FINAL REPORT

AUSTRALIAN MEAT PROCESSOR CORPORATION

Meat Industry Services

Shelf-life: Improving Beef Colour

Project code:	A.MIS.1002
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Abstract

Meat colour has important implications for consumer acceptability and eating quality, with dark carcases incur economic penalties, and thus negatively impact the meat industry. This study aims to investigate the effect of meat colour at grading on 20 week shelf-life of chilled vacuum packed beef striploins by segregation into one of 3 colour groups (A-light, B- intermediate or C-dark) as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. With storage, all meat colours displayed higher values for lightness, purge and lipid oxidation. Colour stability during retail display was not affected. In conclusion, meat colour at grading displayed differences in pH, purge, carbohydrate content, myoglobin forms. No differences were observed in protein solubility or oxidation.

Executive summary

Meat colour has important implications for consumer acceptability and eating quality. Sensory studies have correlated dark colours with a low desirability score (Jeremiah, Carpenter et al. 1972). The highest level of desirability is associated with beef loins that are a pink or pale red colour. Consumers have described such steaks as “meatier” and darker steaks from various muscles have also been described as having an “off- flavour” and can be “peanutty”, “sour” and “bitter” in comparison to normal steaks (Jeremiah, Aalhus et al. 1997; Wulf, Emnett et al. 2002).

In addition, if meat colour specifications are not met, carcases can incur economic penalties, and thus negatively impact both processors and producers financially. Nationally, this figure is estimated to be in the region of $35 million per annum, with some industry sources describing the penalty for a dark cutting to be approximately $400/ carcase. Hence, optimising the colour of beef muscle is an extremely important topic to consider for both the consumer and the economic impact on the producer.

This study aims to investigate the effect of meat colour at grading on the biochemical properties of beef striploins (Longissimus lumborum). In addition, this study will determine the effect on shelf-life of vacuum packed striploins chilled stored for up to 20 weeks. The relationship of beef meat colour at grading colour on colour stability during retail display will also be investigated.

A total of 160 beef striploins were collected from 3 plants across south-east Queensland and were segregated into one of 3 colour groups (A-light, B- intermediate or C- dark) as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark -0.5°C +/- and sampled at either 0, 2, 8, 12, 16 or 20 weeks. Also at 2, 12 and 20 weeks, retail colour stability was also assessed for a period of 12 days.

At collection, colour measurements are consistent with expectations of the lower AMC scores ≤1C (colour group A) having a lighter, more red appearance and the higher AMC scores ≥4 (colour group C) having a darker, less red appearance. In addition, a comparison of carcase side with different bloom times revealed shorter bloom times were associated with a darker colour (lower lightness values) and lower redness.

Initially, biochemical properties (myoglobin and tocopherol content, protein oxidation and solubility) were measured. No significant differences were observed between colour groups for protein oxidation or solubility. Additionally, no significant volatile compounds were detected in any sample.

Retail display at weeks 2, 12 and 20 revealed meat colour group A had significantly higher ($P<0.001$) lightness (L*) values and meat colour group C had lowest values with colour group B being intermediate. In terms of colour stability with time, storage week 2 appeared to have the longest colour stability. Estimated retail display life for acceptability for storage weeks 2, 12 and 20 were 6, 4 and 3 days respectively. Between colour groups, in terms of colour stability over the display time, no major differences were observed. TBARS across all the storage weeks and 3 storage days (0, 6 and 12) colour groups appeared to have similar values and increased over the retail display timeframe.

At each time point, Hunterlab colour (L*, a*, b* and spectral scan 400-700nm), pH, purge, glucosyl, lactate and TBARS were measured. At all time points, meat colour group C (AMC≥4) displayed lower L*, reflectance, lactate values and higher redness and pH values. With storage, all meat colours displayed higher values for lightness, purge, glucosyl and lactate. Lipid oxidation also seemed to increase with storage time.

Between colour groups, spectral scan data exposed differences in myoglobin forms. The lighter colour group A had higher levels of red oxymyoglobin, whereas the darker colour group C showed higher levels of the purple deoxymyoglobin.

In conclusion, from the data generated in this study meat colour at grading displayed differences in pH, purge, carbohydrate content, myoglobin forms. Colour stability during retail display was not affected.

Contents

1 Background

Problems with the colour of red meat have important implications for consumer acceptability, eating quality and economics of the industry. Consumers are seeking an attractive red colour, and show less preference for steaks that are either extremely dark nor extremely pale, with dark colours in particular showing a low desirability score (Jeremiah, Carpenter et al. 1972). In addition, a dark meat coloured carcass is estimated to cost approximately $50-250/ carcass (industry sources). Thus, the national impact of downgrading carcasses (from industry source values) is estimated to be in the region of $15 to 75 million annually. This has a detrimental impact on one of Australia's largest domestic and export industries.

Meat Standards Australia (MSA) have optimised a grading system known as the chiller assessment language (King 2005) where qualified graders assess the carcass for traits such as meat colour. Carcasses are quartered and allowed to bloom for at least 30 minutes at a low temperature (≤12 °C). The *longissimus thoracis* (striploin muscle) at the $10^{th}/11^{th}$ rib or at the $12^{th}/13^{th}$ rib is graded using colour chips on a colour scale (see figure 1). The scale ranges from 1A to 7 scored by the most predominant colour present in the muscle surface. Carcasses are considered non-compliant and unacceptable by MSA if the colour of the striploin is not in the range of 1B to 3. Colour score 1A is considered to be pale, soft, exudative (PSE) or heat toughened and scores ≥4 are known as "dark cutters" (DC). Dark cutting meat is a considerable difficulty for processors due to unacceptability of colour.

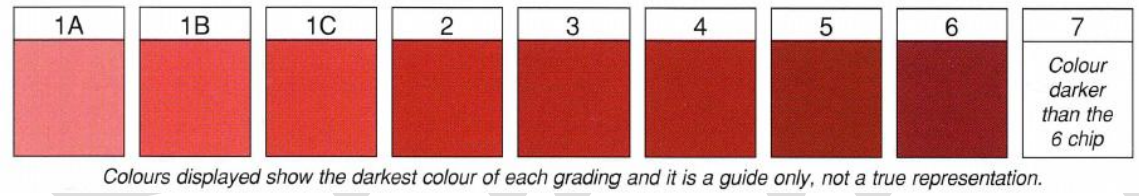


Figure 1. The AUS-MEAT colour scale (King 2005) used for meat colour assessment on beef longissimus muscle.

During early post mortem degradation of the muscle, biochemical mechanisms contribute to the differences observed in the meat quality and overall colour. It is proposed that these differences could influence the shelf life of vacuum packs, especially when stored for export markets, which could be long periods of time (>12 weeks). A previous report (A.MFS.0166) demonstrated that vacuum packed primal could be stored confidently for 26 weeks or more, provided the appropriate conditions were obtained. Thus, this report aims to determine the influence of beef meat colour at grading on the shelf-life of vacuum packed meat. This is relative to the biochemical properties and colour stability during retail display. Recognition and identification of the effect of meat colour on vacuum packed meat is essential to developing solutions to these meat colour issues.

2 Project objectives

The project aims are as follows:

1. Investigate the influence of beef meat colour at grading in terms of the biochemical properties of the meat.
2. Determine the effect of beef meat colour at grading on the shelf-life of vacuum packed striploins chilled stored for up to 20 weeks.
3. Investigate the interaction of beef meat colour at grading on retail display.

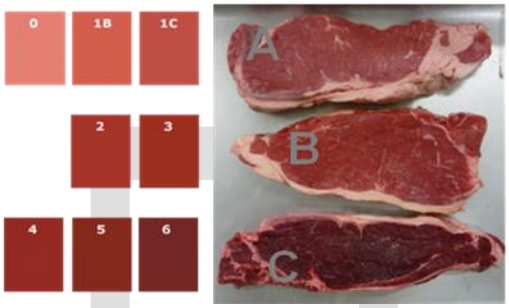
3 Methodology

3.1 Week 0 Collection

3.1.1 Sample collection & colour measurement

During autumn, three plants were visited within south-east Queensland within 3 consecutive days. One plant had to be revisited within the week to obtain all samples. A total of 160 striploins or longissimus thoracis were collected from 80 beef carcases between 1 to 3 days post-mortem. The animals were between 0 to 7 tooth and were either grass or grain fed. Muscles were graded on the right side of the carcase by a qualified assessor using the AUS-MEAT specifications and were segregated to one of 3 colour groups:

Group A: meat colour ≤ 1C
Group B: meat colour 2 and 3
Group C: meat colour ≥ 4



Colour measurements (L* a* b* values and spectral scan from 400-700 nm) were also measured at approximately 10°C. This was conducted using a Hunterlab Miniscan EZ (light source A, observer angle 10°, aperture size 5 cm). Other colour parameters were calculated below (see section 3.4). The left side of the carcase was also quartered and left to bloom for between 30 to 60 minutes prior to measurement. This freshly bloomed side was used to compare those measurements made by the qualified assessor.

