In general, the correlations were stronger with LAB data. For example i-button-TD and LAB ($r = 0.40$, $p < 0.001$) and LogTag-TD and LAB ($r = 0.39$, $p < 0.001$). It should be noted than when the Chinese data are considered separately (without the CSIRO data) these relationships are not significant. The ultimate pH of the VPCB was positively correlated with LAB ($r = 0.32$, $p < 0.001$) and TVC ($r = 0.21$, $p = 0.008$) meaning that VPCB with higher initial pH had higher bacterial growth. It should be noted that the initial pH was all within specification for VPCB.

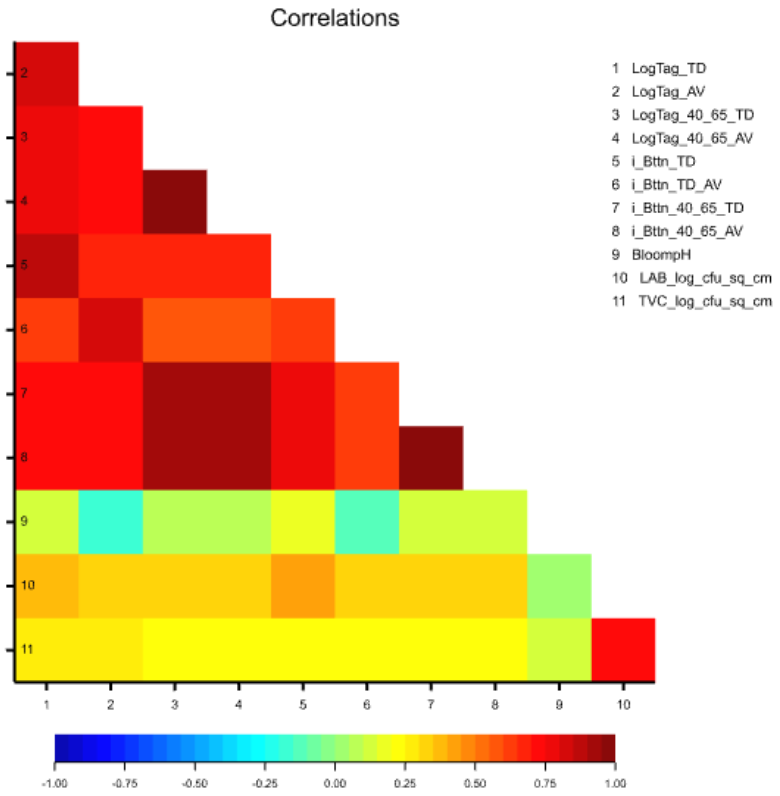


Figure 14: Correlation matrix summarising interaltionshps between temperature deviation parameters, pH, LAB and TVC (n=148).

Relationship between pack sensory scores and temperature deviation

Correlations between the sensory attributes and the various i-button temperature deviation parameters are shown for all the VPCB data together (CSIRO and Chinese) (**Figure 15**). The total temperature deviation (i-Bttn-TD), average i-button total deviation per day (i-Bttn-TD-Av), the temperature deviation between days 40-65 (i-Bttn-40-65-TD) and the average temperature deviation per day between days 40-65 (i-Bttn-40-65-Av) were all more strongly correlated with sensory parameters than with the microbiological counts (LAB, TVC). Similar correlation patterns between the sensory attributes and temperature parameters were found for the LogTag data (Data not shown).

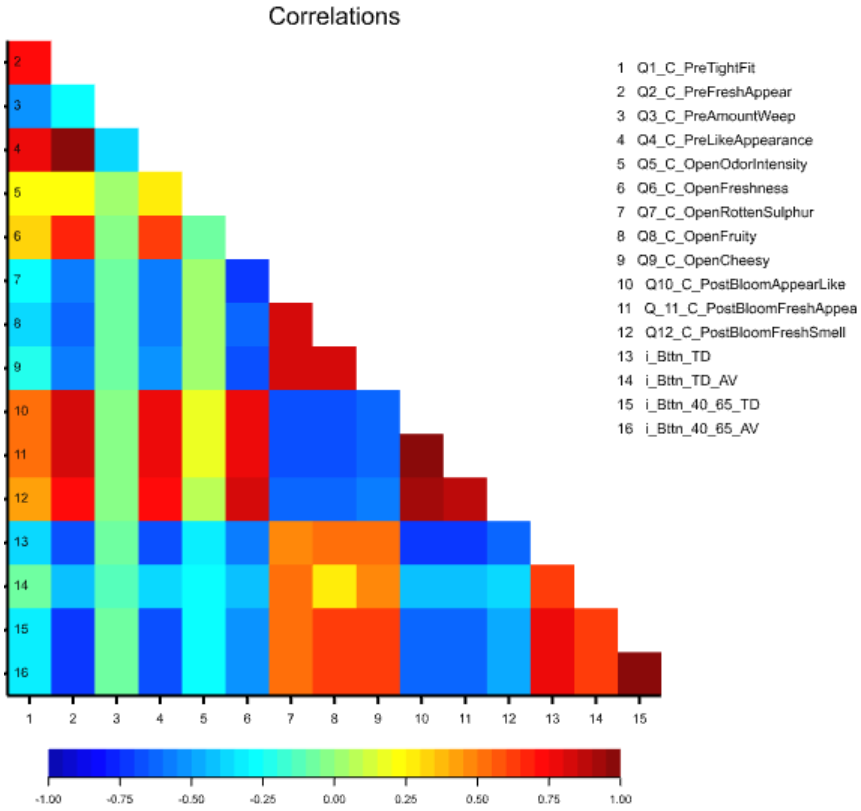


Figure 15: Correlation matrix summarising interaltionshps between sensory attributes and the i-button temperature deviation parameters (n=148)

Open pack freshness odour (Q6) (*r* = -0.56, *p* < 0.001) and post bloom freshness smell (Q12) (*r* = - 0.65, *p* < 0.001) were both negatively correlated, and decreased as the i-Butt-40-65-TD increased. Rotten/sulphur (*r* = 0.53), fruity (*r* = 0.53) and cheesy/fermented odour (*r* = 0.62) were all positively correlated or increased as the size of the temperature deviation increased (*p* < 0.001). Other weaker correlations were also apparent. When the Chinese sensory data were considered separately (no CSIRO data), significant but weaker correlations were measured; i-Butt-40-65-TD and (Q6) open freshness smell (*r* = 0.257, *p* = 0.007); i-Butt-TD and (Q12) post-bloom freshness smell (*r* = 0.54, *p* < 0.001).

