Final Report

AUSTRALIAN MEAT PROCESSOR CORPORATION

Meat Industry Services

Effect of initial muscle biochemical composition on microbiological growth and eating quality of long aged beef, destined for export markets

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Effect of initial muscle biochemical composition on microbiological growth and eating quality of long aged beef, destined for export markets

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Abstract

Previous vacuum-packaged beef shelf-life studies indicated that microbial growth did not follow the expected pattern and peaked at a lower level than measured in earlier studies. The current project aimed to determine if initial biochemical properties on sensory properties and microbiological growth. CSIRO collected samples of vacuum-packed beef striploins from carcases of three different meat colour score ranges – 1b and 1C, 2 and 3, and ≥4, from three export abattoirs in Queensland. The primal cuts were stored at -1°C for up to 20 weeks and opened for assessment by analysis of microbiological and biochemical properties, and visual and sensory examination at 0, 2, 8, 12, 16 and 20 weeks after packing.

The growth of lactic acid bacteria in the vacuum packs was most highly correlated with objective measurement of meat colour, muscle pH and total glycogen level. Samples with a pH below 6.1 and a meat colour score less than 4 were acceptable after 20 weeks storage. Samples with a pH of less than 5.7 had a lower level of microbial growth.

Executive summary

Striploin samples were collected from beef carcases of three different meat colour score ranges from three export plants in southern Queensland with the overall aim of determining if intrinsic biochemical properties of the meat influenced the microbiological growth and hence the shelf life of the vacuum-packaged product.

Half striploin samples from carcases with meat colour scores 1A, 1B, 2,3 and ≥4 were vacuum-packed at the plants, cooled overnight and then transported under refrigeration to CSIRO at Coopers Plains, Queensland and stored at -1°C ±0.5°C for up to 20 weeks. Samples were opened for assessment at 0, 2, 8, 12, 16 and 20 weeks from time of packing and assessed by an experienced panel and sampled for microbiological and biochemical analysis. Samples were also collected at 2, 12 and 20 weeks for sensory assessment by a MSA consumer panel.

The main conclusions drawn from the study were:

- There were minimal differences between plants for appearance of the packs, acceptability of the odour on opening and microbiological quality. Product from plant 2 had the highest scores for MSA sensory assessment and had minimal growth of the spoilage organism *Brochothrix thermosphacta* compared with the other plants.

- No samples exhibited any signs of greening spoilage, although high-pH beef has been implicated in instances of this in the past.

- Samples with a pH above 6.1 were acceptable at 16 weeks but the odour on opening packs at 20 weeks was only marginally acceptable and MSA scores for flavour had declined. All beef samples with a pH below 6.1 were acceptable at 20 weeks.

- Some samples from the group with a meat colour score of 4 and above were of normal pH (~5.4), indicating that either the carcases were incorrectly graded or the darker meat colour was due to some other cause.

- Microbial growth was faster and peaked at a higher level for samples with a pH of 5.7 and above and a dark meat colour. The mean TVC count for samples with a pH below 5.7 was 1.5 – 2.0 $\log_{10}$ units lower than for samples with a higher pH.

- The growth of lactic acid bacteria (the dominant microflora) was most highly correlated to objective measurement of meat colour (L* and a*), pH and total glucose, after 8 weeks storage.

- MSA sensory panellists gave generally increasing scores as storage time increased.

Contents

Background

The shelf life of Australian vacuum-packaged beef was investigated in a project conducted by CSIRO in 2009. This demonstrated that under optimal storage conditions Australian chilled beef had a shelf life in excess of 20 weeks. Initial contamination levels were very low and that the growth of bacteria during storage did not follow the expected patterns found in studies from the 1970s and 1980s. The total viable count (TVC) and lactic acid bacteria (LAB) grew at a slower rate than expected and peaked at a lower level (10^5 per cm^2) which has since been observed in other studies funded and co-ordinated by MLA.

A number of changes have taken place in the Australian meat processing industry since the 1970s which may impact initial bacterial load and also bacterial growth. These changes include production techniques such as a shift away from saleyard selling and a shift to more grain-finishing of cattle. A faster pH fall has been observed and an associated lower ultimate pH. There has also been improved hygiene during slaughter and dressing, less washing of carcases, better chilling and a trend to lower storage temperatures.

The overall aim of the current project was to determine if there are some intrinsic biochemical properties of the meat which limit microbial growth. It is well known that high-pH meat provides a less hostile environment for microbial growth, so consequently dark-cutting meat is not normally vacuum packed for markets that demand a long shelf life, but there may be other biochemical properties that also affect microbial growth.

1 Project objectives

1. Determine the effects of initial biochemistry on the sensory qualities and microbiological growth of long aged (20 weeks) beef striploin destined for export.

2. Determine the effect of biochemical aspects of beef primal cuts on the growth rate and limits of growth of microorganisms on vacuum-packaged beef.

2 Methodology

2.1 Sample collection

Arrangements were made with three large export abattoirs (coded 1, 2 and 3) in South-east Queensland to purchase vacuum-packed striploins from carcases with different meat colour scores. At each plant, nine bodies were selected by the chiller assessor in each of the meat colour score ranges:

1B, 1C,
2, 3, and
4, 5

A numbered and coloured tag was attached to the striploin of each side to identify it through the marshalling and boning process. The sides were then generally processed through the normal boning chain and the deboned striploins transferred to a separate table where they were cut into two and each labelled with a numbered and coloured tag to indicate the meat colour and storage time. The tags were allocated to the samples based on a predetermined random plan (Appendix 1). Carcases were from cattle produced under a range of regimes. Those at plant 1 were all 100-day grain fed steers implanted with hormonal growth promotants, while those at plant 2 and

3 were mainly grass fed with and without HGPs. A summary of the cattle types is provided in Appendix 2.

Immediately after treatment allocation each half striploin was placed in a vacuum bag of the type normally utilised by the plant and the samples processed as a group through the evacuation and shrinking process. After exiting the shrink tunnel, the samples were packed into cartons according to the time of storage with six half striploins in each carton. Samples allocated to Day 0 were transported to the CSIRO laboratory at Coopers Plains in insulated Eskies with freezer blocks with the maximum transport time being 1.5 hours. The remaining cartoned samples were labelled, some fitted with I-Button temperature loggers, and chilled according to the standard plant procedures and then transported to Coopers Plains via commercial refrigerated road transport several days later.

Details were also obtained from the plant of the breed and source of stock, transport time their processing procedures such as slaughter method, electrical inputs, whether there was a microbial intervention, carcase chilling procedure, vacuum bag specifications and carton chilling regime. This information is also presented in Appendix 2.

On arrival at the laboratory the cartons of samples were immediately placed on a pallet in a chiller operating to maintain the meat temperature at -1°C ±0.2°C. Six cartons were fitted with Type T thermocouples attached to a Squirrel Model 2020 data logger to record the meat temperature.

2.2 Sample assessment

2.2.1 Assessment of packs

After storage periods of 0, 2, 8, 12, 16 and 20 weeks, six half-striploin packs from each colour group from each processor (total 54) were removed from storage for assessment. Each pack was weighed prior to opening and with the exception of packs from week 0, assessed by a 6 to 9-member informal sensory panel. Each panel member scored each pack for vacuum integrity, appearance of the intact pack and confinement odour on opening using a 9-point scale (Appendix 3).

Packs for microbiological analysis were sampled soon after assessment. The samples of surface tissue excised for microbiological analysis were weighed and the primal cut patted dry with a paper towel and weighed. The vacuum bag was washed out and dried before weighing. Where drip keepers were used, a sample of 10 unused drip keepers was obtained from the plants for calculation of the average weight of the dry drip keeper. The weight of weep in each pack was calculated from the difference between the total weight of the pack less the weight of the dry bag and the average weight of a dry drip keeper and the weight of the dry primal cut plus the microbiological sample.

$$wt\ of\ weep = total\ wt - (wt\ of\ dry\ cut + wt\ of\ micro\ sample + wt\ of\ bag + wt\ of\ drip\ keeper)$$

$$Percent\ weep - wt\ of\ weep + (total\ wt - (wt\ of\ bag + wt\ of\ drip\ keeper)) \times 100$$

2.2.2 Microbiological assessment

Three striploins from each group from each plant (total 27) were sampled for microbiological analysis by excising 4 x 10 cm^2 samples – two from the subcutaneous fat surface and two from the lean surface on the other side. The four pieces, each 2 – 4 mm thick were placed into a

stomacher bag to which 100 mL of 0.85% saline was added and stomached for two minutes. A decimal dilution series was prepared for each sample in 0.85% saline, and these plated onto Petrifilm Aerobic, Petrifilm *E. coli* / coliform and STAA plates for TVC, *E. coli* and coliforms, and *Brochothrix thermosphacta* counts respectively. The dilutions were also prepared in MRS broth and plated onto Petrifilm Aerobic according to the Petrifilm method for the enumeration of Lactic Acid bacteria. Petrifilm Aerobic were incubated at 25°C ± 1°C for 72 ± 3 h; Petrifilm *E. coli* / coliform were incubated at 35°C ± 1°C for 18 ± 2 h; STAA plates were incubated aerobically at 22°C ± 1°C for 48 ± 3 h; one set of LAB films were incubated anaerobically at 25°C ± 1°C for 120 ± 3 h, while a second set were incubated aerobically at 25°C ± 1°C for 120 ± 3 h.

2.2.3 Biochemical analyses

The pH of a sample of each striploin was measured after using a TPS Model WP80 pH meter fitted with an Ionode IJ44 combination pH probe. The colour of a 25 mm thick steak from each sample was measured using a Hunterlab Miniscan EZ immediately after slicing and again after allowing to bloom for 60 minutes at 3°C. Further samples from each cut were frozen in liquid nitrogen and stored at -80°C for later analysis for:

2.2.3.1 Thiobarbituric acid reactive substances (TBARS)

The concentration of TBARS was determined in 27 striploin samples from each time point (Witte, Krause et al. 1970). Samples were capped and cooked in 75°C water bath for 20 minutes and subsequently cooled for 30 minutes at 5°C prior to extraction. The concentration of malondialdehyde equivalents (mg/kg muscle) was calculated from absorbance readings at 530 nm, using 1,1,3,3- tetraethoxypropane as a standard.

2.2.3.2 Glucose and glycogen content

The protein fraction of frozen muscle samples (2 g) was removed by homogenisation (1:10 w/v) in 30 mM HCl using an Ultra-turrax 22,000 rpm for 2 x 15 second bursts. Samples were centrifuged (3,000 rpm, 4°C, 10 minutes) and supernatants containing glycogen were frozen -20°C until assay could be performed. Thawed samples were analysed for total glycosyl units and free glucose content by incubating 50 µL (37°C, 90 minutes) with or without the addition of 500 µL of hydrolysing enzyme amyloglucosidase (1:200 in 40 mM acetate buffer pH 4.8). The concentration of glucose (g/100 g muscle) was determined in duplicate using a glucose assay kit (Sigma GAGO-20) and glucose as a standard. The absorbance of both samples and standards was measured at 540 nm.