After boning, the muscles were halved and randomly allocated to one of 6 time points; 0, 2, 8, 12, 16 or 20 weeks. Muscles were vacuum packed and were placed in cartons and subjected to plant standard packing and chilling regimes. Apart from week 0 samples, cartons were delivered to the laboratory using a chilled storage vehicle and stacked on a pallet in the dark at -0.5°C +/- 0.5°C for the allocated time frame (see image 1).

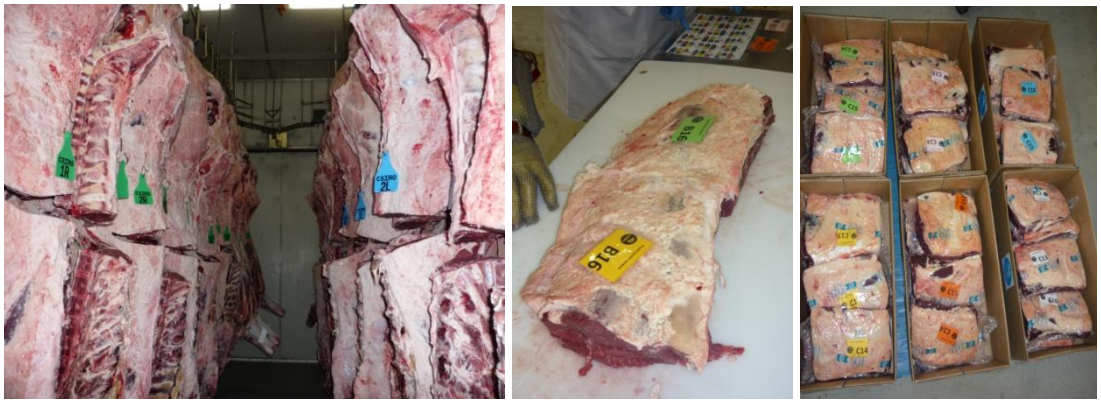


Image 1. Far left: carcase identification and tagging. Centre: striploin halves were labelled and allocated into cartons for transport (far right).

Week 0 samples (54 striploin halves) were transported back to the laboratory in eskies and processed on the same day. Core samples were taken for microbiological analysis from 27 striploin halves (as part of a secondary experiment- schedule 4). Sub-samples were taken from

all samples for protein extraction, lipid oxidation and glucose content and were frozen using liquid nitrogen and subsequently stored at -80°C until analysis could be completed. Steaks for retail display were also cut at weeks 2, 12 and 20 only. The pH, purge and colour were measured (see section 3.4).

3.2 Week 0 Analysis

3.2.1 Myoglobin content

Myoglobin content of the muscle samples was determined spectroscopically (Krzywicki 1982; Trout 1989) on week 0 samples only for standardisation. Muscle samples (5g) were homogenised over ice in 25ml ice-cold 40mM potassium phosphate buffer (pH 6.8) using an Ultra-turrax (13,500rpm, 10 seconds). Homogenates were placed on ice (4°C, 60 minutes) and subsequently centrifuged at 5,000 rpm (4°C, 30 minutes). Supernatants were filtered (whatman #1) and absorbance was measured using a Cary spectrophotometer at 525, 572 and 700nm with phosphate buffer as a blank. The concentration of myoglobin (mg/ml) and percent of metmyoglobin was calculated from the absorbance values.
α- tocopherol content

3.2.2 α- tocopherol content

The α- tocopherol content of muscle was measured using the method of Liu and Scheller with modifications (Liu, Scheller et al. 1996). Duplicate samples of minced muscle (0.5g) were weighed into glass test tubes. Each tube received addition of 0.25g of ascorbic acid and 2mls of 20% potassium hydroxide dissolved in HPLC grade methanol. Tubes were flushed with nitrogen and saponified at 65°C for 30 minutes in a shaking water bath. After 15 minutes in the water bath, samples were removed, 6mls of distilled water was added to the tube and contents were homogenised using a glass homogeniser. After homogenising, samples were immediately returned to the waterbath to complete the incubation period. Once homogenates were removed from the waterbath and had cooled, 8mls of diethyl ether was added and vortexed for lipid extraction. The ether extract was removed into a second set of test tubes and the ether extraction was repeated. Extracts were combined and subsequently washed twice with 8mls of distilled water. Excess water was removed from the extracts by addition of 1-2g of sodium sulphate. Extracts were then transferred into scintillation vials and were dried under nitrogen at 37°C. Dried extracts were then resuspended in 1ml of ethanol, filtered (0.45µm filter) and injected (20µl) onto a Waters C18 resolve guard column. A two solvent gradient program was run at 2ml/min using a 20 minute run time (A: 97% methanol, B: 100% methanol, 0-2mins 100% B; 2-19mins 100% A; 19-20mins 100% A). An α-tocopherol standard (sigma, T3251) was prepared to approximately 4µg/ml and both standard and samples were measured using a fluorescence detector (excitation 295nm, emission 325nm). The α-tocopherol content of samples was expressed as µg tocopherol/ g of sausage batter.

3.2.3 Protein extraction & solubility

Muscle samples (from plants 2 and 3 for storage week 0 only) were removed from -80°C storage and tempered for approximately 30 minutes. Duplicate 2g samples were extracted and the total and sarcoplasmic proteins were extracted (Warner, Kauffman et al. 1997). The protein concentration (mg/g) of total and sarcoplasmic protein fractions was determined spectroscopically using the Biuret method with bovine serum albumin as a standard (Gornall, Bardawill et al. 1949). The myofibrillar proteins were purified (Warner, Kauffman et al. 1997) and subsequently solubilised in a 1.1M NaCl, 0.1M $NaHPO_4$ (pH 7.2) for protein concentration determination. Proteins were prepared to 2mg/ml in this buffer and then were either diluted 1:1

with a final sample buffer (Laemmli 1970) with or without dithiothreitol to generate reducing or non-reducing conditions respectively. The protein (10ug) was loaded onto a Bio-Rad criterion Tris-HCl gel (4-20% linear gradient & 5% resolving gel, catalogue # 345-0027 and 345-0001 respectively) using a broad range molecular weight standard (6.5 to 200kDa, catalogue # 161-0317) for SDS-PAGE. Images were captured and viewed using a Bio-Rad GS-800 densitometer using Quantity One software for analysis.

3.2.4 Carbonyl content

Carbonyl groups in myofibrillar extracts were measured using OxiSelect™ Protein Carbonyl ELISA Kit (Jomar Bioscience Pty Ltd, product number STA-310) using the manufacturers recommended procedure. Duplicate 10µl extracted myofibrillar preparations (100µg/ml) were added to each well, and incubated overnight at 4°C. After washing, wells were treated with 0.04mg/ml 2, 4 dinitrophenylhydrazine or DNPH (Oliver, Ahn et al. 1987) and incubated at room temperature in the dark (45 mins). After washing, blocking and incubation with primary (anti-DNP) and secondary (horseradish peroxidise conjugate) antibodies, the absorbance of the reacted protein hydrazones was measured spectroscopically at 450nm. The concentration of carbonyl groups (nmol of DNPH fixed/mg of protein) was calculated using bovine serum albumin (BSA) as a standard.

3.2.5 Sulphydryl content

Extracted myofibrils (1mg/ml) were analysed for total sulfydryl content using 5,5-dithiobis-2-nitrobenzoic acid or DTNB reagent (Ellman 1959). A stock solution of cysteine (10mM) was diluted in the range of 15.6 to 1000µM was used to verify the linearity of the calibration curve at 412nm. The concentration of sulfydryl groups (moles/ 10^5 g of protein) was calculated using the standard curve.