Overall, the results indicate that the temperature deviation data were strongly correlated (either negatively or positively) to most of the pack sensory attributes, whereas the microbiological count data were only moderately correlated. As a whole, the findings indicate that the absolute microbiological counts (LAB, APC) were not as good predictors of the VPCB sensory attributes as the temperature deviation. Anecdotal and published data have also shown a poor relationship between microbial growth and meat organoleptic properties. The microbiological data strongly suggest that all the measured time points occurred outside exponential growth phase which may explain the weak correlations.

Chilled beef storage colour changes

Replicate L* (lightness), a* (redness) data were subjected to ANOVA using destination and time as fixed factors. Post-bloom L* values differed for destination and time ($p < 0.001$) however no obvious time-dependent trends were measured (**Table 12**). The CSIRO samples had overall significantly lower L* values, however all L* values were within acceptable limits for beef VPCB.

The redness (*a) of the CSIRO samples was significantly higher than Chinese samples ($p < 0.001$) (**Table 13**). The effect of time was also significant ($p = 0.03$); a* generally increased slightly, before decreasing at the last time point. All a* values were within an expected range for VPCB (Hughes, McPhail, Kearney, Clarke, & Warner, 2015).

Table 12: Mean (n=10) L values for each destination and time point. Differences (p < 0.05) for destination×time point comparisons shown by superscripts.*

	84-days	98-days	120-days	140-days
CSIRO	34.78^{a}	37.7^{b}	35.08^{a}	34.67^{a}
Fuzheng	36.77^{b}	37.47^{b}	36.56^{b}	37.44^{b}
Huadong	36.68^{b}	38.15^{c}	36.77^{b}	37.65^{c}
SDAU	36.3^{b}	38.39^{c}	35.75^{a}	37.34^{b}

Table 13: Mean (n=10) a values for each destination and time point. Differences (p < 0.05) for destination×time point comparisons shown by superscripts.*

	84-days	98-days	120-days	140-days
CSIRO	21.66^{a}	21.21^{a}	21.83^{a}	21.32a
Fuzheng	18.92^{c}	20.04^{b}	19.99b	19.09b
Huadong	18.93^{c}	19.85^{b}	19.63b	18.88c
SDAU	19.63^{b}	19.5^{b}	20.46b	18.9c

Total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) is an objective chemical test that can measure the degree of freshness/spoilage in meat products. Although originally developed for an indicator of fish freshness, it has been adapted to other meat products including chicken, pork and beef. It is widely used by Chinese and meat researchers and recognised by Chinese authorities as an important meat freshness/spoilage parameter. The National Standard of the People's Republic of China - GB 2707-2016 - "Hygienic Standard for Fresh (Frozen) Meat of Livestock" states that the TVB-N in fresh or frozen beef must be less than 15 mg/100g to be acceptable for human consumption.

Recent Chinese literature demonstrates the widespread application of TVB-N for meat freshness or spoilage, including chilled beef; (Khulal, Zhao, Hu, & Chen, 2016; Li, Wang, Sun, Zhao, & Huang, 2016; Zhu, Yao, Duan, Ma, & Tang, 2016) (Pan, 2016). TVB-N measurements were conducted on the Chinese VPCB samples (by SDAU) and also on the CSIRO samples according to the Chinese standard method (**Table 14**). Nearly all of the Chinese samples slightly exceeded the limit of 15 mg/100 g.

In contrast, for the VPCB samples stored under near ideal conditions at CSIRO, the TVB-N values were well below 15 mg/100g at all time points and would be deemed acceptable for consumption according to Chinese limits. It should be noted that a Korean study recommended a TVB-N shelf-life limit of 20 mg/100 g for beef and a lower TVB-N limit of 16.5 mg/100 g for pork (BYUN, MIN, KIM, KIM, CHUNG, & LEE, 2003). Hence, if the proposed 20 mg/100 g TVB-N limit was applied, all of the Chinese VPCB samples would be considered compliant at 120-days, and only the SDAU samples would be slightly over the 20 mg/ 100g threshold at 140-days. This was in accordance with the UTas model prediction.

Table 14: Means (n=10) for volatile basic nitrogen (TVB-N mg/100g) at the different time points (84, 98, 120 and 140-days) for VPCB from the three Chinese supply chains (Fuzheng, SDAU, Huadong) and CSIRO samples. Differences ($p < 0.05$) for destination×time point comparisons shown by superscripts.

	84-days	98-days	120-days	140-days
CSIRO	4.8^{a}	4.8^{a}	5.1^{a}	7.7^{b}
Fuzheng	16.0^{c}	16.6^{d}	18.6^{e}	19.4^{e}
Huadong	14.8^{c}	16.2^{c}	16.8^{d}	19.6^{f}
SDAU	17.0^{d}	17.5^{d}	18.8^{e}	20.6^{f}

A recent Chinese study found that TVB-N exceeded 15 mg/100 g in modified atmosphere packaged beef only after 14 days storage at 0 °C (Lyu, Shen, Ding, & Ma, 2016). In another Chinese study (Pan, 2016) chilled overwrapped beef reached 15 mg/100g TVB-N after only 8-days storage at 4 °C, showing how critical temperature control is for maintaining a low TVB-N. Given that the temperature histories of the Chinese VPCB were not typical, it is reasonable to assume that without the temperature excursions at the SIP, all of the TVB-N values would have been well below the 15 mg/100g threshold at 140-days. On the balance of evidence, we propose that the current Chinese limit of 15 mg/100g is not an appropriate shelf-life threshold for VPCB. Since other groups have previously indicated that 15 mg/100 g is too low for VPCB (BYUN, MIN, KIM, KIM, CHUNG, & LEE, 2003), we suggest that a future limit of 20 mg/ 100 g TVB-N limit would be more appropriate. Dr. Zhang from SDAU made enquiries to the Chinese Commodity Inspection and Testing Bureau regarding circumstances when TVB-N testing is performed on imported VPCB. They replied "TVB-N is not a compulsory detected parameter. Usually, the quality of [chilled beef] samples are evaluated by visual observation, microbial tests and some related parameters. TVB-N would be tested for those samples with obvious defective appearance, such as bad colour or frozen-thawing appearance, etc." Hence, it is assumed that TVB-N testing is only routinely performed if visual inspection fails.

Changes in meat lipids during storage and TBARS

Changes in the lipids within the IMF in the raw meat at zero time and after the different storage times are shown (**Table 15**). Decreases were measured for some fatty acids from zero time to the first time point (84-days). After 84-days, there were no further significant loss of lipids. The very low TBARS values measured in the samples at each storage time point may have indicated that oxidation was not the primary cause of lipid loss. The lipid may have been utilised as a carbon source, between zero time and the first time point (84-days). Some LAB and other bacteria are known to metabolise and transform lipid material (Kishimoto, Yamamoto, Toraishi, Yoshioka, Saito, Masuda, et al., 2003; Kishino, Takeuchi, Park, Hirata, Kitamura, Kunisawa, et al., 2013; Signorini, Ponce-Alquicira, & Guerrero-Legarreta, 2003). Notably, significant losses of the polyunsaturated fatty acids, EPA, DPA and DHA were not measured. These findings should be confirmed on a larger number of samples.