2.2.3.3 L-lactic acid content

The L+ lactate determination within the muscle was conducted in accordance with the method of Noll (Noll and H.U.Bergmeyer 1985) and using enzyme concentrations as outlined by Bond (Bond and Warner 2007). The lactate content (µmol/g) of the muscle was determined stiochiometrically by measuring the absorbance of NADH at 340nm (extinction coefficient = 6.22 mM/cm).

2.2.4 MSA sensory assessment

Also at 2, 12 and 20 weeks of storage portions approximately 100 mm long from four striploin samples of each colour category from each processor (total 36) were re-vacuum packaged and the packed into polystyrene insulated boxes with freezer packs. These were shipped by overnight express air freight to Coffs Harbour airport for collection by Cosign Pty Ltd. The samples were prepared by Cosign using standard MSA procedures and frozen for later sensory panel assessment by Sensory Solutions.

2.2.5 Statistical analysis

Data analysis was completed using Genstat 15th edition.

3 Results and discussion

3.1 Sample assessment

3.1.1 Assessment of packs

Panellists scored slight differences between plants for integrity of vacuum with a gradual reduction with storage time (Figure 1). There were no 'leaker' packs. Scores for plant 3 were possibly lower due to the absence of a drip soaker pad and the accumulation of weep gave the appearance of a looser pack. There were no differences between colour score groups for quality of vacuum.

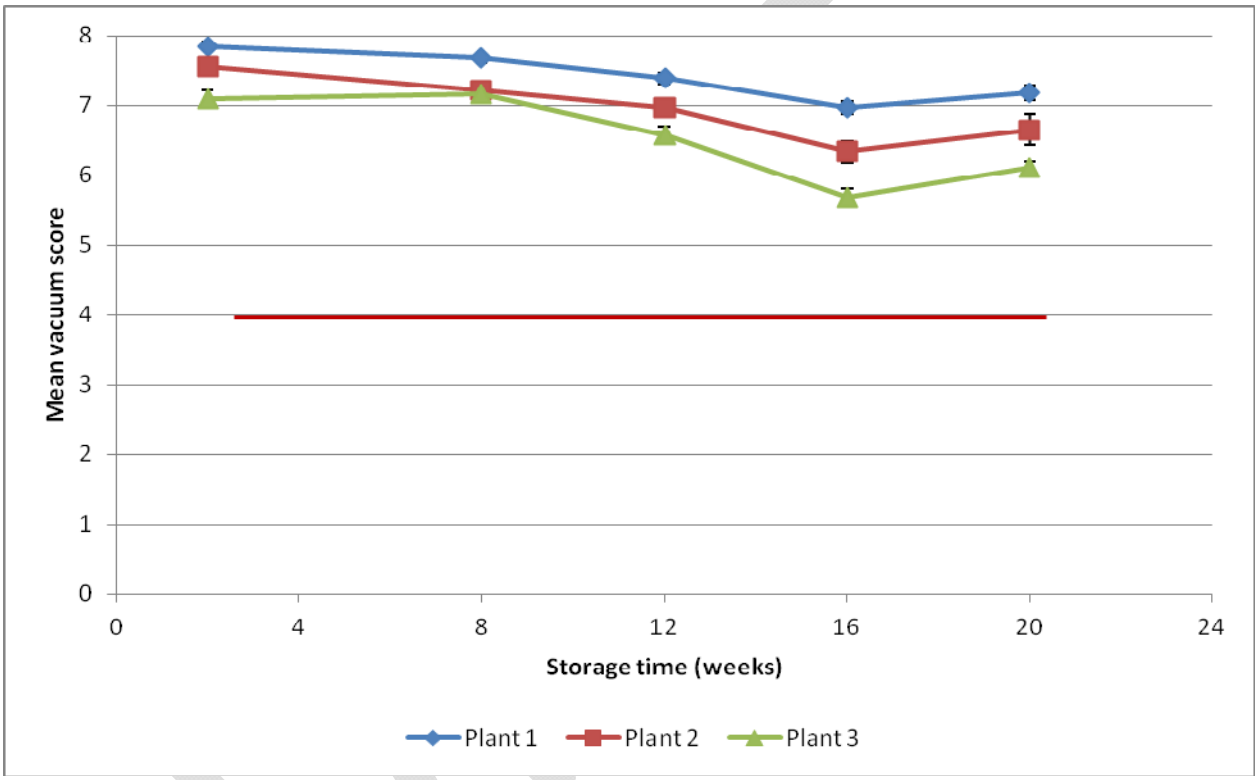


Figure 1: Mean vacuum scores for all samples from each plant (0 – unacceptable, 4 – acceptable, 8 – excellent). Error bars show standard error of the means

Vacuum packs scored highly for visual appearance with a gradual reduction in acceptability after week 8 (Figure 2). There was little difference between plants and no effect of meat colour category or pH on appearance score (data not shown). High-pH beef has, in the past, been implicated in the appearance of greening of vacuum-packaged product but there was no indication of any greening on any of the samples after storage times of up to 20 weeks. It is suspected that the low storage temperature of -1°C prevented growth of the organisms responsible for greening.

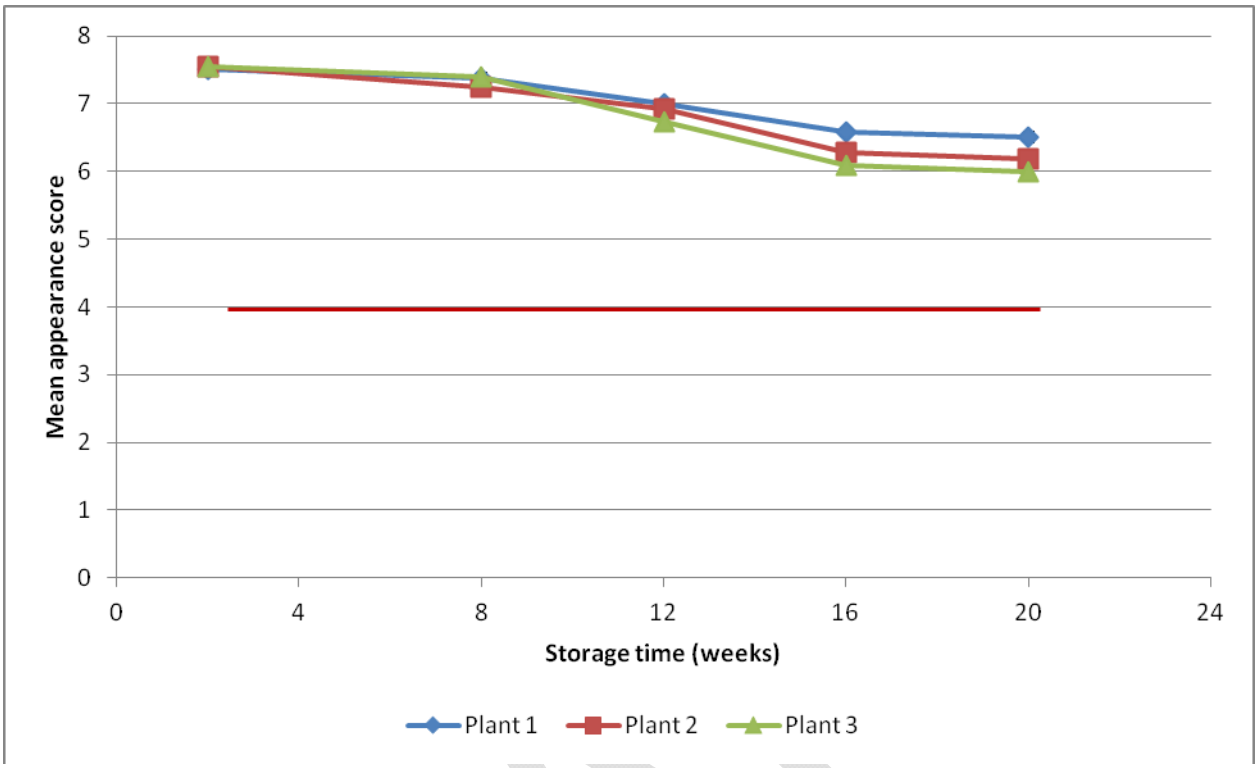


Figure 2: Mean visual appearance scores for all samples from each plant (0 – unacceptable, 4 – acceptable, 8 – excellent). Error bars show standard error of the means

The odour immediately on opening a vacuum pack is the best practical method of assessing the wholesomeness of the product. Vacuum packaged meat that has been aged for several weeks will develop a confinement odour that soon dissipates. After lengthy storage times this can develop into cheesy type odour due to the growth of lactic acid bacteria and eventually an unpleasant off aroma.

The mean scores for odour are presented in figure 3 and shows the development of the odour with time and little difference between plants. A score above 4 indicates that the product would b considered acceptable. Although scores ere lower after 20 weeks of storage, on average the product was still considered acceptable.

When the scores are related to the meat colour categories, differences do emerge, with the darker samples considered less acceptable (figure 4). One dark sample was considered unacceptable at week 20 and several more were marginally acceptable. All of the samples from colour score 1B to 3 were acceptable.

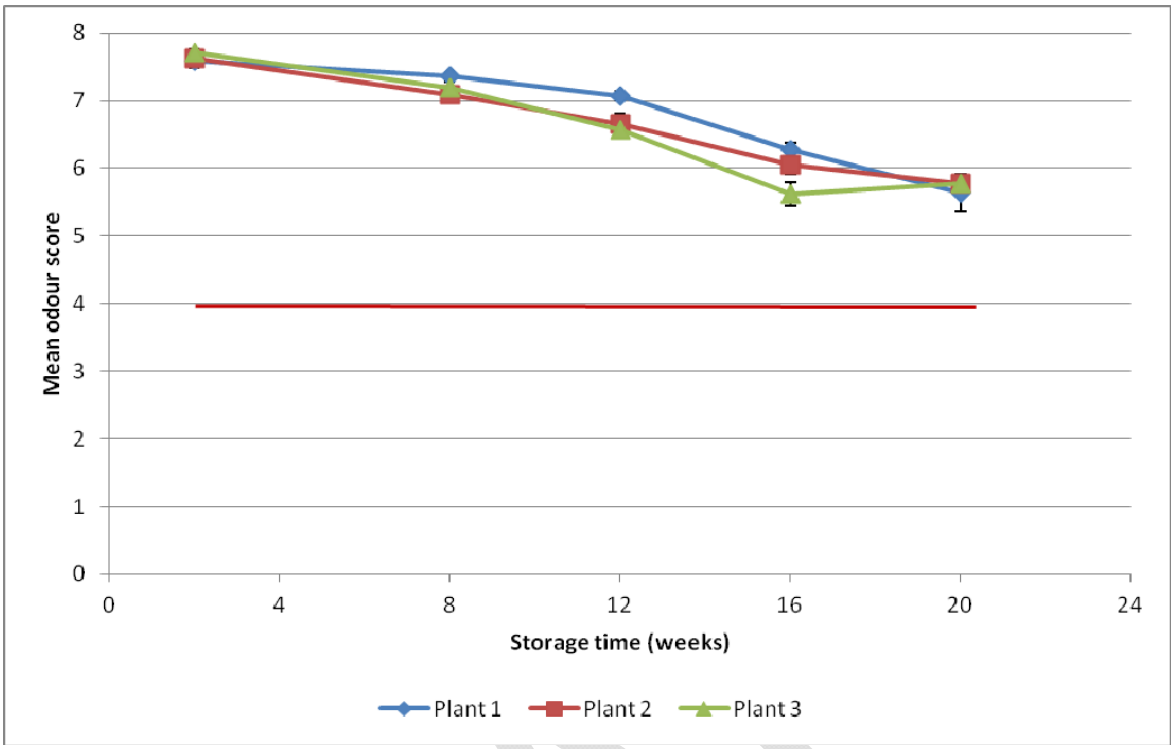


Figure 3: Mean odour scores for all samples from each plant (0 – unacceptable, 4 – acceptable, 8 – excellent). Error bars show standard error of the means