3.2.6 Volatile analysis

The frozen samples were transported from Coopers Plains to Werribee and stored at -80 °C until required for analysis. Prior to analysis, the meat sample was removed from storage and, while frozen, was finely sliced (slice thickness – 1 mm maximum). A portion of the meat (5 g) was placed into a 20 ml headspace and sealed with a Teflon®/steel cap. Each sample was measured in duplicate. The volatile compounds in the headspace were sampled using solid-phase microextraction (SPME) with a CombiPAL autosampler. The vial and its contents were heated at 70 °C for 2 min when a 50/30 µm Carboxen®/polydimethylsilicone/divinylbenzene SPME fibre was inserted into the headspace and held for 30 min. The fibre was withdrawn and inserted into the injector of an Agilent Model 6890 gas chromatograph. The injector was in the splitess mode and heated at 230 °C. The volatile compounds were separated using a HP-VOC capillary column (length = 60 m, diameter = 320 µm, inner diameter = 1.8 µm). The column oven was initially held at 40 °C for 2 min and heated to 220 °C at a rate of 5 °C min^{-1} and then held for a 7 min. Helium was used as the carrier gas (flow rate = 1.9 ml min^{-1}). The interface was held at 280 °C. The mass spectrometer operated in the scan mode, acquiring data from 35 to 350 amu. The acquisition was performed in electron impact mode (70 eV). The response of the mass spectrometer was optimised using the auto-tune function. Identification of the volatile profile was made by comparison of the mass spectra of the unknown with those in a commercial mass spectral database.

3.3 Week 2, 12 and 20 analysis

3.3.1 Retail display

After steaks were bloomed and colour measured, they were transferred into black snopak foam trays (205 x 130 x 12mm) were wrapped with cling wrap and photographed using an Olympus

digital SLR camera, model number E330, under 4 light struts angled at +/- 45° to the sample surface. Samples from each colour score group (A, B & C) were then placed on the top, middle and bottom shelves perpendicular to the light source (see image 2). The light source in the cabinet was illuminated 24 hours a day and provided by 3 light tubes (Osram Natura L 36W/76) which were on the top and sides of the interior of the cabinet. These provided the highest intensity of light on the top shelf (1081 lux), medium level of light on the middle shelf (between 350-721 lux) and lowest intensity on the bottom shelf (290 -474 lux). Steaks from each colour group were rotated between top, middle and bottom shelves on a daily basis.

Steaks were stored for a maximum of 12 days and at each day photographs were taken (as above) and colour measurements as previous. At the end of the storage time, steaks were cut in half transversely and photographed through the depth of the steak to view the depth of browning.

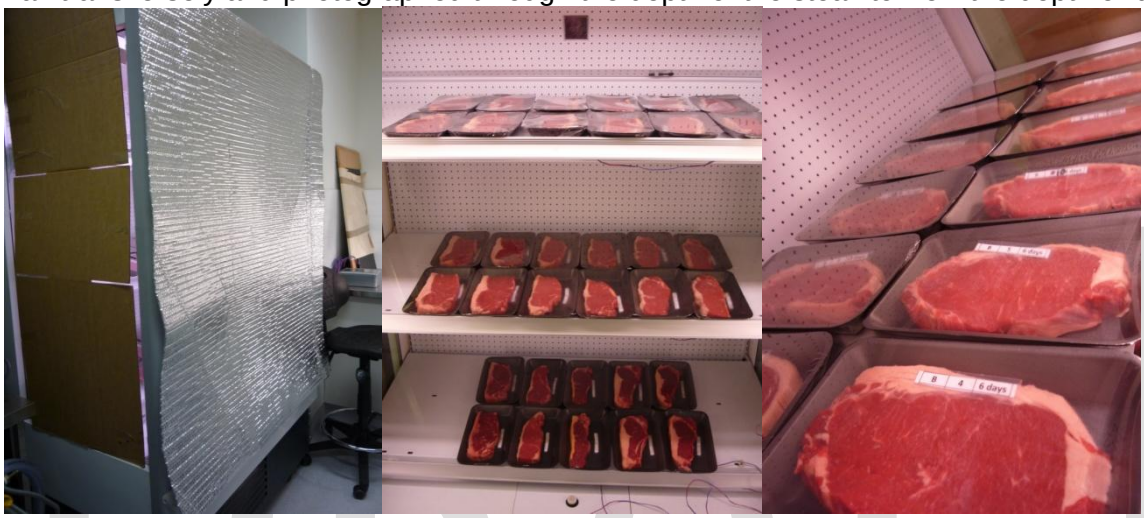


Image 2. Far left: retail display cabinet covered to minimise interference from external light. Centre: cabinet containing steaks across all 3 shelves and top shelf (far right).

3.3.2 Retail display TBARS

The concentration of thiobarbituric reactive species (TBARS) was determined from day 0, 6 and 12 in the retail display samples from weeks 12 and 20 only (Witte, Krause et al. 1970). Week 2 retail display samples were only measured at day 12. 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 530nm, using 1,1,3,3- tetraethoxypropane as a standard.

3.4 All weeks (0, 2, 8, 12, 16 & 20) analysis

3.4.1 Colour measurement

Triplicate colour measurements (L* a* b* values and spectral scan from 400-700 nm) were measured at approximately 10°C. This was conducted using a Hunterlab Miniscan EZ (light source A, observer angle 10°, aperture size 5 cm). This was conducted on a freshly cut 25mm steak after 60 minutes blooming at 5°C.

Colour parameters were measured as follows:

- hue = [arctangent (b/a)]. Distinguishes colour families e.g. red, green, blue etc
- chroma = $(a^{*2} + b^{*2})^{1/2}$. Indicates degree of saturation.
- R630/580 = reflectance @ 630nm/ reflectance @ 580nm. Indicator of redness.

In addition, the relative proportions of each myoglobin form (MMb – metmyoglobin; DMb – deoxymyoglobin; OMb- oxymyoglobin) were calculated as follows (Krzywicki 1979):
Reflectance (R) was converted to reflex attenuance (A) using equation 1:
Equation 1: A =log 1/R
Where R was the reflectance at a specific wavelength expressed as a decimal fraction.
Equation 2: % MMb = (1.395 – ((A572 –A700)/(A525 – A700))) * 100
Equation 3: % DMb = (2.375 * (1-((A473 – A700)/(A525 – A700)))) *100

Equation 4: % OMb = 100 – (%MMb + % DMb)

3.4.2 Purge

The purge for each striploin was calculated based on a weight loss basis only. Samples were weighed prior to opening and post microbiological samplings. The weight of empty bag, drip keeper and microbiology samples were subtracted from initial weight. The weight difference or purge (g) was expressed as a percentage of the initial weight, as follows:
Purge (%) = [(wt striploin with drip /wt striploin no drip)/ wt striploin with drip] * 100

3.4.3 pH

The pH of the samples was measured at the sampling week, as was for the procedure for week 0 samples. This was completed using a TPS Model WP80 pH meter fitted with an Ionode IJ44 combination pH probe.

3.4.4 Thiobarbituric reactive species (TBARS)

The concentration of thiobarbituric reactive species (TBARS) was determined in 27 striploin samples from each time point (Witte, Krause et al. 1970) as outlined in section 3.3.2..

3.4.5 Glucosyl content

The protein fraction of frozen muscle samples (2g) was removed by homogenisation (1:10 w/v) in 30mM HCl using an Ultra-turrax 22,000rpm for 2 x 15 second bursts. Samples were centrifuged (3,000 rpm, 4°C, 10 minutes) and supernatants containing free glucose and glycogen were frozen -20°C until assay could be performed. Thawed samples were analysed for total glucosyl units by incubating 50µl (37°C, 90 minutes) with the addition of 500µl of hydrolysing enzyme amyloglucosidase (1:200 in 40mM acetate buffer pH 4.8). The concentration (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 540nm.