Table 15: Changes in individual fatty acids (measured as FAMEs) in VPCB at zero time and after different storage times. (mg/ 100g raw meat). Differences ($p < 0.05$) for destination×time point comparisons shown by superscripts.

Fatty acid	Common name	Zero	84-days	98-days	120-days	140-days	LSD	p value
TBARS		0.32	0.36	0.39	0.37	0.37	0.11	0.701
C12:0		7.1	4.2	4.2	5.0^{b}	5.2^{b}	1.7	0.003
C14:0		183^{a}	71^{b}	74^{b}	97^{b}	93^{b}	32.2	<0.001
C16:0	palmitic	1330^{a}	887^{b}	909^{b}	952^{b}	972^{b}	140	<0.001
C16:1		423^{a}	241^{b}	228^{b}	283^{c}	269^{bc}	52	<0.001
C18	stearic	873^{a}	535^{b}	548^{b}	602^{b}	569^{b}	101	<0.001
C18:1	oleic	2602^{a}	1560^{b}	1653^{b}	1827^{b}	1781^{b}	287	<0.001
C18:1	vaccenic	15	8	9	11	11	6	0.125
C18:2	linoleic	192	158	179	169	184	28	0.098
C18:2	CLA	33^{a}	19^{b}	24^{b}	26^{b}	24^{b}	7	<0.001
C18:3	linolenic	627^{a}	462^{b}	492^{b}	500^{b}	508^{b}	89	0.003
20:5, n-3	EPA	105	105	92	100	109	41	0.925
22:5, n-6	DPA	361	288	329	329	318	51	0.086
22:6, n-3	DHA	107	96	115	106	103	18	0.452

Changes in non-volatile compounds

Each non-volatile compound was interpreted as a marker for distinctive biochemical processes (**Table 16**). The 5'-ribonucleotide, adenosine monophosphate (AMP) may be considered an indicator of ongoing bacterial activity in the packs. Inosine monophosphate (IMP) is a major breakdown product of the degradation of adenosine triphosphate (ATP, not measured) that rapidly forms in meat post-mortem (Tikk, Tikk, Tørngren, Meinert, Aaslyng, Karlsson, et al., 2006). IMP was abundant at zero-time, but decreased markedly over storage. The major breakdown product of IMP, hypoxanthine, increased over the storage period. Hypoxanthine is a well-known meat flavour enhancer (Ichimura, Nakamura, Yoshida, & Hattori, 2017). Free amino acids and the meat peptide carnosine, were interpreted as indicators of meat protein breakdown or proteolysis. In most cases, these proteolysis markers increased significantly with storage time. For many of these proteolysis markers, a peak concentration occurred at 120-days and decreased thereafter. As many free amino acids contribute directly to the taste of meat (Frank, Ball, Hughes, Krishnamurthy, Piyasiri, Stark, et al., 2016; C. Pereira-Lima, Cambero, Garcia De Fernando, Hierro, & Ordonez, 1998; C. I. Pereira-Lima, Ordonez, de Fernando, & Cambero, 2000) and indirectly to the formation of aroma, it may be expected that the flavour intensity of the cooked or grilled meat may have increased over the extended VPCB over the storage time. Whether these changes lead to perceivable (desirable or undesirable) sensory changes cannot be ascertained, as sensory descriptive profiling and consumer liking studies were not conducted in this project. Creatine, a non-amino acid nitrogenous compound, increased to day-120 then decreased. Carnitine, important in fatty acid metabolism, did not change over time. Lactic acid increased to a maximum at day-98 and is most likely a product of ongoing LAB activity in the VPCB. Succinic and fumaric acids are intermediates in bacterial metabolic pathways and increased over time. Both organic acids have flavour impacts (Cambero, Pereira-Lima, Ordonez, & de Fernando, 2000; Frank, Ball, et al., 2016). Tyramine is a biogenic amine, formed through the decarboxylation of tyrosine during fermentation or decay of meat and is one of the major biogenic amines formed in bovine meat (Vinci & Antonelli, 2002).

High levels of biogenic amines such as tyramine may be indicators of meat spoilage and may have negative health impacts (Jairath, Singh, Dabur, Rani, & Chaudhari, 2015). The level of tyramine increased over time, reaching a maximum concentration of ~66 mg/kg at 140-days. This concentration of tyramine is within previously reported ranges in matured beef products and does not represent a health risk (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997; Hernández-Macedo, Barancelli, & Contreras-Castillo, 2011).

Table 16: Changes in selected non-volatile metabolites in raw meat at zero time and after different storage times measured by LC-MS/MS. Units are semi-quantitative (mg/kg raw meat).