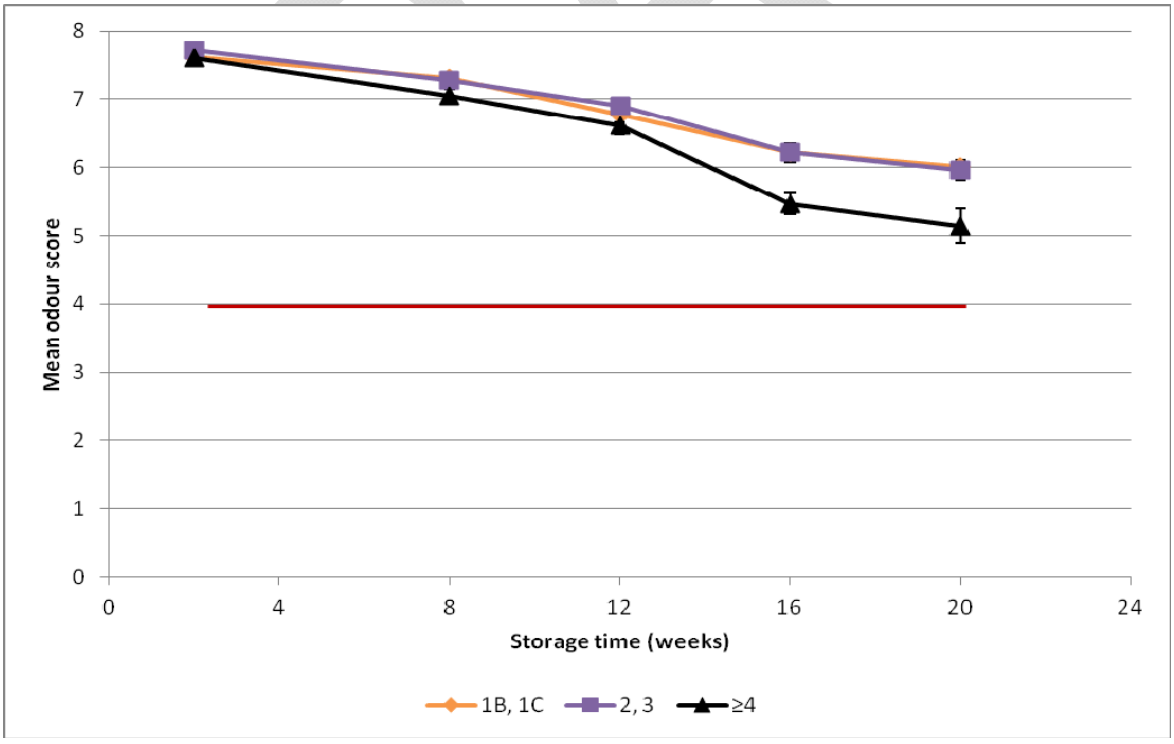


Figure 4: Mean odour scores for all samples for different colour score ranges (0 – unacceptable, 4 – acceptable, 8 – excellent). Error bars show standard error of the means

The acceptability of the odour of high-pH samples dropped markedly at 20 weeks (figure 5) and one of the four samples with a pH above 6.1 was unacceptable. It had a pH of 6.31.

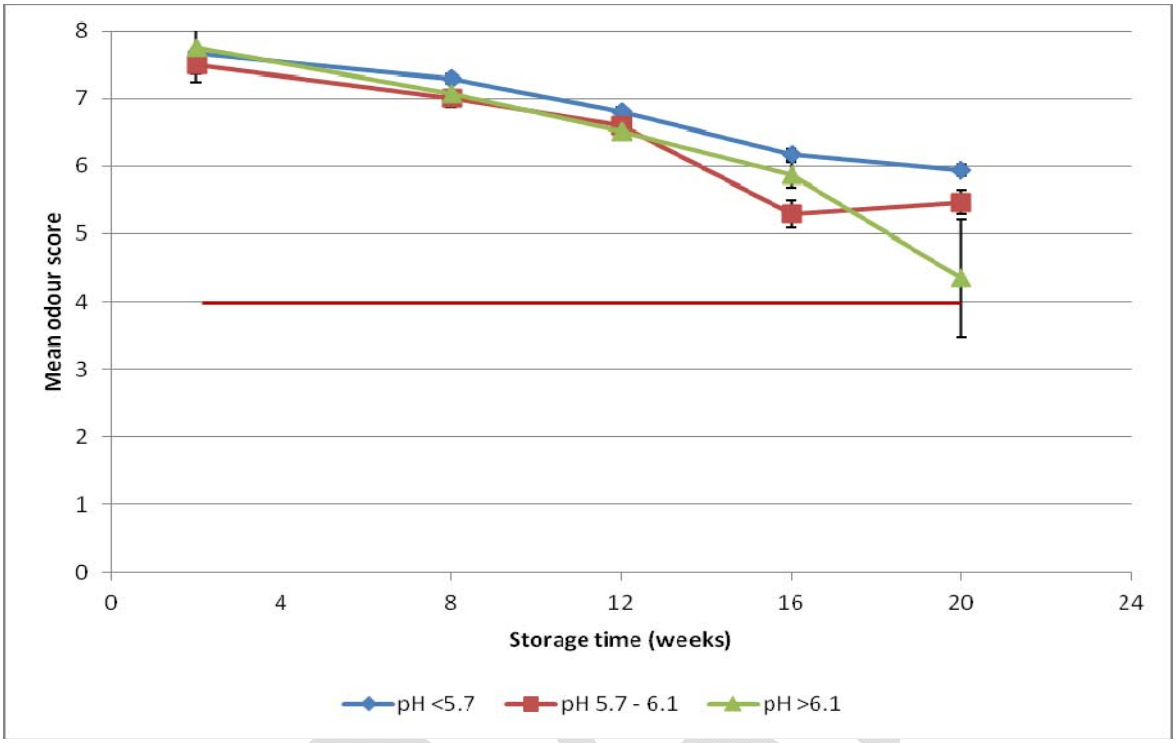


Figure 5: Mean odour scores for all samples for different pH ranges (0 – unacceptable, 4 – acceptable, 8 – excellent). Error bars show standard error of the means

The amount of weep in the vacuum packs increased with storage time up to 3 to 4% by 16 weeks (figure 5). Samples from plant 3 had significantly less weep ($p<$) at most assessment times. Factors that have been considered to influence the amount of weep are carcase cooling rate and meat pH. Plant 3 had modern carcase chillers equipped with spray chilling which should minimise the chance of heat toughening that can increase weep. This plant was also the only one not to use drip keepers in the bags.

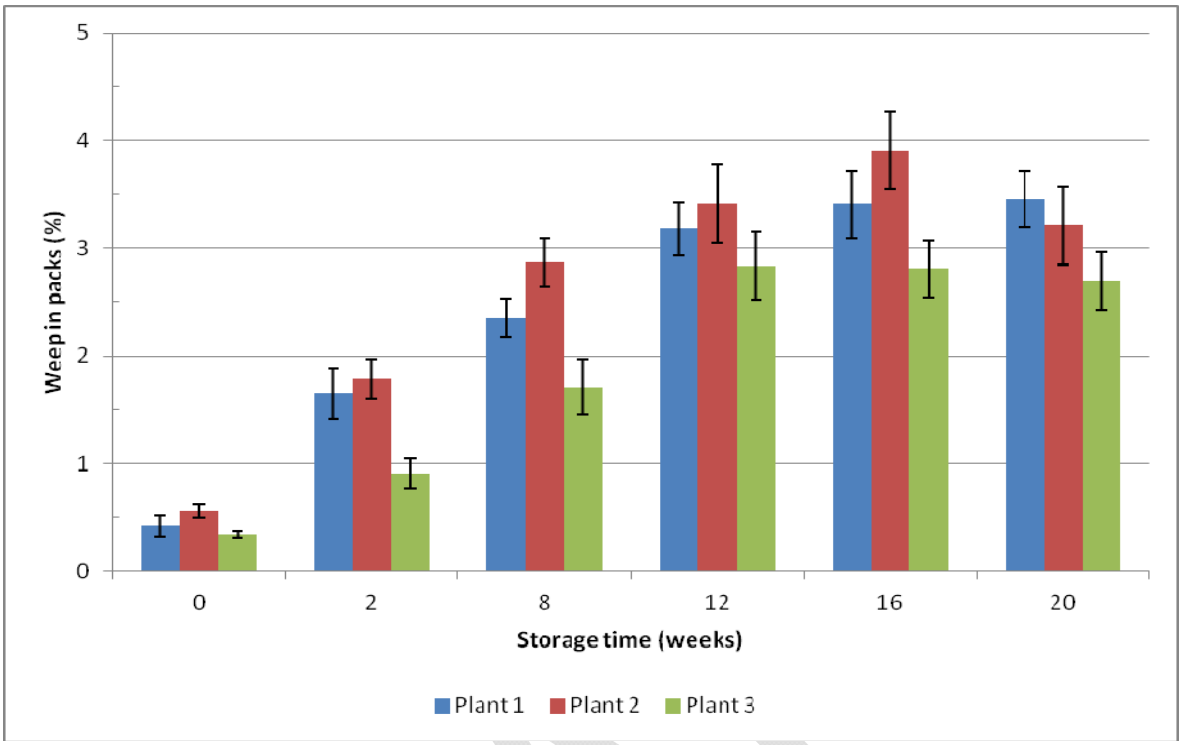


Figure 5: Mean percent weep in vacuum packs for all samples from each plant (error bars show standard error of the means)

The amount of weep at each assessment time was not affected by the meat colour group (Figure 6). However there was an effect of pH with the high-pH (>6.1) samples having significantly less weep than samples of medium and normal pH (Figure 7).