3.4.6 Lactate 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).

3.5 Statistical analysis

Data analysis was completed using Genstat 15th edition. Restricted maximum likelihood (REML) was used for comparison between treatments. Variates for the 630/580 ratio, L*,a* and b* values were compared in the model, using a fixed treatment structure (colour group* storage week) and a random experimental structure of plant/colour group/carcase/side/orientation from the rump

end. For retail display analysis, the model was reconfigured to take into account the day of retail display and tray (fixed treatment: colour group*day and random structure:plant/colour/tray/day). Least standard intervals (LSI) were generated to graphically display differences between colour groups.
Means provided in the analysis are thus predicted means generated from the F-test and similar for the approximate probability values, expressed as F Pr. Approximate probability values were considered to be highly (F Pr <0.001), statistically (F Pr <0.05) or non-significant (F Pr ≥0.05). Standard error of differences (s.e.d) with appropriate numbers of degrees of freedom (df) were reported to describe differences between treatments.
When data variates appeared to be heteroscedastic, a natural log transformation was used in the REML model, and subsequently means were back transformed and reported. Such variates are reported as such.

4 Results and discussion

4.1 Week 0 collection

4.1.1 Carcase data

Cattle were either grass or grain fed depending on the availability at the plant on the day of collection. Grain fed animals had all been fed for 100 days. Animals from plant 3 had Brahman content (Brahman, santa, euro cross), and were recorded as having a hump height of between 45 to 90 mm. Other animals from plant 3 were Angus, shorthorn or Angus, Hereford. Other animals were Santa cross, charolats cross or char cross bullocks (plant 1), however breed data was not available at plant 2. MSA data and vendor information were collected from plant 3 only, as other plants were unable to obtain this data. This meant only specific meat colour scores were obtained from plant 3.

The carcases from each plant were selected based upon the animal grade and dentition score and are summarised in table 1. The majority of animals were steers, although there were a couple of bulls and ox from plant 1 and a couple of females (0-2 tooth) from plant 2. All animals from plant 1 were between 0 to 7 tooth, and from the other 2 plants were 0 to 4 tooth. Fat depth, as measured at the rib site where carcass was quartered, ranged between 6 to 22 mm, with the lowest values being observed in the grass fed animals from plant 2. The carcass weight for this group of animals was also slightly lower compared to the other plants.

Table 1: Carcase data collected from 3 different plants (1, 2 and 3).

Plant	1	2	3	1	2	3
	mean			range		
carcass weight (kg)	348.9	294.5	300	294 to 434	221 to 440	255 to 331
fat depth (mm)	14.6	10.7	14.9	10 to 22	6 to 20	9 to 21
dentition	1.7	2.5	1	0-7	0-4	0-4
feed	grain	grass	grain & grass			

4.1.2 Plant parameters

Some of the processing parameters for each plant are summarised in Table 2. All plants were using the halal slaughter method, using the hidepuller stiffening and spray chilling the carcases. Plant 2 was not using the immobiliser or hot water decontamination unit and plant 3 was not using the bleed rail electrical stimulation. The vacuum bag specification is also highlighted and it appeared that all the carton chilling regimes were below -1°C for at least 15 hours or more. All of these parameters should be contemplated when considering the condition of the tissue.

Table 2. Processing parameters from 3 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

4.1.3 Colour of the carcase side

AUS-MEAT colour assessment was conducted as per standard plant protocol using the left side of the carcase approximately 8 hours prior to our arrival onsite. Due to the long bloom time, we requested the right side of the carcase be cut fresh and we conducted colour measurements after approximately 60 minutes bloom. A summary of the measurements can be observed in table 3. As mentioned previously, only plant 3 was able to provide the full Meat Standards Australia (MSA) grading assessment information, so the L*, a*, b* values could only be described in colour groups, rather than specific AUS-MEAT colour (AMC) scores.
Comparison of blooming times, revealed the differences observed in L* and b* values, and consequently hue and chroma values. When the side had been bloomed for a longer period, colour groups for both L* and b* values were significantly different, whereas this was not the case after blooming for only 1hr. This highlights the importance of allowing a sufficient bloom time for colour development, as carcases graded with a shorter bloom time could be more difficult to allocate into AMC scores. It was therefore important to consider carcase side in the random effects within the REML structure.
From the side graded (LHS), carcases from colour group C (meat colour group ≥4) had significantly lower L*, a* and b* values, indicative of the darker, less red, more blue colour of the meat. This was also reflected in a lower chroma score, however hue scores were not significantly different. The reflectance ratio (R630/580) used as an indicator of redness was also slightly lower but was not significantly different. These colour measurements are consistent with expectations of the lower AMC scores ≤1C (colour group A) having a lighter, more red appearance and the higher AMC scores ≥4 (colour group C) having a darker, less red appearance.

Table 3. Colorimetric measurements of muscles from different meat colour groups, as defined by AUS-MEAT colour (AMC) scores: A- ≤ 1C; B- 2 or 3; C- ≥4, where n=18. The left side (LHS) of the carcase was graded by the qualified assessor and had between 7-9hours blooming at ~5°C, whereas the right side (RS) was bloomed for ~1hour in the same conditions.

	Bloom time 7-9hr (LHS)					Bloom time ~1hr (RHS)				
	A	B	C	s.e.d	F Pr	A	B	C	s.e.d	F Pr
L* value	38.96	37.45	31.03	1.544	0.011	37.18	34.83	31.01	2.292	0.137
a* value	31.28	30.21	27.44	0.8557	0.029	29.95	29.54	25.07	1.174	<0.001
b* value	24.51	23.22	20.2	0.9396	0.006	22.17	21.46	17.22	1.336	0.094
hue	38.07	37.53	36.35	0.6124	0.064	36.42	35.87	34.2	1.257	0.314
chroma	39.73	38.1	34.02	1.111	0.003	37.16	36.5	30.53	1.536	<0.001
R630/580	8.008	7.814	7.234	0.4402	0.241	7.945	8.439	6.97	0.8393	0.29

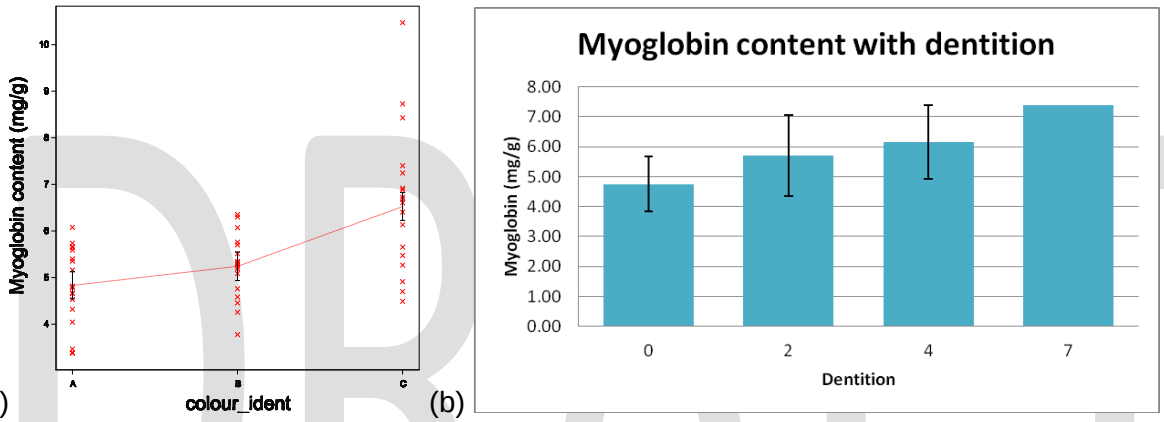
4.1.4 Viability of colour groups

To check the attributes of viability of the colour groups, for each storage week a principle component analysis was conducted (data not shown). Five components were used for the analysis and these were the variates deemed most important for colour formation. These were the raw data for pH, purge, L*, a* and b* values. Overall, there was not clear separation from the 3 meat colour groups, with both meat colour group A and B, and groups B and C overlapping. However, there were clusters which appeared for each colour group, and so each carcase was considered to be in the appropriate group for further analysis.

4.2 Week 0 analysis

4.2.1 Myoglobin content

The myoglobin content (mg/g) for each of the muscles from each colour group is displayed in figure 1. The myoglobin content was found to be significantly different between treatments (P = 0.034). The lighter colour group (A) had the lowest mean myoglobin content (4.835mg/g), followed by the intermediate colour group B (5.242mg/g) and the darker colour group (C) having the highest content (6.525mg/g). This could be due the higher absorbance values at the specified wavelengths (525 and 700nm) for meat colour group C, possibly due to higher purple deoxymyoglobin content of this group.