Compound	Probable Origin/ function	RT (min)	MRM-Transition (m/z)	Collision energy(V)	Trend	Zero	84	98	120	140	SED	p value
adenine	Adenosine breakdown	2.81	136.1 → 119.1	23.7	↑	0.034	0.0328	0.0376	0.0592	0.0684	0.014	0.05
adenosine	DNA breakdown	2.81	268.2 → 136.0	42.1	↓	0.1644	0.0315	0.0204	0.0735	0.054	0.036	<0.001
cytidine	RNA breakdown	5.73	244.0 → 112.0	10.3	↑↓	1.37	2.54	3.22	3.52	2.95	0.356	<0.001
guanine	Guanosine breakdown	5.61	152.0 → 134.9	17.5	↑↓	2.01	1.96	2.88	1.33	0.74	0.47	<0.001
hypoxanthine	Purine degradation	3.01	137.0. → 119.0	20.9	↑	245	469	482	546	505	36.2	<0.001
inosine-MP	DNA breakdown	2.81	269.0 → 137.1	10.3	↓	586	37	88	10	8	12.7	<0.001
adenosine -MP	Bacterial activity	6.29	348.2→ 136.0	42.1	—	1.53	1.83	2.12	1.63	1.84	0.24	0.174
AMP-N^{15}	Internal standard	6.29	353.0 → 141.0	19.3								
alanine	Proteolysis	7.37	90.1 → 72.1	10.3	—	6.8	6.1	7.6	7.4	6.4	1.3	0.413
asparagine	Proteolysis	6.09	133.1 → 87.0	10.3	↑	119	486	766	1075	1014	305.0	<0.001
aspartic	Proteolysis	6.66	134.1 → 74.0	14.9	↑	7.1	79.3	141.3	245.7	261.9	20.2	<0.001
glutamic	Proteolysis	6.66	148.1 → 84.0	14.5	↑	72	298	503	584	611	49.3	<0.001
glutamine	Proteolysis	7.02	147.1 → 130.1	10.3	↑	318	423	488	559	553	44.9	<0.001
isoleucine	Proteolysis	6.10	132.1 → 86.1	10.3	↑	105	448	677	858	812	69.0	<0.001
leucine	Proteolysis	6.03	132.1 → 74.0	10.2	↑	140	507	752	866	818	9.5	<0.001
methionine	Proteolysis	6.16	150.1 → 133.0	10.2	↑	42	151	241	277	265	21	<0.001
proline	Proteolysis	6.30	116.1 → 70.1	15.8	↑	56	176	227	368	370	27	<0.001
phenylalanine	Proteolysis	5.99	166.1 → 120.1	13.4	↑	229	791	1229	1525	1370	114	<0.001
tryptophan	Proteolysis	5.91	205.1 → 187.1	10.3	↑↓	7	29	38	55	51	5.2	<0.001
tyrosine	Proteolysis	6.19	182.1 → 136.0	13.2	↑	118	391	568	686	595	56	<0.001
tyramine	Tyrosine catabolism	7.67	138.1 → 77.1	28.3	↑	0.0	5.9	8.2	39.8	66.4	16.4	<0.001
valine	Proteolysis	6.33	118.1 → 55.1	20.4	↑	92	359	528	697	662	46	<0.001
carnitine	Meat metabolite	6.87	162.1 → 85.0	20.3	—	1222	1219	1281	1333	1222	67	0.44
carnosine	Proteolysis/antioxidant	12.14	225.1 → 110.1	10.3	↑↓	7082	6583	10795	8798	7800	846	<0.001
creatine	Lipid metabolism	6.92	132.1 → 90.1	10.3	↑↓	3262	3089	3819	3450	3192	145	<0.001
glutathione	LAB activity?	6.61	308.1 → 179.0	10.3	—	121	72	138	75	75	14	<0.001
glutamine-N^{15}	Internal standard	7.00	148 → 131.0	10.2								
fumaric	Citric acid cycle	4.65	115.0 → 71.1	10.3	↑	3.8	50.7	77.8	68.3	90.1	11.5	<0.001
lactic acid	LAB activity	2.60	89.1 → 43.2	10.3	↑↓	7475	7547	10137	8461	8548	541	<0.001
succinic acid	TCA cycle LAB activity	1.96	117.0 → 73.2	10.3	↑	21.1	42.4	38.6	33.9	61.5	13.4	<0.001
succinic acid-d4	Internal standard	1.96	121.0 → 77.1	10.2								

Changes in volatile compounds in raw and grilled VPCB

Previous studies have indicated changes in the volatile profiles in vacuum packaged meat associated with spoilage (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015). Differences in the relative concentration of volatile compounds in raw and grilled meat at zero time and after storage are shown in **Table 17.** Volatiles are categorised by chemical class. The rationale for measuring volatiles in both raw and grilled VPCB samples was to see whether any differences in thermally induced volatiles would occur over storage time. Large volatile differences in the grilled samples may indicate potential changes in the flavour.

There is little published information on the volatile changes that take place after such long storage of VPCB. For example, in a recent review a maximum of 30 days vacuum packaged storage is mentioned (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015). In the current VPCB samples 70 volatiles were measured in the headspace of the raw and grilled beef samples by the SPME method. Of these, only 18 volatiles changed significantly over the storage period ($p < 0.05$). Only 13 volatiles changed over the storage time in the grilled samples.

The concentration of 48 volatiles changed significantly with grilling in accordance with the fact that most flavour in meat is formed by thermal processing (p state).

Alcohols are well known products of anaerobic bacterial activity. 1-pentanol and 1-penten-3-ol clearly increased from zero time to 84-days and remained at a similar level for the remaining storage period. Grilling increased the concentration of some alcohols (1-pentanol, 1-hexanol, 1-penten-3-ol), suggesting that thermal pathways accelerate formation of these alcohols. A significant increase in the amount of ethanol was measured between 120 and 140-days in both raw and grilled VPCB, suggesting that ethanol may be an indicator of advanced or end of shelf-life.

Aldehydes are important for grilled beef flavour (Frank, Ball, et al., 2016). Nearly all of the aldehydes increased significantly with grilling. A number of aldehydes are produced through thermal Strecker degradation of free amino acids. For example 2-methylpropanal is produced from valine, 2-methylbutanal from isoleucine and 3-methylbutanal from leucine. These volatiles clearly increased after grilling and also increased significantly over time, mirroring the free amino acid data to an extent (**Table 16**). A maximum amount of volatiles were measured at 98-days and 120-days and then dropped off at 140-days. Similarly, the maximum concentration of free-amino acids were measured at 98-days and 120-days and dropped off at 140-days. Hence, in the case of the grilled beef Strecker aldehydes, a direct relationship between the concentration of total free amino acids and volatiles was evident. Although sensory analysis of the grilled VPCB was not conducted, these volatile data suggest that the optimal grilled beef aroma may be reached at 98 to 120-days, decreasing thereafter. Another group of beef volatile aldehydes are formed through the degradation of lipids, such as β-oxidation of unsaturated fatty acids or lipid auto-oxidation e.g.; hexanal, heptanal, octanal and nonanal. The concentration of these volatiles were higher at each time point compared to zero for both raw and grilled VPCB. Grilling significantly increased these important volatiles, however their concentration was similar at each time point. Acetone increased with time in both raw and grilled samples. 2-octanone increased with time in the grilled samples. 3-hydroxy-2-butanone changed significantly over time in the raw samples, reaching a maximum after 98-days storage and dropping off thereafter. Acetone and 3-hydroxy-2-butanone are generated from lipid degradation.

Methanethiol, methional and 2-acetyl-2-thiazoline are formed from the thermal degradation of the amino acids methionine or cysteine and all increased after grilling. Carbon disulphide decreased after grilling. Dimethyl disulphide peaked in concentration at day-98 decreased in the raw VPCB. Large increases in methanethiol, dimethyl sulphide, dimethyl disulphide or dimethyl trisulphide were not observed over the storage of VPCB. These sulphur volatiles have been shown to be key indicators of meat spoilage, where rapid increases are expected to coincide with spoilage and strong objectionable odours (Mayr, Margesin, Klingsbichel, Hartungen, Jenewein, Schinner, et al., 2003).

Acetic acid, another common fermentation volatile, increased in raw VPCB to day-98 and then decreased. A number of alkane compounds were measured in the VPCB headspace. (Z)-octene (tentative identification) increased significantly from zero time to day-84 and then decreased. Similar changes were also seen for other unidentified alkanes, e.g.; alkane 2.3 m, alkane 2.7 m, aldehyde 37.9 min. The presence of these volatiles may be derived from the breakdown of lipid material.