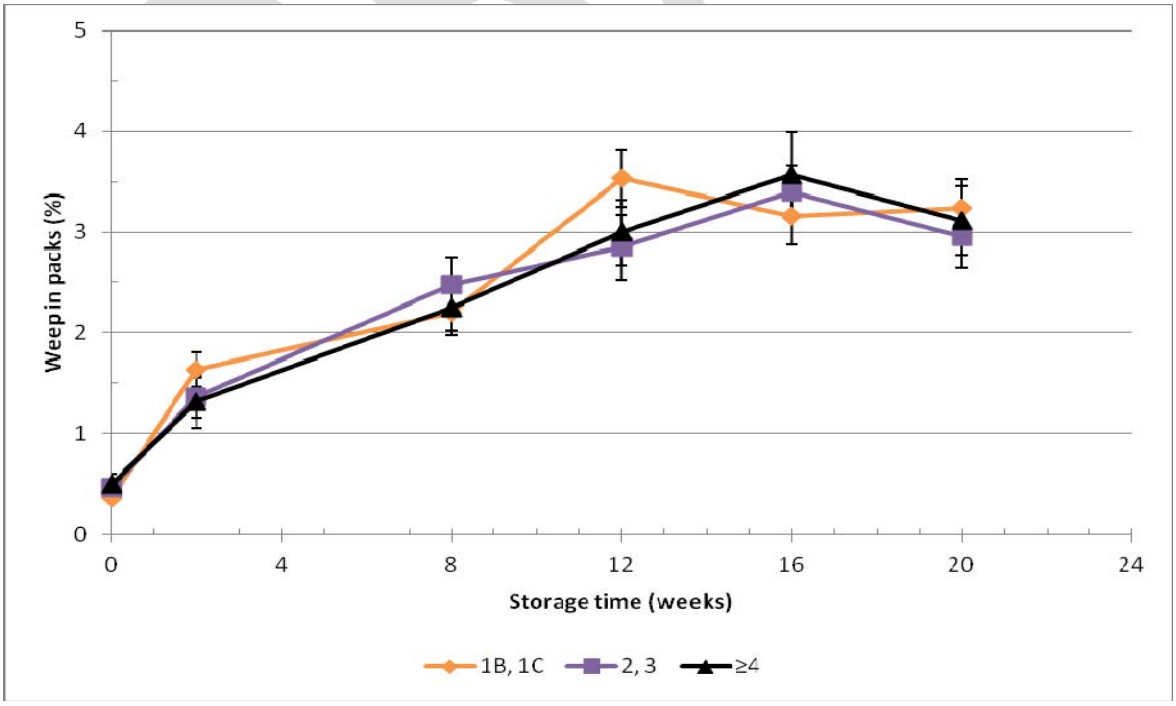


Figure 6: Mean percent weep in vacuum packs for each meat colour score range (error bars show standard error of the means)

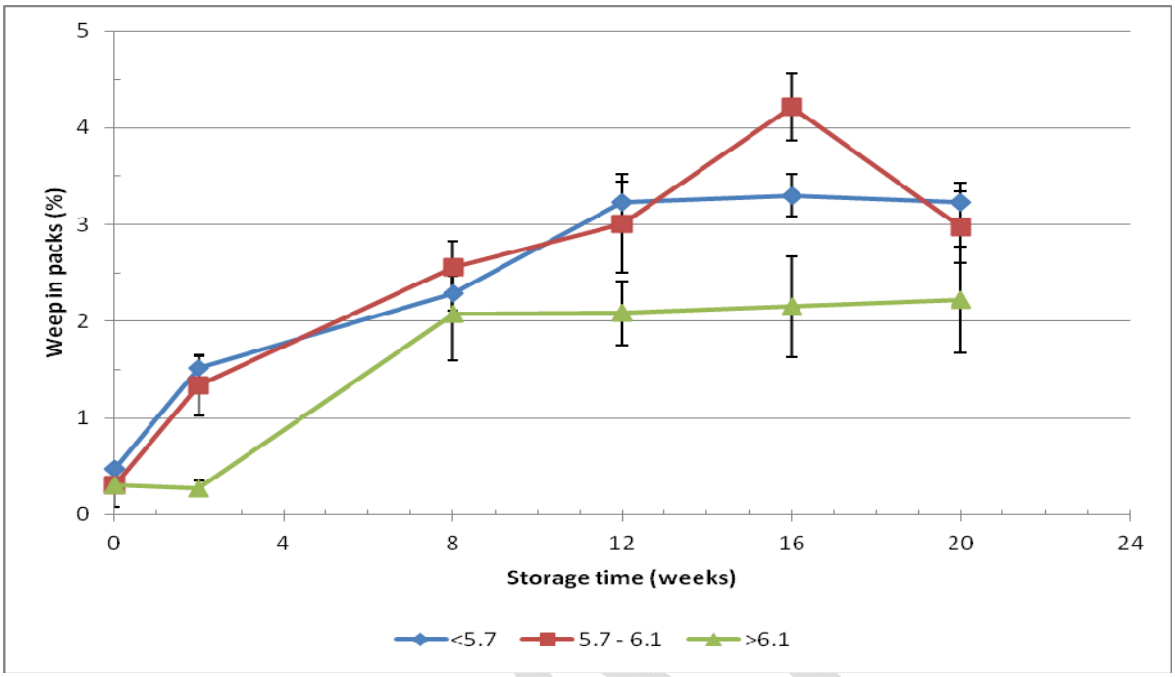


Figure 7: Mean percent weep in packs of different pH range (error bars show standard error of the means)

The primal cuts were selected from carcases based on meat colour score as this is a parameter measured by most plants during chiller assessment. As pH is one of the major factors influencing meat colour, it might have been expected that a range of pHs would have been encountered. This was not the case for the samples from carcases with meat colour scores of 1B through to 3 which were all in the low-pH range (~5.4) for the week zero samples (Figure 8). The pH of the striploins from carcases with meat scores of 4 and above ranged in pH from a normal 5.38 to a high 6.53. Both these carcases were from similar grain-fed animals processed at the same plant. Therefore there were very few genuinely high-pH carcases and there was not an even distribution of meat pHs across all the samples collected. There was only a reasonable relationship (R^2=0.556) between pH and lightness (L*) as measured by the Hunterlab.

The fact that some of the samples from carcases with a high meat colour score were of normal pH would indicate that either the carcases were misgraded or causes other than pH had an influence on meat colour and that there may be scope for some of these carcases to be moved to a lower, higher-value colour score possibly by changes to processes.

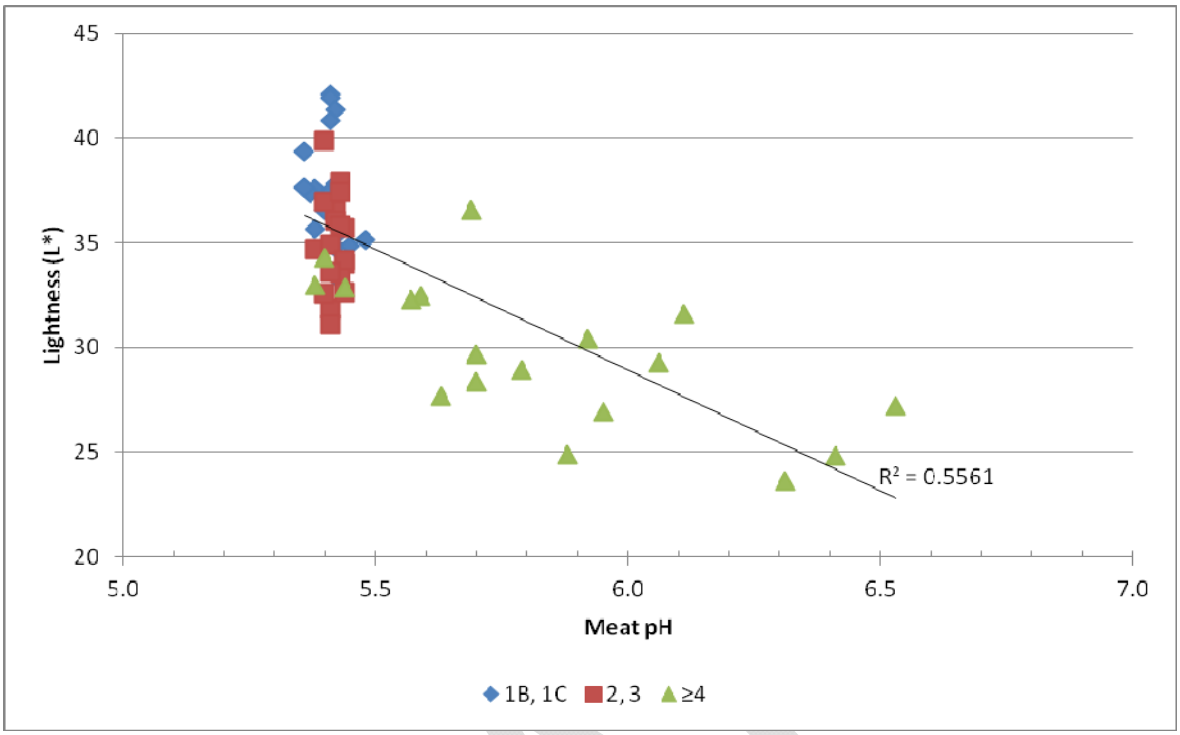


Figure 8: Relationship between meat pH and lightness (L*) at week 0

3.1.2 Microbiological assessment

The mean total viable count (TVC) on the surface for all the cuts rose from 2 to 3 $\log_{10}$ cfu/cm^2 at the time of packing to 5-6 $\log_{10}$ cfu/cm^2 by week 8 and to 6-7 logs by week 20. All three plants followed a similar pattern (figure 9) which is in contrast to the previous study in 2009 (Small et al 2009) where there appeared to be some differences between plants in rate of TVC growth on striploins.

The initial level of contamination on the striploins was approximately the same low level as for the previous study indicating that spray chilling currently utilised has not resulted in any increase in microbial counts.

In a CSIRO study from 1973 (Bensink et al), the total count on the surface of vacuum-packed striploins at a similar time of 6 – 8 weeks after packing but at a much higher level of 7-8 $\log_{10}$ CFU and the product was considered unacceptable to consumers after 13 weeks storage. The main differences in that study were the initial microbial level on the product of 4-6 $\log_{10}$ compared with approximately 2 $\log_{10}$ CFU in this study, a storage temperature of 0°C compared with -1°C and the packaging films.