(a) (b)

Figure 1: Myoglobin content (mg/g) means (+/-) e.s.e.'s for (a) colour group (A, B or C) and (b) means (+/-) s.d. with dentition (only one carcase with dentition score 7).

4.2.2 α-tocopherol content

Due to the variation in feeding regimes between the animals, an analysis of the primary anti-oxidant content of the muscle was measured. Vitamin E (α- tocopherol) content was quantified on frozen muscle samples (where possible measured on the week 0 time point) and are summarised in figure 2. Regardless of colour score, grass fed animals had a significantly higher (P =0.004) tocopherol content (2.275µg/g) within the muscle compared to grain fed animals (3.681µg/g). These results are comparable with other data published (Descalzo and Sancho 2008). There was no difference between colour groups (P = 0.168) or the interaction between colour group and feeding regime (P = 0.689).

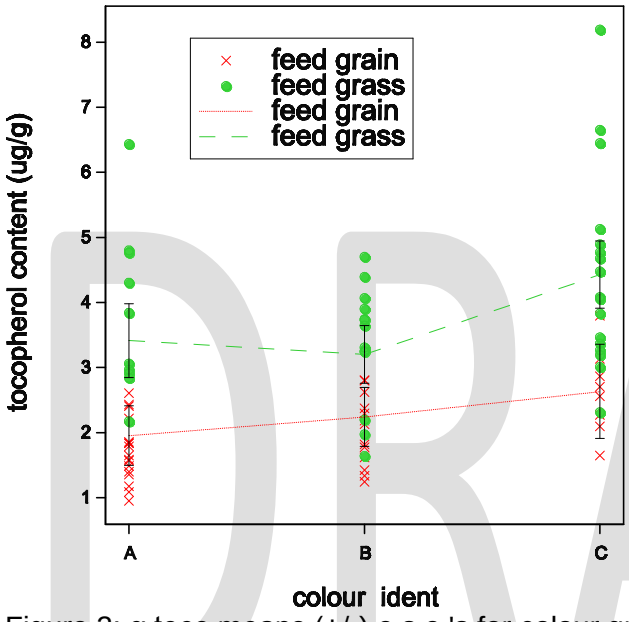


Figure 2: α toco means (+/-) e.s.e.'s for colour group (A, B or C) with different feeding regimes (grass or grain)

4.2.3 Protein extraction & solubility

To evaluate the effect of colour score on protein properties, both the extractability and solubility were assessed (see table 4 and figure 3). There was no difference observed in the total, sarcoplasmic or myofibrillar protein concentration between the 3 colour groups. Others have found lower protein extractability with lighter coloured muscles (Warner, Kauffman et al. 1997), however this was not observed in this study. The reason for this is unknown, although it would be interesting to observe if this is the case using a range of different pH buffers for the extraction process, as this extraction procedure was only completed at a relatively high pH of 7.2.

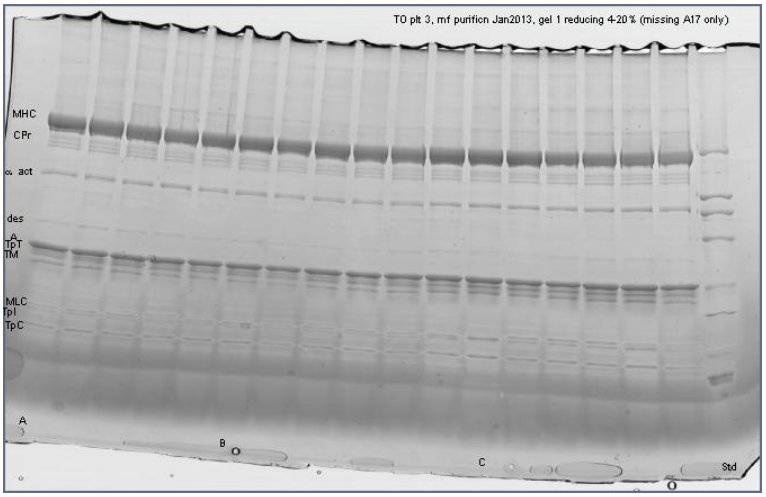
Table 4. Comparison of protein properties from colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively at week 0. Protein analysis was for mean total, sarcoplasmic and myofibrillar protein concentrations (pr concn) and the sulphydryl and carbonyl contents of myofibrillar extracts from striploin muscles.

	A	B	C	sed	chi pr
total pr concn (mg/g)	221.6	223.1	212.7	5.582	0.128
sarcoplasmic pr concn (mg/g)	74.77	73.33	73.76	2.659	0.854
purified myofibril pr concn (mg/g)	94.36	93.68	88.47	15.09	0.913
sulfhydryl content (moles/10^5 g protein)	25.41	25.26	23.73	4.104	0.902
carbonyl content (nmoles/mg protein)	74.2	73.05	70.86	9.608	0.939

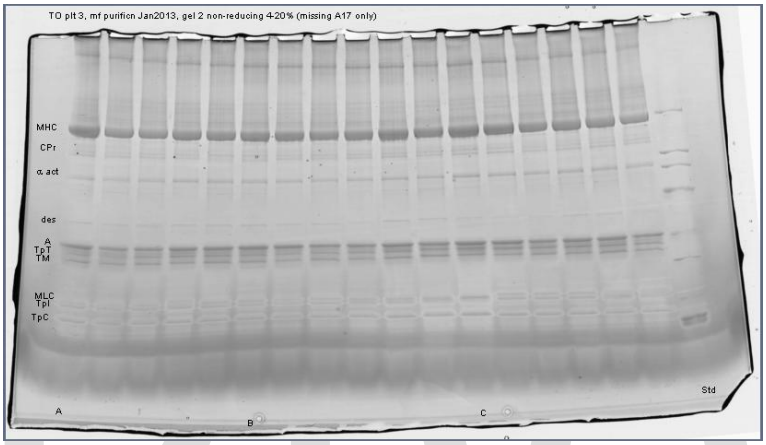
Using the purified myofibril preparations from plant 3 only, the protein degradation products from both reducing and non-reducing conditions was assessed and can be visualised in figure 3. Proteins run on the 4-20% gel (figure 3 (a) and (b)), displayed the full range of myofibrillar proteins. Under reducing conditions, the darker meat colour group C appeared to show slightly more lower molecular weight proteins, such as myosin light chain and troponins I and C. Under non- reducing conditions, as expected, there were more high molecular weight proteins at the top of the gel, but a distinction between colour groups was not apparent. Overall, under both reducing and non-reducing conditions the degradation products appeared to be relatively similar, with no obvious differences apparent.

Proteins run with the 5% gel (figure 3 (c) and (d)) displayed the higher molecular weight proteins more clearly. The darker meat colour group C appeared to have a higher quantity of titin band T2, and possibly more nebulin present. This is consistent with others (O'Halloran, Troy et al. 1997) who have observed less degradation of such high molecular proteins. The integrity of these proteins could be associated with structural differences at the myofibrillar level and influence the rate of myofibrillar shrinkage which occurs during the early post-mortem period.

(a)



(b)



(c)

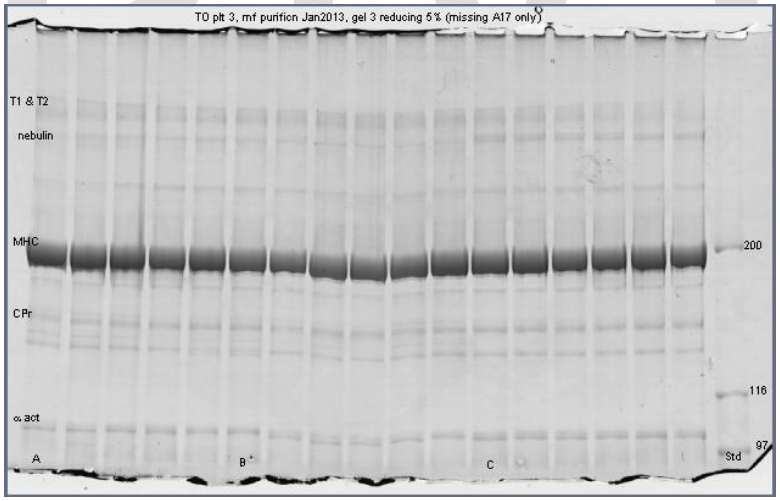


Figure 3: SDS-PAGE by colour group A (lanes 1-5 from left to right), B (lanes 6-11) or C (lanes 12-17) for week 0 myofibrillar extracts. Broad range standard (6.5-200kDa) displayed in far right (lane 18). Gels were either 4-20% (a)-reducing and (b)-non-reducing or 5% (c) polyacrylamide. Myofibril proteins in order of ascending molecular weight are: Troponin C (TpC), troponin I (TpI), myosin light chains (MLC), tropomyosin (TM), troponin T (TpT), actin (A), desmin (des), α-actinin (α-act), C- Protein (C-Pr), myosin (M), nebulin (neb), and titin 1 & 2 (T1 & T2).