Table 17: Semi-quantitative (µg/g) adjusted means for volatile compounds in raw and grilled beef at zero time and after 84, 98, 120 and 140-days storage. Quan = mass/ charge ratio (m/z) for quantitation, ID = method of identification, EI = electron impact mass spectrum

	Quan	ID	Raw							Grilled							
alcohols/diols	**m/z**		**0**	**84**	**98**	**120**	**140**	**SED**	**P raw**	**0**	**84**	**98**	**120**	**140**	**SED**	**P grilled**	**P State**
ethanol	45	EI,R	5.0	4.4	7.3	5.0	15.0	2.3	**<0.001**	3.7	3.2	6.2	4.9	11.3	1.9	**<0.001**	0.142
1-butanol	56	EI,R	4.6	3.6	2.6	2.6	2.8	0.9	0.070	3.2	3.1	2.6	2.0	2.5	0.9	0.642	0.097
1-hexanol	56	EI,R	1.6	6.6	8.0	4.2	6.2	2.4	0.302	13.4	17.9	9.9	6.4	13.7	7.8	0.636	0.01
1-heptanol	56	EI	0.8	2.1	2.4	2.0	2.1	0.5	0.119	1.7	6.4	4.4	2.7	5.1	2.2	0.235	0.002
1-pentanol	55	EI, R	2.8	11.7	10.4	14.0	8.7	3.0	**0.013**	11.4	32.2	25.0	23.9	24.0	8.6	0.226	<0.001
1-penten-3-ol	57	EI	77.6	82.8	83.0	83.3	82.8	1.2	**<0.001**	79.3	81.5	82.5	83.6	83.3	1.4	**0.012**	0.872
1-octanol	56	EI, R	3.6	5.6	6.5	6.2	5.8	1.2	0.231	3.4	8.1	5.9	4.1	6.4	2.0	0.177	0.862
2-ethylhexanol	57	EI, R	7.4	8.9	5.6	4.4	7.3	2.9	0.421	2.2	4.2	5.3	1.9	3.0	2.0	0.341	0.004
1,4-butanediol	57	EI	0.5	0.5	0.6	0.6	0.6	0.2	0.783	0.6	1.0	1.1	1.1	1.2	0.7	0.765	<0.001
aldehydes/dienals																	
2-methylpropanal	72	EI, R	0.1	0.2	0.2	0.2	0.3	0.1	0.669	2.6	5.4	7.7	11.5	5.3	2.8	**0.017**	<0.001
2-methylbutanal	57	EI, R	0.3	0.5	1.1	0.9	1.4	0.565	0.191	26.1	66.2	86.9	144.9	58.4	34.5	**0.007**	<0.001
3-methylbutanal	58	EI, R	0.3	0.7	2.7	2.1	3.1	0.9	**0.024**	13.0	25.1	35.4	40.4	24.9	10.9	**0.019**	<0.001
(E,E)-2,4-hexadienal	81	EI, R	0.0	1.3	2.6	1.9	1.9	0.8	0.090	0.7	2.4	3.3	5.3	3.9	2.67	0.440	0.039
hexanal	56	EI, R	1.1	37.9	45.9	55.1	47.1	19.6	0.097	42	102	120	103	98	50.0	0.543	<0.001
heptanal	70	EI, R	0.0	4.4	4.0	4.3	3.8	1.3	**0.005**	6.3	19.4	19.9	12.8	14.5	11.5	0.745	0.002
octanal	56	EI, R	1.2	3.8	3.5	3.8	3.3	0.8	0.047	5.5	12.0	10.9	9.4	10.8	4.2	0.569	<0.001
nonanal	57	EI, R	5.8	25.4	26.4	25.6	22.9	4.2	**<0.001**	22.4	39.0	36.9	31.1	32.3	10.3	0.535	0.003
benzladehyde	105	EI,R	2.2	2.0	2.5	2.0	2.8	0.5	0.503	8.0	13.5	11.6	13.4	11.2	3.4	0.461	<0.001
2,5-dimethylbenzaldehyde	134	EI	10.9	46.6	45.6	42.5	44.5	11.4	0.081	13.8	43.8	30.7	31.8	31.4	11.8	0.190	0.097
ketones																	
acetone	43	EI,R	25.9	39.2	52.5	51.8	54.4	10.0	**0.030**	25.4	39.0	46.0	54.9	55.1	11.4	**0.038**	0.921
2-butanone	43	EI,R	28.3	23.9	25.6	21.1	21.8	5.9	0.278	35.8	20.2	25.8	26.0	25.0	4.2	**0.013**	0.21
2,3-butanedione	43	EI, R	50.4	49.4	61.2	43.5	46.0	9.6	0.215	55.4	38.9	48.6	41.7	46.8	7.5	0.218	0.416
2-octanone	58	EI, R	0.0	0.0	0.2	0.0	0.1	0.0	0.074	0.3	0.7	0.7	0.8	0.8	0.3	0.362	<0.001
3-hydroxy-2-butanone	45	EI, R	84.3	116.4	157.6	98.6	72.8	29.0	**0.027**	144.9	111.5	232.2	80.8	121.8	94.2	0.415	0.115
sulfur																	
methanethiol	**48**	EI, R	0.0	0.2	0.3	0.2	0.2	0.1	0.291	1.0	1.1	1.7	3.1	2.4	1.1	0.194	<0.001
carbon disulphide	76	EI, R	104.2	27.2	81.0	52.5	67.2	41.1	0.168	42.9	20.4	24.8	16.6	16.3	12.6	0.153	<0.001
dimethyl sulphide	62	EI, R	4.2	5.2	8.7	5.4	3.4	2.0	**0.004**	3.6	5.3	8.7	6.7	4.9	2.0	0.078	0.456
dimethyl disulphide	94	EI, R	0.0	2.3	0.8	0.6	0.5	1.2	0.364	0.5	0.7	1.4	2.0	1.1	0.6	0.105	0.318
dimethyl trisulphide	126	EI, R	0.0	1.7	0.4	0.4	0.4	0.9	0.321	0.1	0.4	0.4	0.4	0.4	0.1	0.091	0.326
methional	104	EI, R	0.00	0.00	0.00	0.00	0.00	nd	na	0.06	0.31	0.42	0.81	0.53	0.30	0.117	<0.001
2-acetyl-2-thiazoline	61	EI, R	0.00	0.00	0.00	0.00	0.00	nd	na	0.29	0.04	0.06	0.06	0.00	0.07	**<0.001**	<0.001
acids																	
acetic acid	61	EI, R	4.3	17.3	21.4	14.8	17.2	4.2	**0.030**	10.4	13.6	12.5	11.6	17.5	3.4	0.174	0.308
2-ethyl butanoic acid	88	EI, R	0.1	2.2	2.4	3.7	1.5	1.5	0.240	1.1	4.2	3.8	5.5	3.8	2.3	0.359	0.034
hexanoic acid	60	EI, R	2.4	5.8	7.1	5.4	6.0	1.3	0.122	2.6	14.4	9.1	6.1	6.4	5.7	0.393	0.321