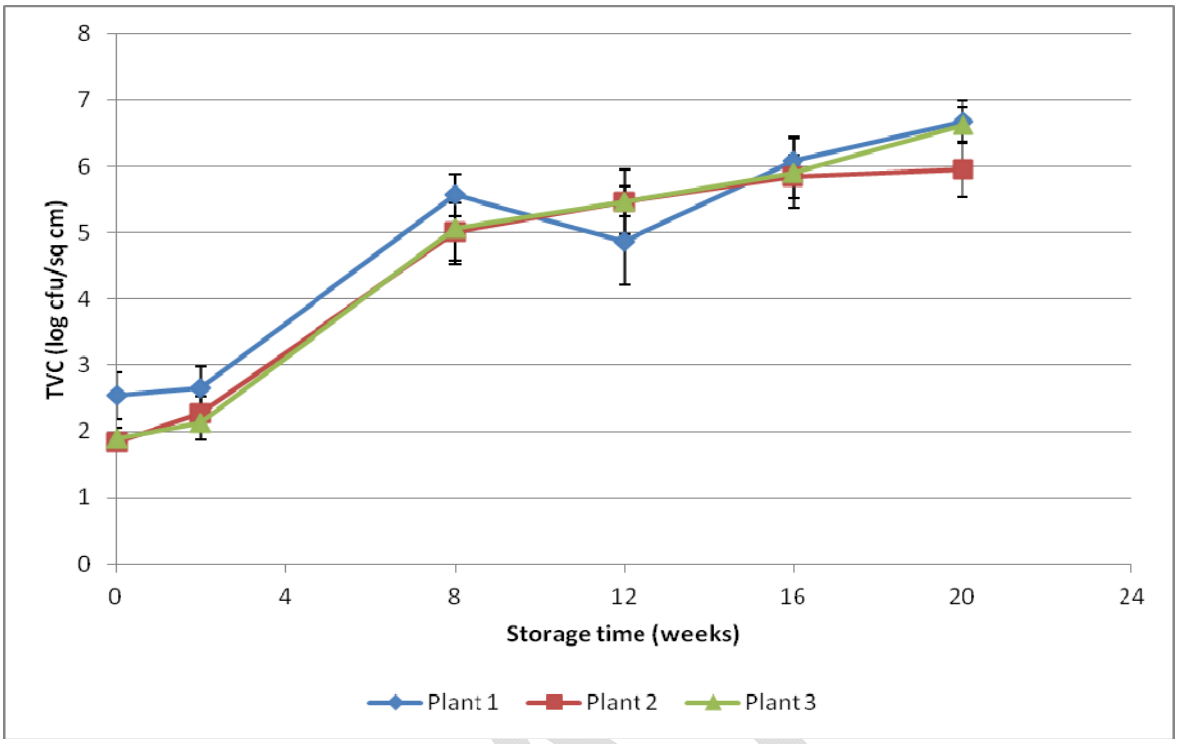


Figure 9: Growth of TVC (mean $\log_{10}$ cfu/cm^2) for the three plants (error bars show standard error of the means)

When the growth of TVC is related to each of the meat colour score ranges for all plants (Figure 10), it is clear that growth is faster on the darker meat. This would be expected as the lighter colour-score samples were of a similar pH range while the darker samples range up to pH 6.5.

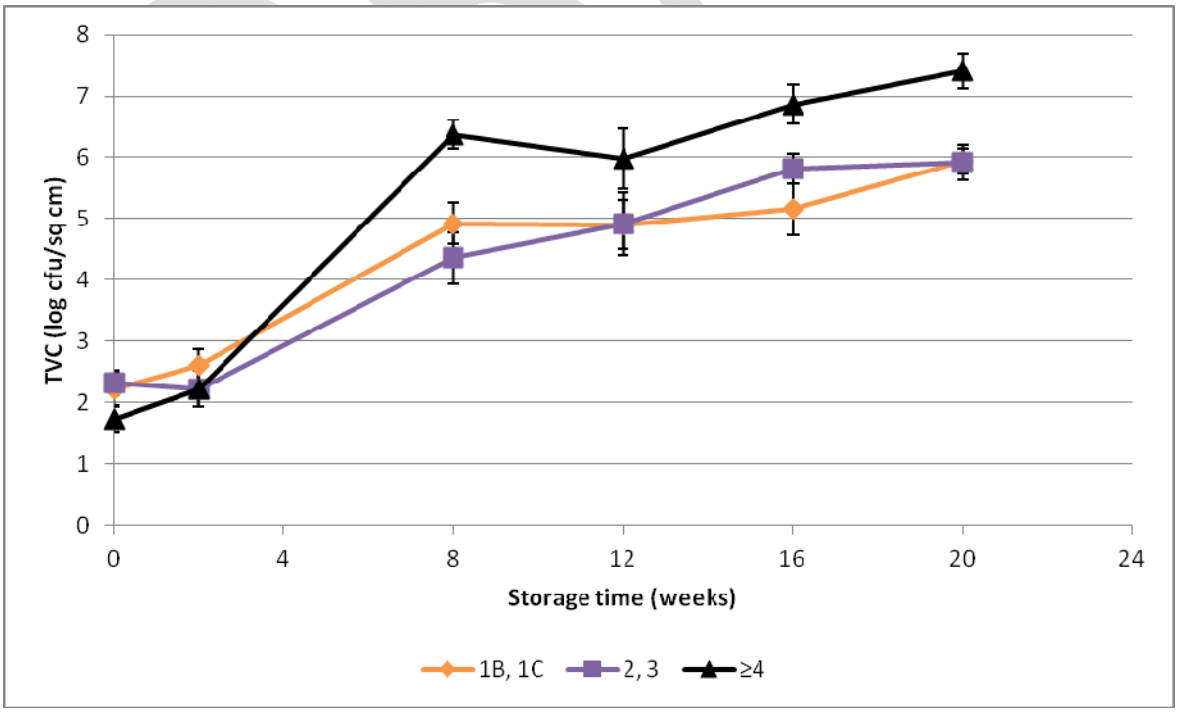


Figure 10: Growth of TVC (mean $\log_{10}$ cfu/cm^2) for each colour score range (error bars show standard error of the means)

It can be seen from figure 11 that pH has a major influence on the TVC growth where samples with a pH of 5.7 and above had a higher growth than on the low-pH meat. The growth rate of the low-pH samples is similar to that of the lighter-coloured samples in figure 10 above because all the high-pH samples were from carcases with a colour score of 4 or higher.

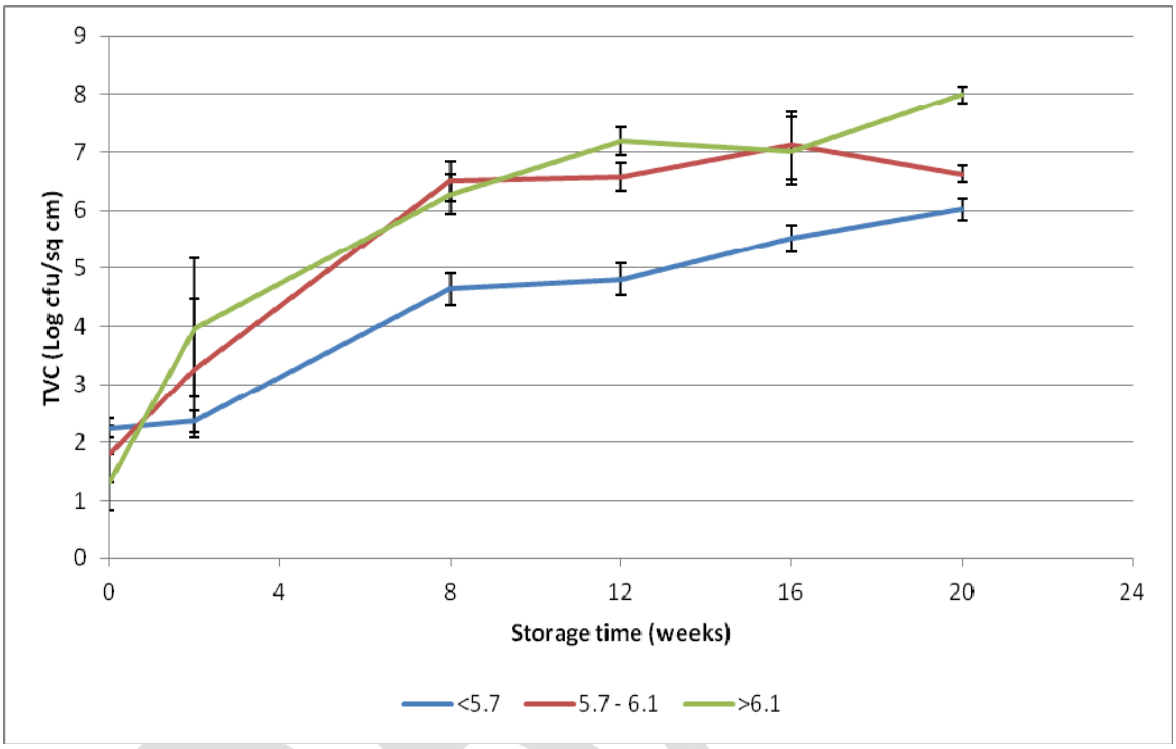


Figure 11: Growth of TVC (mean $\log_{10}$ cfu/cm^2) for samples of different pH range (error bars show standard error of the means)

Lactic acid bacteria (LAB) are normally the predominant organism on vacuum packaged meat. Therefore, their growth as expected followed a similar pattern to the growth of TVC with minimal difference between plants (figure 12) and similarly affected by the meat pH.

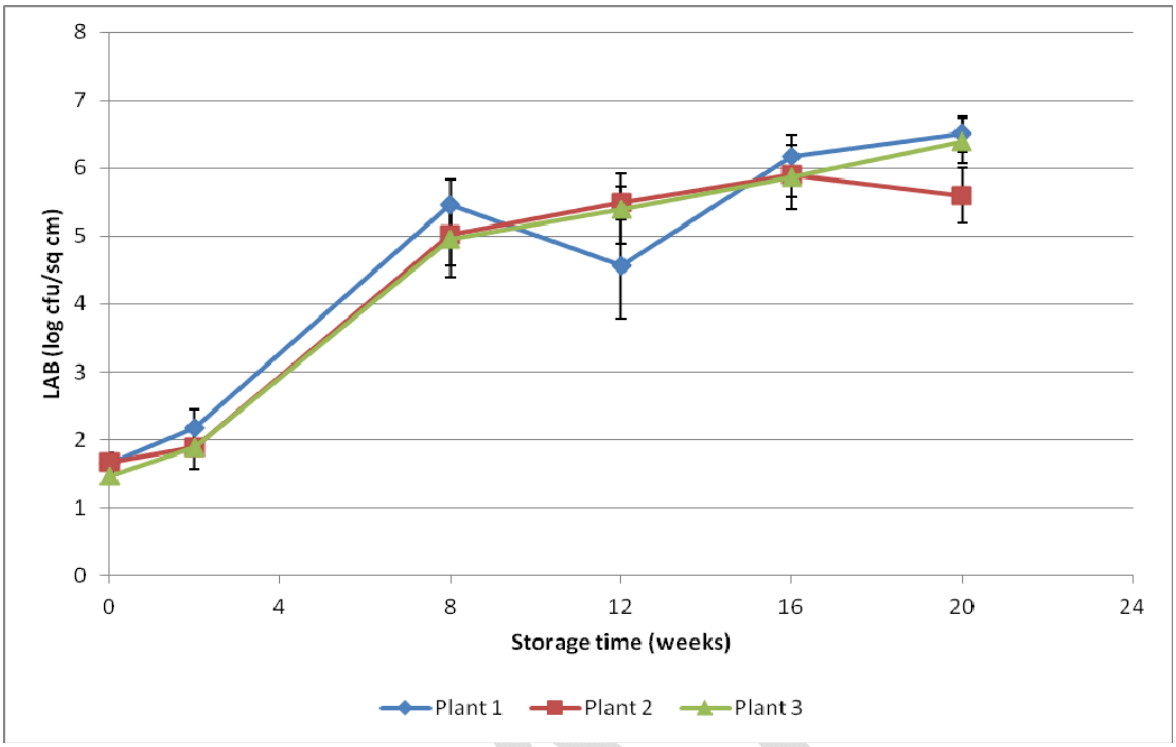


Figure 12: Growth of LAB (mean $\log_{10}$ cfu/cm^2) for the three plants (error bars show standard error of the means)

In contrast to the growth of TVC and LAB, there appeared to be an effect of processing plant on the growth of *Brochothrix thermosphacta*. Figure 13 illustrates that there were much lower number of *B. thermosphacta* isolated from samples from plant 2 than from the other two sites.