4.2.4 Protein oxidation (carbonyl and sulphydryl content).

Measures of myofibrillar protein oxidation were measured as either an increase in carbonyl content or a reduction in sulphydryl content. Neither of these indicators were observed between colour groups (table 4) and hence no difference in protein oxidation was found between different meat colour groups.
The relationships of colour and pH attributes with protein solubility, oxidation and anti-oxidant content were investigated using a correlation table (see table 5). As would be expected, highly significant correlations were observed between the colour attributes (L*, a* and b* values) and pH. Lightness was positively correlated to myofibrillar protein concentration, suggesting proteins from of lighter coloured meat had a higher solubility in the high salt buffer. Myofibrillar protein concentration was also positively associated with an increase in sulphydryl content, which indicates less protein oxidation. However, the myofibrillar protein solubility was also positively correlated to TBARS thus indicative of more lipid oxidation in these samples. In addition, total protein concentration was negatively correlated to metmyoglobin concentration (*r=-0.048,* data not shown). Together these findings illustrate a relationship between protein solubility, protein oxidation and lipid oxidation which needs to be further investigated.

Table 5: Correlation table depicting correlation coefficients (*r*) where n=18 for protein solubility, protein oxidation and anti-oxidant measures. Probability values illustrated in *italics* (statistically significant, where p<0.05) and **bold** (highly significant, where p<0.001).

L* value	1										
a* value	2	*0.71*									
b* value	3	**0.72**	**0.99**								
pH	4	**-0.74**	**-0.87**	**-0.83**							
total protein (mg/g)	5	0.42	0.23	0.19	-0.40						
myofibril protein (mg/g)	6	*0.52*	0.33	0.31	-0.44	**0.93**					
sarcoplasmic protein (mg/g)	7	-0.35	-0.31	-0.37	0.20	-0.01	-0.38				
sulphydryl content	8	-0.03	-0.04	-0.07	-0.05	*0.50*	*0.48*	-0.05			
carbonyl content	9	-0.27	-0.14	-0.17	0.04	0.17	0.15	0.04	**0.78**		
TBARS	10	0.36	0.24	0.19	*-0.49*	*0.52*	*0.55*	-0.18	-0.09	-0.12	
tocopherol (ug/g)	11	-0.11	-0.08	-0.06	0.10	*-0.57*	*-0.58*	0.13	*-0.56*	-0.17	-0.12
		1	2	3	4	5	6	7	8	9	10

4.2.5 Volatile analysis

The volatile profile of each sample from 0 and 20 weeks was measured by GC-MS. No significant volatile compounds were detected in any sample. This was surprising since it was anticipated that there would some present in the profile yet this was not the case. One reason for this could have been the extraction temperature (70 °C) which might not have been sufficient to release the volatile compounds from the meat, but was selected to be comparable to the TBARS analysis. However, the extraction conditions used in this study were reported, and successfully deployed (Vasta, Luciano et al. 2011).These workers found a number of different compound classes (acids, alcohols, hydrocarbons and others) in beef which might have been expected to be present in these samples. It is likely though that there will be volatile compounds in the samples used in this study but at concentrations were not detected with the experimental conditions used for this study.

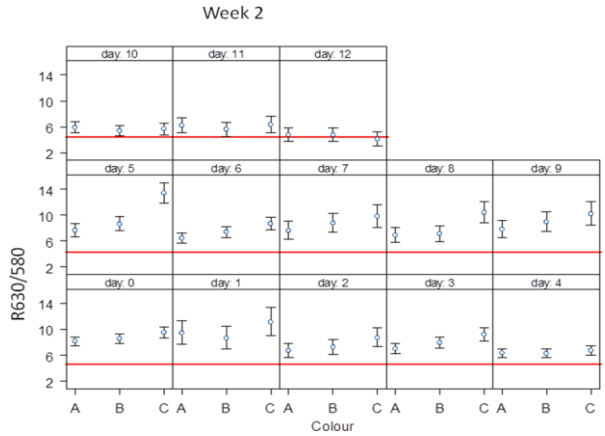
4.3 Week 2, 12 and 20 analysis

4.3.1 Retail display

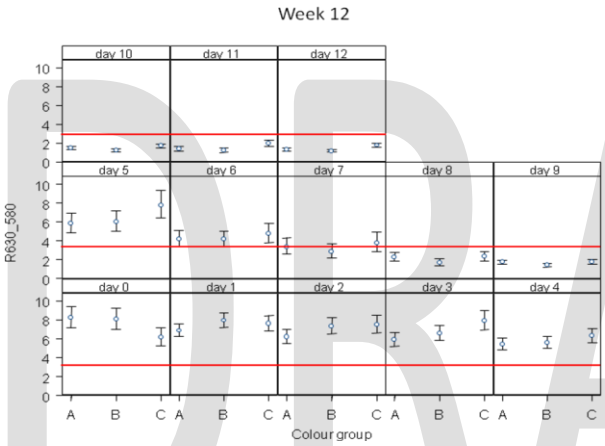
Analysis of colour groups from retail display steaks was investigated for various colour attributes. For all weeks, lightness (L*) values were significantly higher (P<0.001) in meat colour group A and lowest in meat colour group C, with colour group B being intermediate (data not shown). The reflectance ratio (R630/580) was considered to be of primary interest, due to the reported relationship with consumer acceptability score in other studies (Morrissey, Jacob et al. 2008). In these studies, lamb longissimus colour was reported as being unacceptable when R630/580 values were below a cut point of 3.5. For the purpose of this study, the back transformed R630/580 means with LSI were graphed for each day at each storage week (figure 4) and this acceptability value was displayed as a red indicator line.

In terms of colour stability with time, storage week 2 appeared to have the longest colour stability with all colour groups displaying R630/580 >3.5 until day 12. In comparison, at weeks 12 and 20 this value was reduced to 7 and 4 days respectively. Although, this was the case, steaks were visually unattractive before this time, mainly as a result of discolouration to surrounding fat and browning at the edges of the steaks. Therefore, the final day for acceptability for weeks 2, 12 and 20 were 6, 4 and 3 days respectively. Typical images for steaks at week 20, day 3 are displayed in image 3 below. Further research into consumer acceptability could be conducted in order to confirm such findings.

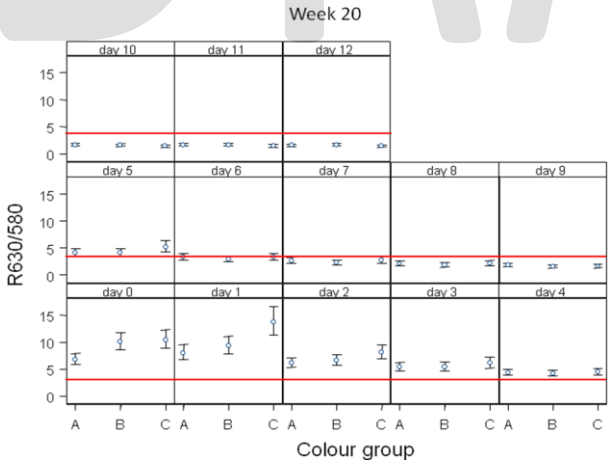
Between colour groups, in terms of colour stability over the display time, no major differences were observed. Meat colour group C tended to display higher R630/580 but for the majority of days this was non-significant. At week 20, group C displayed lower chroma (P<0.001) and hue (P<0.05) values, indicating the lower saturation of the meat. At week 2, meat colour group A had a significantly lower brown metmyoglobin value for days 0 and 1, although after this time values were similar to other groups. For the same week, no significant differences were observed for purple deoxymyoglobin or red oxymyoglobin levels between colour groups. In short, steaks from all colour groups appeared to have similar trends in terms of colour stability.