octanoic acid	60	EI, R	1.7	1.9	3.5	3.3	2.9	1.3	0.590	1.6	5.4	4.1	2.1	2.2	3.0	0.714	0.866
alkanes/ alkenes																	
(E)-octene	55	EI	3.0	36.2	25.7	15.8	13.2	18.1	**0.047**	2.1	22.0	22.9	11.3	7.7	10.5	0.030	0.291
a-pinene	93	EI	51.3	58.8	37.7	42.7	37.1	18.1	0.542	47.6	46.5	38.8	40.7	32.3	20.5	0.929	0.648
alkane (7.9 m)	57	EI	30.6	62.1	69.6	57.1	47.7	14.2	0.167	21.4	44.0	35.2	32.8	40.3	7.8	0.050	<0.001
pentadecanal (37.5 m)	57	EI	0.4	2.4	3.1	2.9	3.0	0.56	**<0.001**	0.3	1.8	1.9	2.1	2.1	0.4	**<0.001**	<0.001
dimethylhexane (2.3 m)	57	EI	0.4	16.7	9.8	6.1	6.0	4.1	**0.006**	0.5	8.6	8.7	4.3	3.5	3.3	0.063	0.175
dimethylhexane (2.7 m)	57	EI	2.5	31.3	25.5	15.7	17.0	8.6	**0.047**	9.4	29.0	35.8	31.0	29.0	11.2	0.142	0.032
alkane (5.53 m)	57	EI	15.8	28.8	22.7	14.8	16.7	9.6	0.385	2.4	13.2	12.1	6.0	7.8	5.9	0.354	0.003
alkane (9.76 m)	57	EI	2.8	7.6	7.6	6.5	5.9	1.8	0.193	2.3	4.9	4.5	3.7	4.4	0.8	0.017	<0.001
b-pinene	93	EI	14.5	16.4	8.9	9.5	8.1	7.1	0.666	14.6	13.2	9.5	10.9	7.7	7.9	0.882	0.98
esters																	
butylformate	56	EI	3.2	3.8	4.4	3.9	3.4	1.4	0.581	15.3	15.3	13.7	14.8	13.9	2.6	0.946	<0.001
methyl acetate	43	EI, R	20.1	15.0	21.1	15.0	13.8	4.1	0.917	18.1	6.9	21.5	6.3	8.6	9.3	0.298	0.15
methyl butanoate	74	EI, R	8.4	19.3	19.2	18.5	17.7	4.4	**0.050**	22.6	16.7	19.7	17.4	18.6	5.0	0.794	0.233
methyl-2- methylbutanoate	88	EI	0.643	0.7	0.8	0.8	0.7	0.2	0.910	8.1	5.3	6.7	5.7	6.4	2.0	0.665	<0.001
methyl pentanoate	74	EI	0.8	1.8	1.9	2.1	2.3	0.5	0.097	0.1	0.2	0.2	0.1	0.1	0.1	0.800	<0.001
methyl hexanoate	74	EI, R	10.0	28.3	22.5	16.0	21.6	7.6	0.135	0.9	4.1	3.0	1.2	1.5	1.6	0.201	<0.001
methyl heptanoate	74	EI	3.9	24.3	25.4	26.7	26.8	5.5	**<0.001**	8.5	23.7	23.4	24.9	23.2	5.7	**0.021**	0.589
methyl nonanoate	74	EI	8.4	8.2	6.3	7.9	8.8	3.2	0.942	0.4	0.3	0.4	0.2	0.1	0.2	**<0.001**	<0.001
ethyl nonanoate	88	EI	0.1	0.1	0.1	0.2	0.2	0.1	0.552	0.6	1.1	1.0	1.1	1.0	0.3	0.194	<0.001
methyl octanoate	74	EI	3.6	5.7	6.6	5.8	5.1	1.6	0.504	0.5	0.4	0.5	0.0	0.2	0.8	0.346	<0.001
methyl propanoate	57	EI	3.9	2.4	3.1	2.2	2.2	1.3	0.255	18.6	9.3	10.3	7.6	10.1	4.1	0.061	<0.001
pyrazines																	
methylpyrazine	94		0	0	0	0	0	nd	na	3.9	3.4	3.6	4.7	3.0	1.2	0.583	<0.001
2,3-dimethylpyrazine	108	EI, R	0	0	0	0	0	nd	na	1.1	0.8	1.0	1.0	0.6	0.4	0.489	<0.001
trimethylpyrazine	122	EI, R	0	0	0	0	0	nd	na	8.7	6.3	6.3	9.3	5.6	2.3	0.298	<0.001
2-ethyl-5-methylpyrazine	121	EI, R	0	0	0	0	0	nd	na	2.4	1.4	1.2	2.1	1.1	0.6	0.109	<0.001
2-ethyl-6-methylpyrazine	121		0	0	0	0	0	nd	na	2.9	2.2	3.2	5.4	3.1	1.3	0.128	<0.001
2,6-dimethylpyrazine	108		0	0	0	0	0	nd	na	9.0	4.6	2.9	4.7	3.9	3.1	0.282	<0.001
2-ethyl-3,5-dimethylpyrazine	135		0	0	0	0	0	nd	na	6.1	5.7	5.2	8.3	4.9	0.6	0.407	<0.001
3-ethyl-2,5-dimethylpyrazine	135		0	0	0	0	0	nd	na	1.5	1.4	1.3	2.1	1.2	0.6	0.407	<0.001
diethylmethylpyrazine	149		0	0	0	0	0	nd	na	0.2	0.2	0.2	0.4	0.2	0.2	0.455	<0.001
Miscellaneous																	
trimethylamine	58	EI, R	0.5	0.7	1.1	0.9	0.9	0.2	0.126	2.6	1.5	1.7	2.1	1.4	0.5	0.130	<0.001
2-pentylfuran	81	EI, R	0.2	1.9	2.8	0.7	1.6	0.7	0.045	0.7	2.1	2.9	3.9	3.2	2.0	0.473	0.04
butyrolactone	86	EI	12.8	7.0	7.4	5.4	6.8	1.4	**<0.001**	11.8	23.0	15.0	12.4	10.4	14.1	0.879	0.095
4-methylphenol	107	EI, R	15.3	19.7	14.9	15.9	13.3	1.6	0.560	10.6	12.3	10.8	9.7	8.8	1.1	0.831	0.002
2-methylphenol	107	EI	5.6	7.7	5.9	6.3	5.5	5.1	0.427	4.0	5.1	4.5	4.4	3.9	2.7	0.752	0.004

Methyl esters are also markers of microbial metabolism (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015), however they are mainly observed in samples stored aerobically. Only methyl butanoate and methyl heptanoate increased significantly in raw VPCB from day zero to 84-days. Increases in methyl pentanoate and methyl hexanoate approached significance. Most of the esters increased with grilling, indicating some are thermally induced.