Campbell *et al* (1979) reported that *B. thermosphacta* will only grow on vacuum-packaged meat when the pH is 5.8 or lower. The results from these trials indicate that growth still occurred on the normal pH samples but was slower than for the higher pH product particularly for the first 8 to 12 weeks of storage (figure 14).

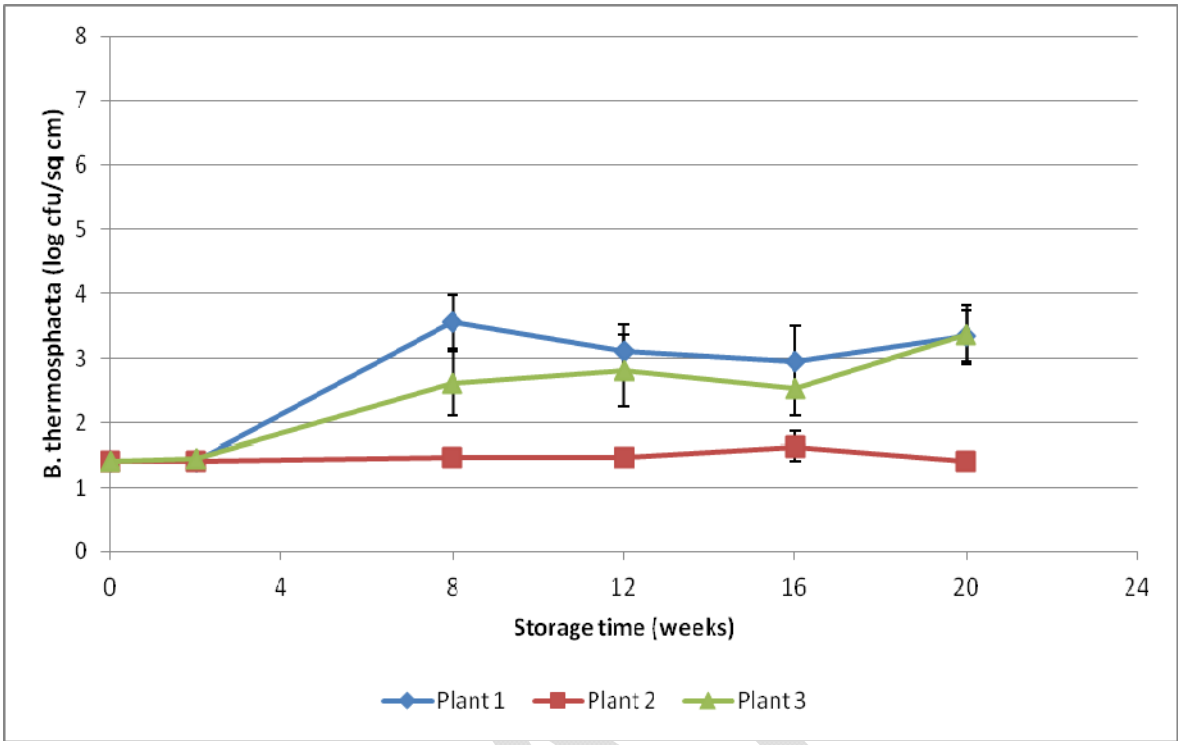


Figure 13: Growth of *Brochothrix thermosphacta* ((mean $\log_{10}$ cfu/cm^2) for the three plants (error bars show standard error of the means)

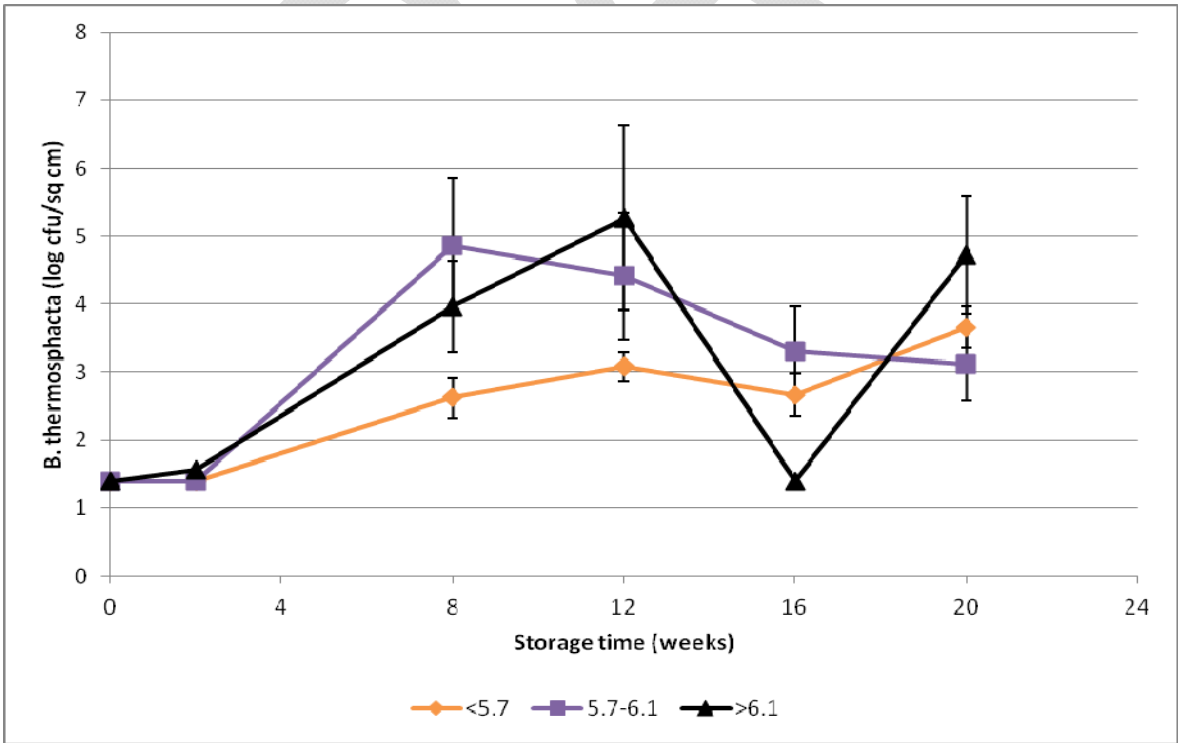


Figure 14: Growth of *Brochothrix thermosphacta* ((mean $\log_{10}$ cfu/cm^2) at different pH ranges (excl. plant 2), error bars show standard error of the means)

3.1.3 Biochemical analyses

3.1.3.1 pH

The mean pH for the vacuum-packed samples was higher during storage by about 0.1 to 0.2 pH units compared with that measured at time zero (Table 1). The range in pH values was much higher for the darkest meat colour group with some samples at each assessment considered to be of normal pH.

Table 1: Mean pH of beef striploin of each colour score group after vacuum storage (range in brackets)

Meat colour	Storage time (weeks)					
	0	2	8	12	16	20
1B, 1C	5.41 (5.36-5.48)	5.53 (5.36-6.65)	5.53 (5.42-5.67)	5.58 (5.52-5.66)	5.60 (5.51-5.70)	5.60 (5.52-5.69)
2, 3	5.42 (5.38-5.44)	5.59 (5.49-5.74)	5.56 (5.43-5.77)	5.63 (5.56-5.82)	5.62 (5.53-5.68)	5.64 (5.54-5.80)
≥4	5.84 (5.38-6.53)	5.89 (5.49-6.44)	5.95 (5.63-6.34)	5.88 (5.57-6.26)	5.90 (5.61-6.59)	5.90 (5.58-6.31)

3.1.3.2 TBARS, L-lactate and total glucose

The mean results for analysis for TBARS, L-lactate and total glucose for each colour group at each vacuum pack opening occasion are presented in Table 2. The L-lactate and glucose levels were lowest for the samples from the darkest meat colour group but changed little through the storage period.

Table 2: Mean values for TBARS, L-lactate and total glucose for each meat colour category after storage times of 0, 2, 8, 12, 16 and 20 weeks at -1°C

	Meat colour	Storage time (weeks)					
		0	2	8	12	16	20
TBARS (mg/kg)	1B, 1C	0.22	0.13	0.18	0.16	0.27	0.26
	2, 3	0.32	0.13	0.14	0.24	0.28	0.29
	≥4	0.15	0.15	0.20	0.15	0.27	0.31
L-Lactate (μmol/g)	1B, 1C	22.05	22.24	20.13	20.26	17.25	21.89
	2, 3	22.43	22.59	21.09	19.85	16.92	21.66
	≥4	19.86	19.45	17.21	17.11	15.08	18.86
Total glucose (μmol/g)	1B, 1C	36.72	41.54	53.04	53.33	51.91	48.32
	2, 3	38.35	43.07	46.39	51.24	49.67	50.96
	≥4	31.23	27.28	20.92	30.52	24.96	33.73

3.1.3.3 Effect of biochemical properties on microbial growth

The main biochemical properties of the meat that affected microbial growth are demonstrated by the correlation coefficients presented in table 2. Week 8 was selected as this is the stage at which microbial growth commenced to plateau. The correlations indicate that the growth of lactic acid bacteria, the predominant organism on chilled meat, is influenced mainly by pH and colour lightness (L*) and redness (a*) and total glucose. Meat colour and pH are highly correlated as expected. Tables of correlations for weeks 2, 12, 16 and 20 are presented in Appendix 5. There are no significant correlations between microbial growth and biochemical properties at 2 weeks but at other storage times, LAB is most strongly correlated with pH and total glucose.

There is no correlation between microbial growth and percent weep in the packs.