(a)



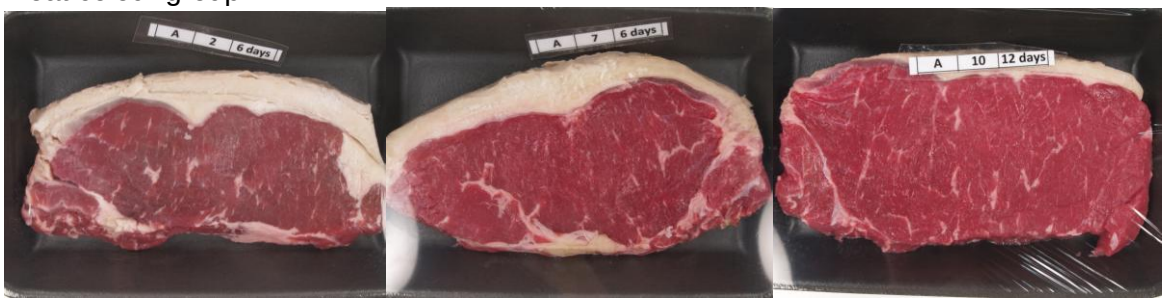
(b)



(c)

Figure 4: Comparison of backtransformed means (+/- LSI) R630/580 retail display steaks from colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for 2,12 or 20 weeks. The steaks were cut and bloomed for 60 minutes at 5°C, then placed in black trays and over-wrapped and stored in the retail display cabinet for a total of 12 days. Red marker line indicates cut-point (R630/580=3.5) which is required for consumer acceptability.

Meat colour group A



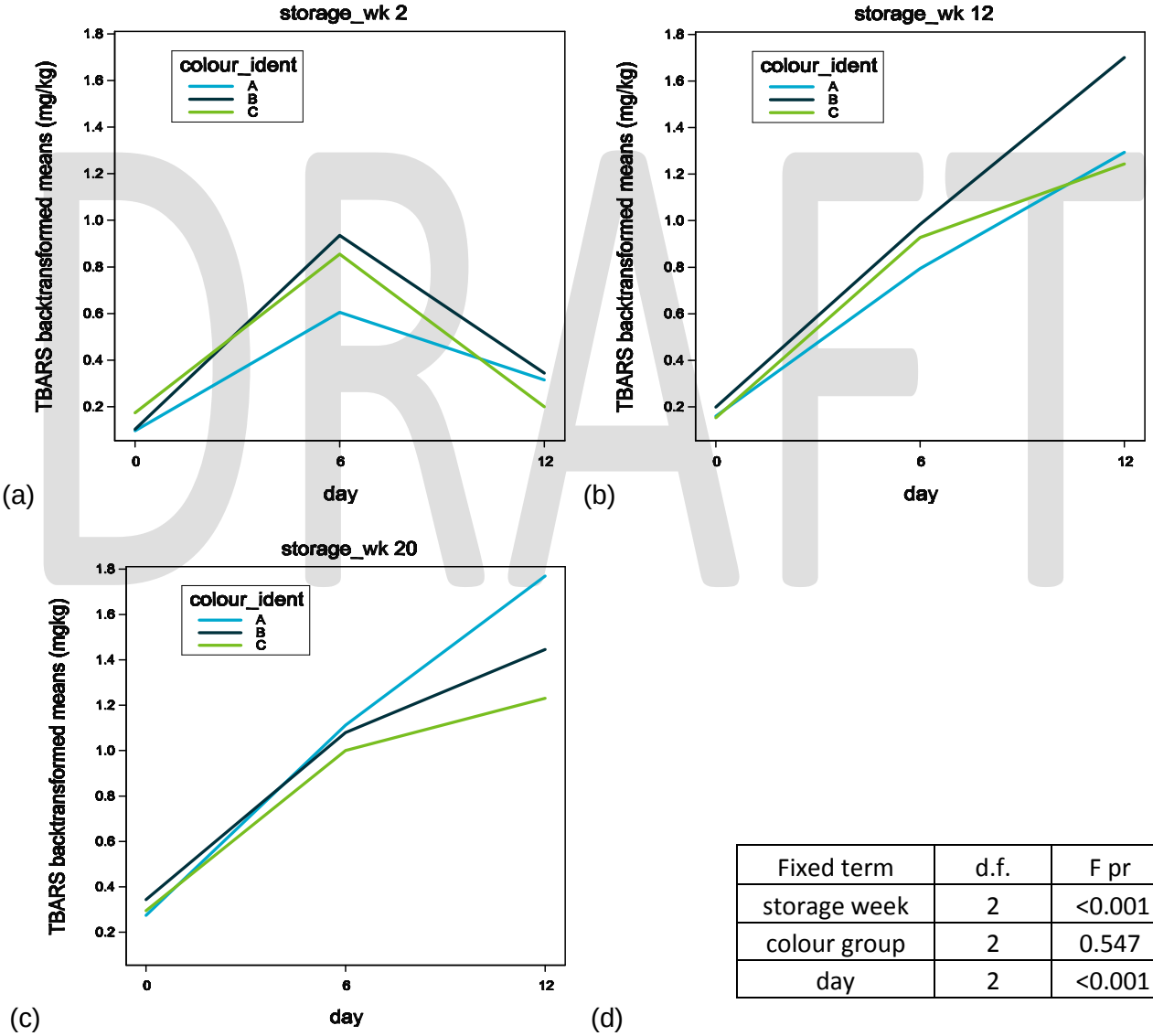
Meat colour group B

Meat colour group C

Image 3. Comparison of representative retail display steaks from colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for 20 weeks. The steaks were cut and bloomed for 60 minutes at 5°C, then placed in black trays and over-wrapped and stored in the retail display cabinet for a total of 12 days. These steaks are after 3 days storage in the cabinet.

4.3.2 Retail display TBARS

The data for TBARS across all the storage weeks and 3 storage days (0, 6 and 12) was observed to be heteroscedastic, and so was transformed using a natural log, for the purpose of the REML analysis and back-transformed for the trellis plot (figure 5). For all weeks, colour groups appeared to have similar values (P=0.547), with colour group A tending to show slightly lower values. Compared to weeks 12 and 20, storage week 2 displayed a different trend, with values for all colour groups decreasing at day 12. This could be either an error in the measurement, or could be due to the measured reactive species becoming utilised in other chemical reactions. At storage weeks 12 and 20, lipid oxidation increases with retail display day, which could be associated with myoglobin oxidation (data not shown). In short, both day and storage week displayed highly significant differences, whereas colour groups did not.



Fixed term	d.f.	F pr
storage week	2	<0.001
colour group	2	0.547
day	2	<0.001

Figure 5. Trellis plot and summary table with a comparison of groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively with back-transformed means (n=6) retail display TBARS at storage week 2 (a), 12 (b) and 20 (c). Retail display was conducted on steaks over a total of 12 days, with TBARS being conducted on samples at 0, 6 and 12 days. REML fixed terms (d), degrees of freedom (d.f.) and F statistic probability (FPr) are also displayed.

4.4 All weeks (0, 2, 8, 12, 16 & 20) analysis

4.4.1 Basic colour attributes

The basic colour attributes (lightness, chroma, hue and redness- as indicated by R630/580) from each colour group and storage week are summarised in table 6. Both the lightness and chroma values were highly significant between both treatments (colour group and storage week), with steaks being lighter and having more saturation in colour group A, compared to B and C. As expected, meat colour group C were darker and displayed lower chroma and hue values, indicative of the lower intensity of a more red steak. This is also confirmed in the significantly higher (P=0.03) redness indicator (R630/580) of the group.

In terms of storage week, all four variates showed highly significant (P<0.001) differences over the allocated time period. With storage time, all colour groups were lighter and less red (higher hue, lower R630/580) and also displayed an increase in chroma, indicative of more saturation in the primary hue of the sample. Such observations could be a result of a reduction in muscle fibre diameter, increased extracellular space and associated with purge generation during the ageing process. It is interesting to note, all colour groups displayed a R630/580 well above the consumer acceptability minimum score of 3.5 (Morrissey, Jacob et al. 2008), indicative of a visually acceptable product.