A range of pyrazine compounds are essential for producing desirable grilled beef aroma (Frank, Ball, et al., 2016). As these compounds are formed through heating, pyrazines were only detected in the grilled samples. In general, the effect of storage time did not have a significant impact on pyrazine formation, with similar amounts formed at zero time and each storage time point. It was of interest, however, that the highest pyrazine concentration tended to occur at day-120 and drop off at day-140, in accordance with the free amino acid data. 2-pentylfuran increased significantly in raw VPCB and is formed through lipid oxidation. In general, few volatile changes were measured in VPCB after 84-days, implying that no major microbiological or biochemical changes occurred. The largest and clearest volatile changes with time were associated with ethanol. The pack sensory assessments did not indicate abrupt or obvious deterioration in the odour quality of the VPCB samples.

Rapid volatile profiling by PTR-MS

Proton transfer reaction mass spectrometry (PTR-MS) allows measurement of volatile compounds in real time (in contrast to GC-MS). PTR-MS can instantaneously measure the concentration of volatiles and provide a quality fingerprint. PTR-MS may provide a more accurate measurement of the true concentration of many low molecular mass species compared to SPME GC-MS. In some cases PTR-MS can measure volatiles that are not easily measured by GC-MS. Slurries (20 g) of raw VPCB samples (1 raw meat: 2 part Milli-Q water) were placed in a Schott bottle (100 mL) and sealed with a Teflon lined fitting with a gas inlet and outlet. After equilibration to 37 ^{o}C (40 minutes), the samples were attached to the inlet of the PTR-MS (Ionicon, Innsbruck, Austria). A number of key fragments changed significantly over time. The most concentrated fragments are shown in **Table 18**. Most fragments could be logically matched to volatiles previously identified in the headspace of VPCB by SPME (previous section) or as reported elsewhere (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015).

For example a 3-fold increase in ethanol (*m/z* 47) at 140-days was measured, consistent with the SPME GC-MS data. Acetaldehyde (*m/z* 45) increased significantly with storage time (r = 0.43, p = 0.003). Methanethiol (*m/z* 49) increased over time (r = 0.62, $p < 0.001$) as did acetone (*m/z* 59, r = 0.53, $p < 0.001$) and trimethylamine (*m/z* 60, r = 0.53, $p < 0.001$). Trimethylamine is one of the basic nitrogen volatiles measured in the TVB-N assay, amongst others. The total sum of volatiles approximately doubled from time zero to 140-days. The PTR-MS data also indicated that future shelf-life prediction may be possible through rapid measurement of indicator volatiles in packs.

The PTR-MS data generally supported the SPME-GC-MS volatile data, especially the abrupt change in ethanol concentration at 140-days. A rapid rise in ethanol may indicate the approach of biochemical changes in the VPCB that correspond to a change in microbial growth and may signal a loss in flavour and quality. As meat consumer acceptance studies were not performed on the samples in the current report, this remains speculative.

Table 18: Selected PTR-MS ions (m/z) that changed significantly with storage time of raw VPCB. Most likely volatile identified (ppbv/g). Differences ($p < 0.05$) for time point comparison shown by superscripts.

m/z	most likely ID	zero time	84-days	98-days	120-days	140-days	LSD	*p* value
35	**hydrogen sulphide**	4	6	11	13	8	8	0.126
45	**acetaldehyde**	768[a]	2716[a]	1886[a]	3165[b]	4177[b]	2254	0.04
47	**ethanol**	505[a]	468[a]	624[a]	535[a]	1546[b]	430	<0.001
49	**methanethiol**	3[a]	5[b]	6[b]	6[b]	9[c]	2	<0.001
57	**1-hexanol/alcohols**	90[a]	169[b]	204[b]	200[b]	173[b]	76	0.024
59	**acetone**	8742[a]	13365[b]	13480[b]	16762[b]	16409[b]	4574	0.006
60	**trimethylamine**	306[a]	457[ab]	469[b]	583[b]	572[b]	158	0.006
61	**acetic acid**	59	67	77	100	111	38.8	0.054
63	**dimethyl sulphide**	258[b]	243[b]	412[a]	193[b]	154[b]	134	0.01
83	**hexanal**	23[a]	222[a]	369[b]	776[c]	479[b]	331	<0.001
85	**1-octanol/alcohols**	16[a]	21[a]	22[b]	29[c]	22[b]	6	0.006
	Total volatiles	10777[a]	17743[b]	17565[b]	22369[b]	23663[c]	4948	<0.001

Collaboration with SDAU

Dr. Yimin Zhang and Pr. Xin Luo from the Meat Science group at Shandong Agricultural University were the main Chinese scientists involved in this project. Dr. Zhang is a senior lecturer and also coordinates numerous postgraduate research projects. A number of postgraduate students were involved in performing tests on the VPCB and their contribution is gratefully acknowledged. Dr. Zhang and Pr. Luo organised all aspects of sample management within China and facilitated access to the laboratory facilities at SDAU. The collaboration on this project has built on existing links with SDAU and CSIRO and strengthened ties between Chinese and Australian meat researchers. CSIRO and SDAU intend to build on this collaboration in the future. SDAU already has interaction with Dr. David Hopkins from the Department of Primary Industries New South Wales. Dr. Zhang and Prof. Lou visited Australia as part of the initial project and Dr Zhang visited to discuss the results with the industry stakeholder and to discuss the data analysis. Dr. Zhang and Dr. Frank (CSIRO) visited the industry stakeholder and DPI-NSW (Cowra) to discuss potential future collaborations between CSIRO and SDAU, which will benefit Australian meat researchers and the Australian red meat industry (**Figure 16**).

Figure 16: Left to right: Damian Frank (CSIRO), Yimin Zhang (SDAU), David Hopkins (NSW-DPI, Cowra) and staff member at DPI-NSW Cowra.

7.0 CONCLUSIONS & RECOMMENDATIONS

In general, the cold supply chain during shipping and transport in China was able to maintain appropriate temperature control for extended shelf-life of vacuum packaged beef (up to 140-days). Despite longer than expected holding times at customs clearance in the Shanghai International Port and associated temperature deviations, nearly all the VPCB achieved a 140-day shelf-life, according to the UTas model. The UTas prediction model confirmed that the CSIRO samples could be expected to easily achieve a 140-day shelf-life, possibly up to 180-days in some cases. TVB-N was identified as a potential objective index of Australian VPCB freshness/spoilage. The TVB-N data consistently increased over storage time, unlike the microbiology data, hence making TVB-N a potentially useful measure of VPCB freshness in future shelf-life studies.