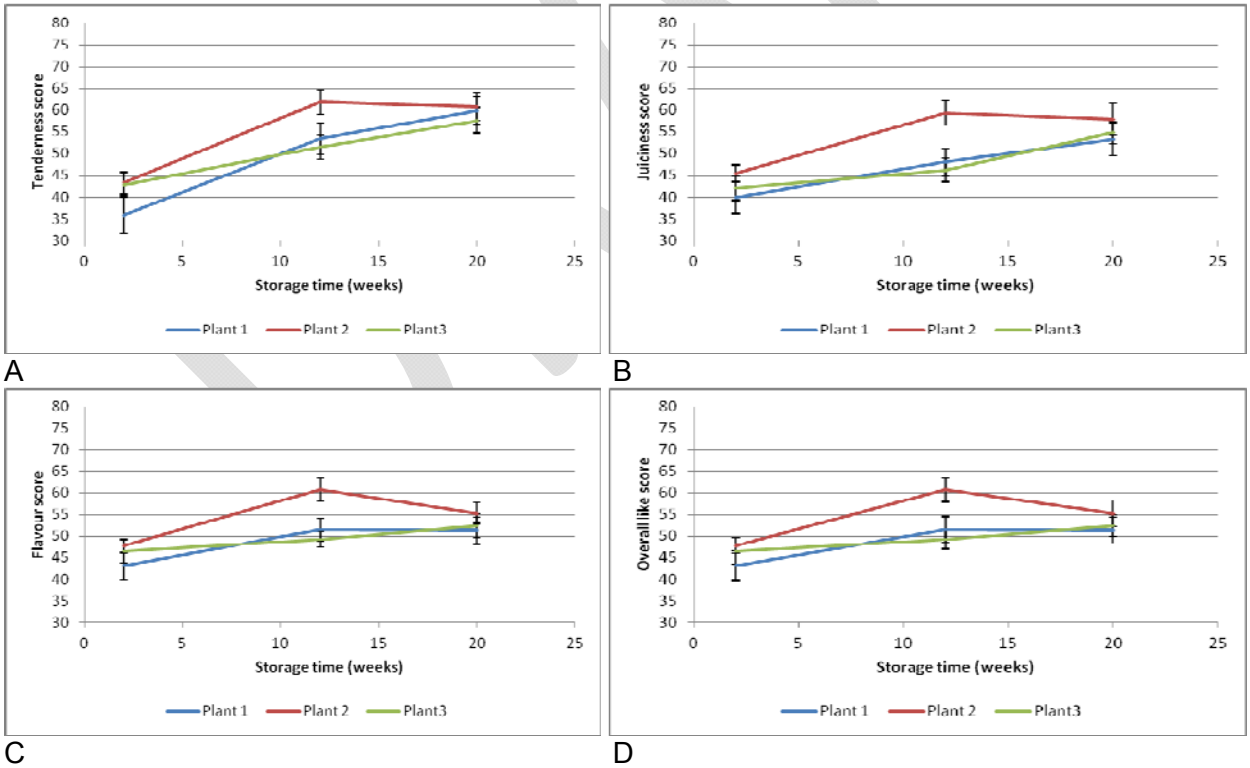
Table 2: Correlation coefficients for chilled beef microbiological and biochemical properties after 8 weeks storage at -1°C

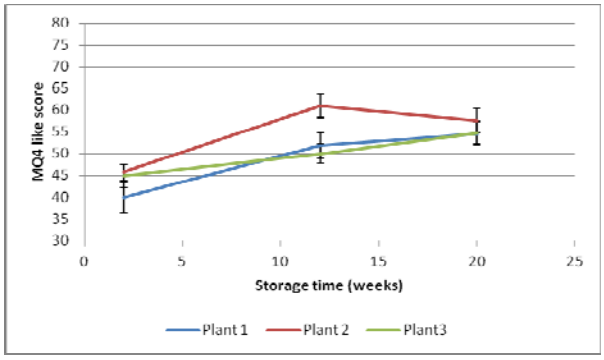
		1	2	3	4	5	6	7	8	9
TVC	1	-								
LAB aerobic	2	0.966	-							
Brochothrix	3	0.533	0.451	-						
Post bloom L*	4	**-0.570**	**-0.570**	-0.335	-					
Post bloom a*	5	**-0.590**	**-0.541**	**-0.579**	0.808	-				
pH	6	**0.559**	**0.548**	0.260	-0.884	-0.798	-	-		
Percent weep	7	-0.176	-0.208	-0.329	0.130	0.146	-0.07	-	-	
Total glucose	8	**-0.556**	**-0.568**	**-0.384**	0.769	0.674	-0.72	-0.2	-	-
L-Lactate	9	-0.271	-0.296	0.194	0.595	0.403	-0.76	-0.2	0.556	-

Correlation coefficients (r) for microbiological properties in bold are significant (p <0.05)

3.1.4 MSA sensory assessment

The results of MSA sensory panel assessment of samples stored for 2, 12 and 20 weeks, indicate that there were improvements in tenderness, juiciness, flavour, overall liking and consequently MQ4 score between 2 weeks and 12 weeks storage but little further change between 12 and 20 weeks. Samples from plant 2 were preferred over that of plants 1 and 3 at 12 weeks of storage but there was little difference at 2 and 20 weeks (figure 15 A-E).





E

Figure 15: Effect of processing plant on MSA scores for A – tenderness, B – juiciness, C- flavour, D – overall liking, and E – MQ4 (error bars shoe standard error of means)

These results are possibly unexpected as there was no difference between plants for other attributes from this study. Samples from plant 1 were all from high quality grain-fed cattle, whereas those from plant 3 were a mixture and 3 were all grass fed. Possibly panellists displayed a preference for the attributes of grass-fed beef.

The colour category of the beef samples had no effect on any of the MSA sensory scores. Dark firm and dry (DFD) beef is variously defined by the pH being above 5.8 (Viljoen *et al* 2002), 6.0 (Yancey *et al* 2005) or 6.2 (Lesiow & Ockerman 1998). Viljoen *et al* (2002) found that consumers were unable to detect differences in sensory attributes of cooked DFD (pH 6.15 – 6.37) and normal-pH steaks. In this study, less than 50% of the samples with a meat colour score of 4 or above had a pH above 5.8, so it is unsurprising that little difference was detected. The results are presented in the Appendix 4.

When the effect of meat pH is considered, there was minimal difference in scores at all storage times for the normal and medium pH samples but the high-pH meat scored higher at all storage times for tenderness and juiciness and other attributes at 2 and 12 weeks. Results are presented in the Appendix 4.

4 Conclusions

Vacuum-packaged beef striploin samples of different meat colour scores from three export plants located in south-east Queensland were stored at -1°C ±0.5°C for up to 20 weeks. They were opened for evaluation after 0, 2, 8, 12, 16 and 20 weeks from packing. The main conclusions from the study were:

- There were minimal differences between plants for appearance of the packs, acceptability of the odour on opening and microbiological quality. Product from plant 2 had the highest scores for MSA sensory assessment and had minimal growth of the spoilage organism *Brochothrix thermosphacta* compared with the other plants.

- No samples exhibited any signs of greening spoilage, although high-pH beef has been implicated in instances of this in the past.

- Samples with a pH above 6.1 were acceptable at 16 weeks but the odour on opening packs at 20 weeks was only marginally acceptable and MSA scores for flavour had declined. All beef samples with a pH below 6.1 were acceptable at 20 weeks.

- Some samples from the group with a meat colour score of 4 and above were of normal pH (~5.4), indicating that either the carcases were incorrectly graded or the darker meat colour was due to some other cause.

- Microbial growth was faster and peaked at a higher level for samples with a pH of 5.7 and above and a dark meat colour. The mean TVC count for samples with a pH below 5.7 was 1.5 – 2.0 $\log_{10}$ units lower than for samples with a higher pH.

- The growth of lactic acid bacteria (the dominant microflora) was most highly correlated to objective measurement of meat colour (L* and a*), pH and total glucose, after 8 weeks storage.

- MSA sensory panellists gave generally increasing scores as storage time increased.

5 Reference list

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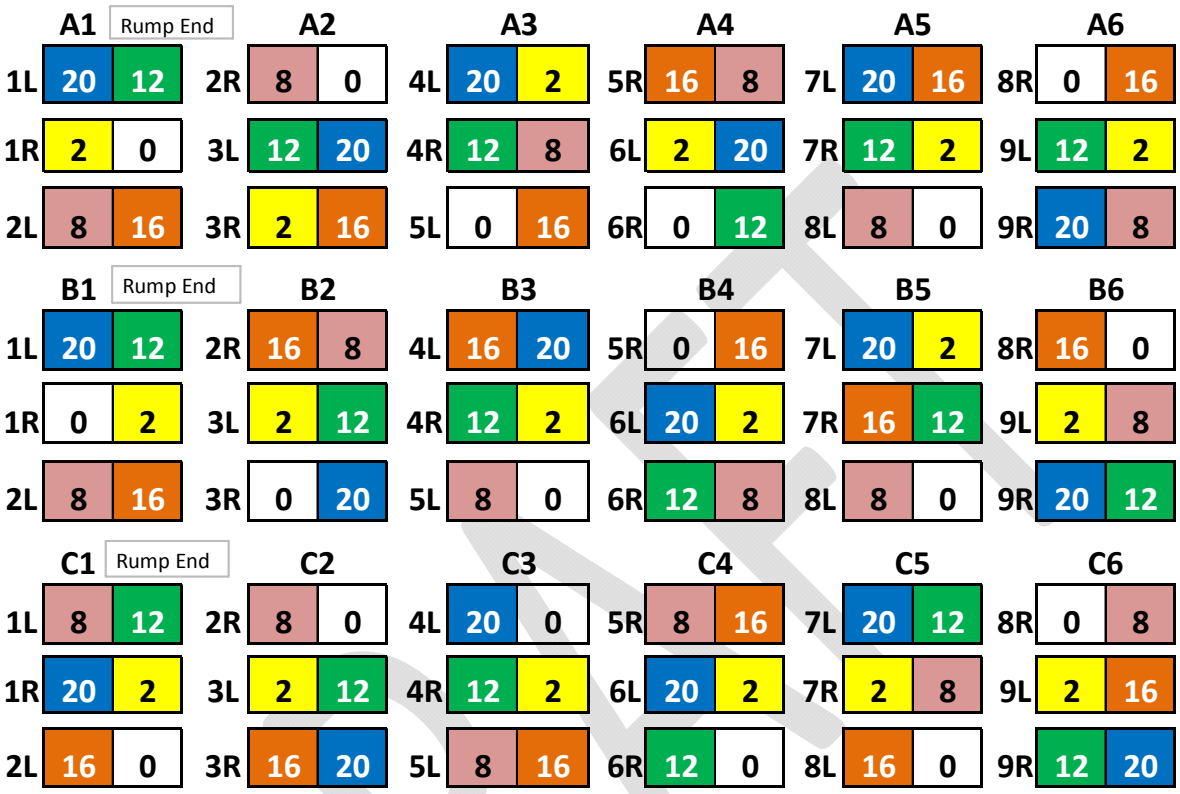
6 Appendices

6.1 Appendix 1: Example of sample collection plan for plant 1



6.2 Appendix 2: Cattle and processing parameters

Table 2-1: Cattle from which meat samples were selected

Plant	Meat colour	Feed	Sex	Dentition	HSCW	HGP implant
1	1B, 1C	100 day GF	M	0-2	303 – 434	Y
	2, 3	100 day GF	M	0-2	314 – 424	Y
	4, 5	100 day GF	M & F	0-7	294 – 359	Y
2	1B, 1C	Grass	M & F	0-4	272 – 408	N
	2, 3	Grass	M & F	2	247 – 446	N
	4, 5	Grass	M & F	0-4	221 – 329	Y
3	1B, 1C	70-day GF	M	0	255 – 323	Y
	2, 3	Grass & grain	M	0-2	273 – 340	Y
	4, 5	Grass	M	2-4	246 – 328	Y