Extensive biochemical measurements were conducted on the CSIRO VPCB samples, including changes in the fatty acid composition, volatile generation in raw and grilled meat and changes in key non-volatile molecules. Although none of these changes were indicative of spoilage, some chemical parameters (e.g.; TVB-N, ethanol, acetaldehyde, acetone, and tyramine) may act as objective markers of shelf-life in future predictive models and may also serve as non-microbiological biomarkers for quality control tests.

As a whole, the findings indicate that the absolute microbiological counts (LAB, APC) were not as good predictors of the VPCB sensory attributes as the temperature deviation. This supports the validity of the UTas model, which takes into consideration the initial APC and the time×temperature profile of individual VPCB packs. This indicates the importance of temperature monitoring during the transportation process. The relationship between the sensory data obtained for raw VPCB and the eating quality of grilled beef is unknown. Future studies should be conducted to understand this relationship and define appropriate sensory cut-off scores. Maintaining low temperature at all steps in the cold supply chain is critical to maintain high quality Australian VPCB and positive consumer experiences.

9.0 APPENDICES

9.1 Appendix 1 _ Sensory Assessment Ballot sheet – English version

1). PRE-OPENING PACK ASSESSMENT – to be performed on UNOPENED packs

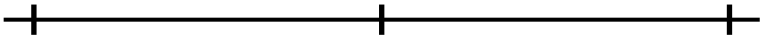
Assessor name: **Date:**

Sample code: B10

Q1.) Pack vacuum integrity (tick one):

☐	☐	☐	☐
Vacuum broken	Very lose, good vacuum	Slight loose, good vacuum	Tight fit, good vacuum

Q2.) Freshness - (how fresh does the product look in the pack?)

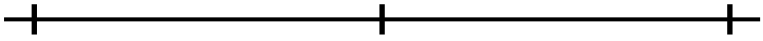


Not Fresh **Very Fresh**

Q3.) Amount of weep/liquid in pack - (how much liquid is visible in the pack?)

None **a lot**

Q4.) Overall liking appearance (how much do you like the appearance of the meat?)



Dislike Extremely **Like Extremely**

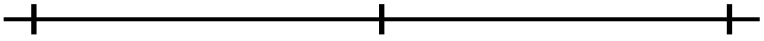
Other remarks:

2). POST-OPENING PACK ASSESSMENT – to be performed on OPENED packs at ~10 ºC
WEAR LATEX GLOVES WHEN HANDLING PACKS/MEAT.

Assessor name: **Date:**

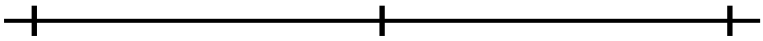
Sample code: B10

Q5.) Overall odour intensity (rate the overall strength of the odour)



None **Very Strong**

Q 5.) Freshness (the smell of fresh raw meat)

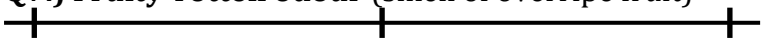


Not fresh **Very fresh**

Q6.) Rotten- Sulphur odour (the smell of rotten meat/Sulphur)

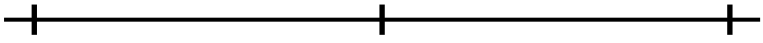
Not rotten/sulphur **Very Rotten/Sulphur**

Q7.) Fruity-rotten odour (smell of overripe fruit)



Not fruity **Very fruity**

Q8.) Cheesy/fermented (dairy fermented, cheesy smell)



Not cheesy/ Fermented **Very cheesy/ Fermented**

Other remarks:

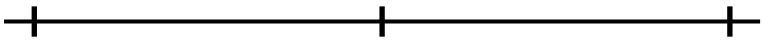
3). POST-BLOOMING – to be performed after blooming period (after at least 30 minutes/ 4°C).

BLOOMED MEAT will be removed from the packs and placed on a clean tray.

Assessor name: **Date:**

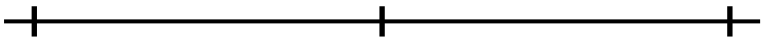
Sample code: B10

Q9.) Overall liking appearance (how much you like the appearance of the meat)



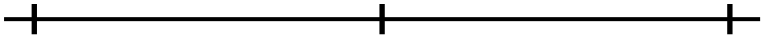
Dislike Extremely **Like Extremely**

Q 10.) Freshness appearance – (how fresh the meat looks)



Not fresh **Very fresh**

Q 11.) Fresh odour – (how fresh the raw meat smells after blooming)



Not fresh **Very fresh**

9.2 Appendix 1 _ Sensory Assessment Ballot sheet – Chinese version

1). 打开包装前评估-----在未打开包装的情况下进行

评审员姓名：　　日期：

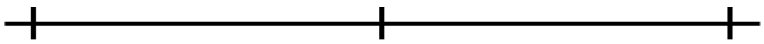
样品代码：

①真空包装完整度：（打√）

真空被破坏　　（ ）　完全松散，真空度完好（ ）

轻微松散，真空度完好（ ）　非常紧密，真空度完好（ ）

②外观新鲜度（产品在包装中的新鲜程度）

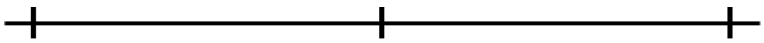


不新鲜　　非常新鲜

③汁液渗出量/包装内汁液量（包装中可视的汁液渗出有多少）

没有　　很多

④外观总体喜爱度（你对肉的外观的总体喜爱程度）



非常不喜欢　　非常喜欢

其他意见：

2). 打开包装后评估----在 10℃下戴乳胶手套处理包装和肉（主要是通过嗅觉来感受气味）

①总体气味（气味的强弱程度）

无异味　　异味强烈

②气味新鲜度（新鲜肉味的强度）

不新鲜　　非常新鲜

③腐烂的硫磺气味（肉腐烂/硫磺的气味）

无腐烂/硫磺气味　　非常强烈的腐烂/硫磺

④腐烂的水果气味（水果过熟的气味）

无腐烂的水果气味　　非常强烈的腐烂的水果气味

⑤干酪/发酵气味（乳制品发酵/干酪气味）

无干酪/发酵气味　　非常明显的干酪/发酵气味

其他意见：

3). 发色后----在发色（16℃下至少 30min）后进行评估

从包装中转移到托盘中进行发色

①外观总体喜爱度（你对肉样外观的总体喜爱程度）

非常不喜欢　　非常喜欢

②外观新鲜度（肉看上去的新鲜程度）

不新鲜　　非常新鲜

③气味新鲜度（发色后肉气味的新鲜程度）

不新鲜　　非常新鲜

其他意见

10.0 BIBLIOGRAPHY

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