Table 2-2: processing parameters at the three export plants

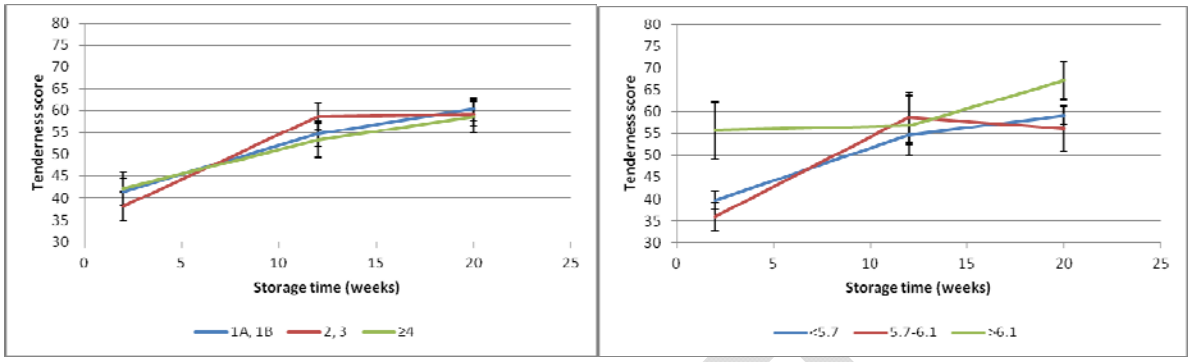
Parameter	Plant 1	Plant 2	Plant 3
Slaughter method	Halal	Halal	Halal
Immobilisation	Y	N	Y
Bleed rail ES	Y	Y	N
Hidepuller stiffening	Y	Y	Y
Intervention	HW decontamination	Nil	HW decontamination
Carcase chilling	Spray	Spray	Spray
Vacuum bag	Cryovac Newteq®	Packsys FME ST	Cryovac Newteq®
Carton chilling	-5°C for 15 h Lids on	-2°C for 24 h Lids off	-1°C for 22 h Lids off

6.3 Appendix 3: Visual and odour evaluation of vacuum packs

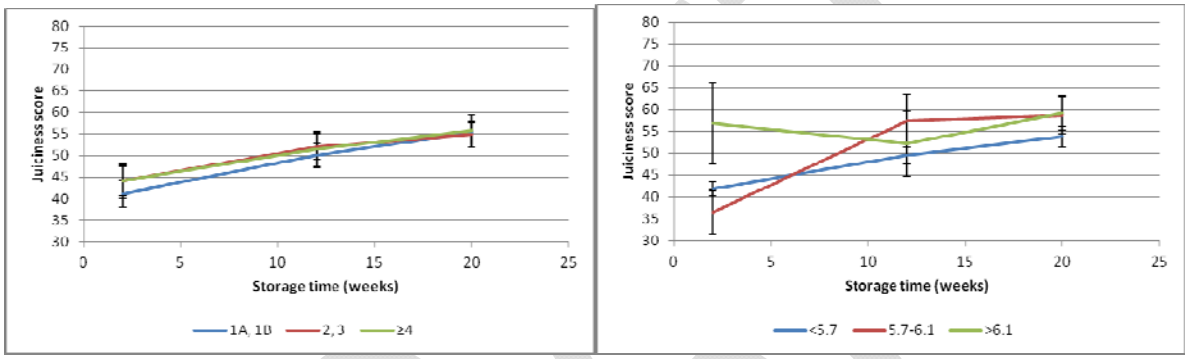
Date: .....

Sample number	Attribute			Comments
	Vacuum 8 = complete vacuum, tight package adhesion 6 = good vacuum 4 = moderate vacuum 2 = poor vacuum 0 = no vacuum, probable leaker	**Appearance** 8 = very fresh, no discolouration 6 = fresh, slight discolouration 4 = good, acceptable 2 = poor 0 = severe discolouration	**Odour** 8 = fresh, no off odour 6 = slight off odour 4 = medium odour 2 = strong off odour 0 = extreme off odour	
		Intact pack	**On opening**	

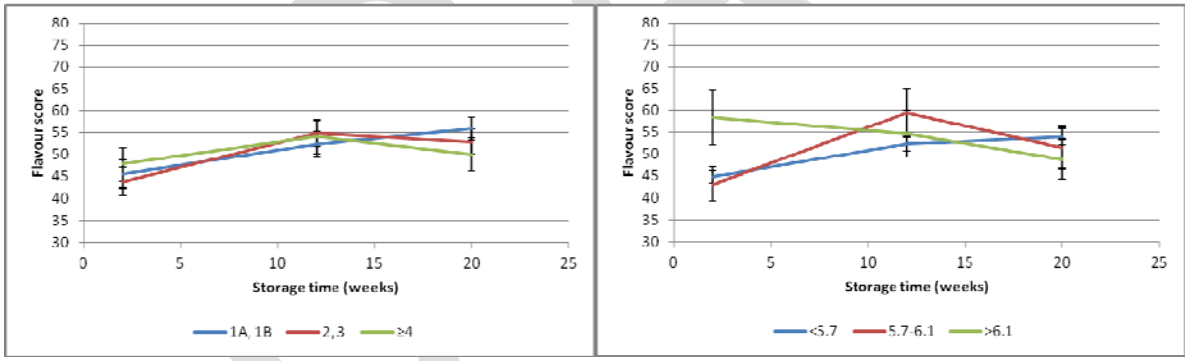
6.4 Appendix 4: Effect of colour score and pH on MSA attributes



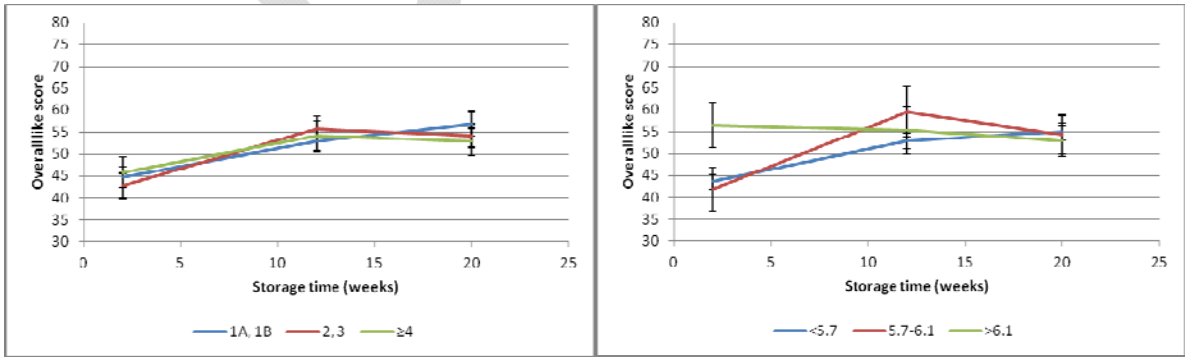
Tenderness



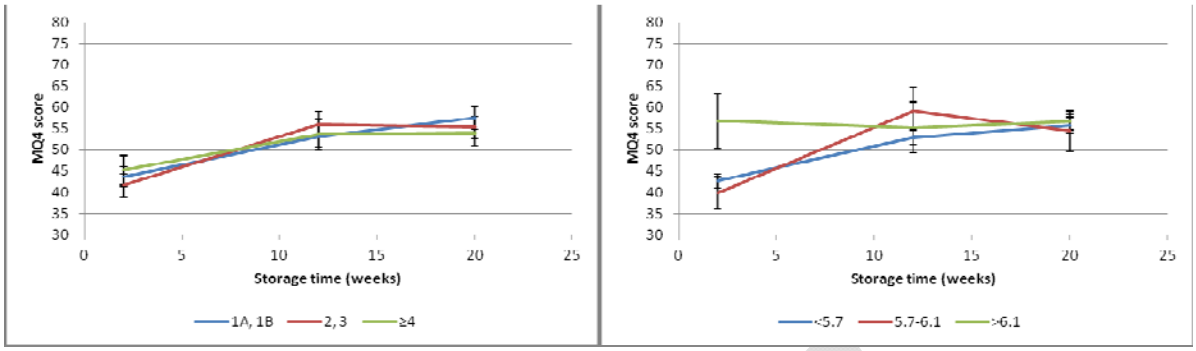
Juiciness



Flavour



Overall liking



MQ4 Score

6.5 Appendix 5: Correlations between microbial growth and biochemical properties of meat

Table 5-1: Correlations after 2 weeks storage at -1°C

		1	2	3	4	5	6	7	8	9
TVC	1	-								
LAB aerobic	2	0.702	-							
Brochothrix	3	0.268	0.424	-						
Post bloom L*	4	0.254	0.016	-0.289	-					
Post bloom a*	5	-0.026	-0.069	-0.163	0.711	-				
pH	6	-0.078	0.217	0.249	-0.852	-0.754	-			
Percent weep	7	0.217	0.010	-0.239	0.365	0.198	-0.433	-		
Total glucose	8	-0.097	-0.197	-0.184	0.535	0.537	-0.594	-0.023	-	-
L-Lactate	9	-0.240	-0.297	-0.349	0.638	0.704	-0.754	0.295	0.520	-

Correlation coefficients (r) for microbiological properties in bold are significant (p <0.05)

Table 5-2: Correlations after 12 weeks storage at -1°C

		1	2	3	4	5	6	7	8	9
TVC	1	-								
LAB aerobic	2	0.975	-							
Brochothrix	3	0.587	0.487	-						
Post bloom L*	4	**-0.402**	-0.354	-0.342	-					
Post bloom a*	5	**-0.466**	-0.353	**-0.699**	0.577	-				
pH	6	**0.587**	**0.518**	**0.496**	-0.883	-0.744	-			
Percent weep	7	-0.145	-0.124	-0.233	0.350	0.368	-0.395	-		
Total glucose	8	**-0.527**	**-0.493**	-0.148	0.586	0.333	-0.661	-0.011	-	-
L-Lactate	9	**-0.472**	**-0.430**	-0.336	0.780	0.570	-0.893	0.301	0.674	-

Correlation coefficients (r) for microbiological properties in bold are significant (p <0.05)

Table 5-3: Correlations after 16 weeks storage at -1°C

		1	2	3	4	5	6	7	8	9
TVC	1	-								
LAB aerobic	2	0.952	-							
Brochothrix	3	0.361	0.319	-						
Post bloom L*	4	**-0.622**	**-0.669**	0.032	-					
Post bloom a*	5	**-0.568**	**-0.572**	-0.189	0.644	-				
pH	6	**0.603**	**0.612**	-0.121	-0.845	-0.785	-			
Percent weep	7	-0.159	-0.067	-0.234	0.101	0.247	-0.318	-		
Total glucose	8	**-0.672**	**-0.715**	-0.026	0.827	0.483	-0.696	-0.081	-	-
L-Lactate	9	-0.270	-0.369	0.133	0.505	0.500	-0.654	-0.025	0.430	-

Correlation coefficients (r) for microbiological properties in bold are significant (p <0.05)

Table 5-4: Correlations after 20 weeks storage at -1°C

		1	2	3	4	5	6	7	8	9
TVC	1	-								
LAB aerobic	2	0.901	-							
Brochothrix	3	0.440	0.397	-						
Post bloom L*	4	**-0.600**	**-0.419**	-0.114	-					
Post bloom a*	5	**-0.720**	**-0.594**	**-0.435**	0.723	-				
pH	6	**0.731**	**0.596**	0.306	-0.894	-0.850	-			
Percent weep	7	-0.260	-0.176	-0.380	0.274	0.370	-0.296	-		
Total glucose	8	**-0.446**	-0.329	-0.083	0.604	0.449	-0.634	-0.044	-	-
L-Lactate	9	**-0.537**	-0.342	-0.064	0.714	0.505	-0.753	0.092	0.585	-

Correlation coefficients (r) for microbiological properties in bold are significant (p <0.05)