When the interaction between colour group and storage week is considered, there appears to be a significant effect on chroma (P<0.001), hue (P=0.026) and R630/580 (P=0.022) but not on lightness (P=0.48). This indicates the interaction appears to be with the a* and b* values, rather than with the lightness variable. Perhaps, the oxidative state of the proteins could be playing a role in this relationship.

Table 6. Colorimetric measurements of striploin muscles from different meat colour groups, as defined by AUS-MEAT colour (AMC) scores: A- ≤1C; B- 2 or 3; C- ≥4. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for between 0 to 20 weeks. At each time point, steaks were cut and bloomed for 60 minutes at 5°C prior to colour measurement. REML analyses displays the standard error of differences (sed) and approximate F-test probability (FPr) values, with 5, 2 and 10 degrees of freedom (df) for storage week, colour group and storage week.colour group interaction, respectively.

week	A	B	C	storage week		Colour group		Storage week. Colour group	
	mean			FPr	sed	FPr	sed	FPr	sed
Lightness									
0	37.53	34.62	29.63	<0.001	0.915	<0.001	0.7066	0.48	0.919
2	41.50	38.08	32.13						
8	41.73	39.03	32.14						
12	42.20	38.88	32.65						
16	42.17	39.22	34.14						
20	42.75	39.15	33.36						
Chroma									
0	36.36	35.16	32.58	<0.001	0.4215	<0.001	0.5795	<0.001	0.8838
2	39.45	38.69	32.63						
8	38.15	38.06	34.76						
12	38.38	38.01	34.75						
16	38.05	37.8	34.4						
20	38.01	38.33	35.72						
Hue									
0	36.32	35.48	34.73	<0.001	0.3725	0.005	0.3404	0.026	0.3752
2	37.33	36.93	34.72						
8	36.96	36.62	35.38						
12	37.12	36.64	35.47						
16	37.03	36.61	35.64						
20	37.03	36.70	35.98						
R630/580									
0	7.58	7.87	8.49	<0.001	0.3589	0.03	0.3429	0.022	0.3614
2	7.58	8.23	7.40						
8	7.23	7.71	8.30						
12	7.01	7.62	8.10						
16	6.92	7.29	7.22						
20	6.69	7.69	7.93						

4.4.2 Spectral colour data

The reflectance spectral data from 400 to 700nm for each storage week is displayed in figure 6. As would be expected, the lowest reflectance values were observed in the wavelength range (530-590nm) associated with green colours, indicative of the maximum absorption at these wavelengths. In comparison, the maximum reflectance occurred at the higher end of the spectrum (>610nm), indicative of minimum absorption of more red wavelengths of light.
Between colour groups reflectance values were similar at the lower end of the spectrum, and the profile of the data, (especially around 570nm) is indicative of minimal metmyoglobin content of the muscles. Towards the higher end of the spectrum, meat colour group C displays a lower reflectance, illustrating the higher absorption of red light. This effect is related to lower light being scattered within the muscle, which allows for a longer light path into the structure and more absorption by myoglobin (Swatland 2004). In comparison, the lighter colour group A displays more light scattering and less absorption, and hence presents higher reflectance values.
When comparing spectrum across the storage weeks, there is little differences observed. Although, week 0 does show slightly lower values compared to the other time points. This could be due to the earlier time post-mortem and could be associated with the structural differences of the muscle. After, 2 weeks ageing the oxygen levels within the pack may have stabilised and

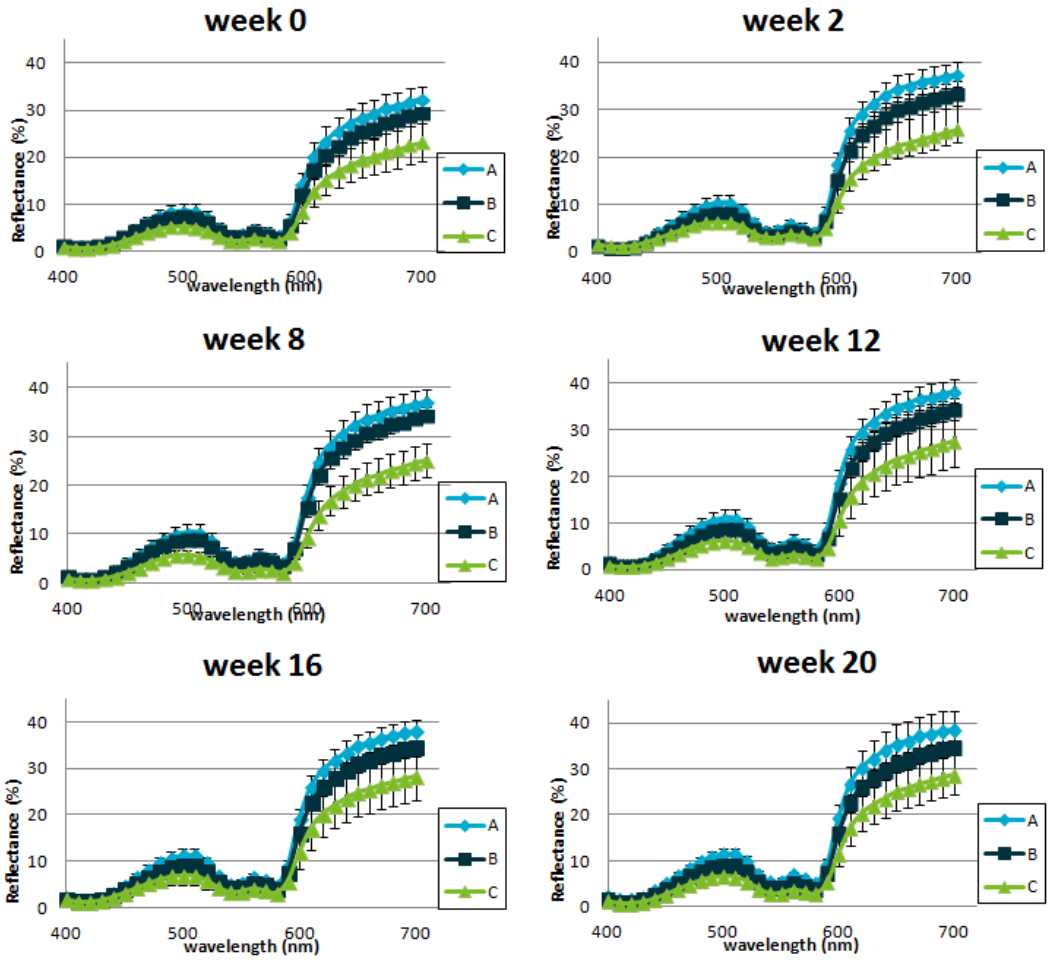


Figure 6: Reflectance (%) means (+/-) s.d. plotted against wavelength (nm) from striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Values generated from 60 minute bloomed steaks using a Hunterlab Miniscan EZ.

4.4.3 Myoglobin colour attributes

The 3 forms of myoglobin (DMb or deoxy-, OMb or oxy- and MMb or met-) were determined for each colour group at each storage week (figure 7) and were compared between treatments (table 7). As would be expected, oxymyoglobin (commonly associated with a red colour) was the predominant form of the pigment and displayed >60% for all colour groups at all storage weeks. The purple deoxymyoglobin was the least prominent form. Metmyoglobin content (commonly associated with more of a brown colour) was intermediate, but did show the lowest standard error of differences and also the most significant probability values.

Between colour groups, both the deoxy- and oxy- form were highly significantly with the lighter colour group A displaying higher levels of red OMb and lower purple DMb contents, whereas the darker colour group C showed the opposing trends. This group displayed higher levels of the DMb and less OMb indicative of less oxygenation. Unexpectedly, the MMb content was highest in this colour group, indicative of more oxidation. In the literature, a darker pork meat colour has been associated with a reduced MMb content (Zhu and Brewer 1998), due to a higher oxygen consumption rate (OCR) and metmyoglobin reducing activity (MRA), so the reason for the difference here is not known.

Between storage weeks, all the myoglobin forms displayed highly significantly different values ($P<0.001$). There were higher levels of DMb and lower levels of OMb present at week 0 compared to the other time points and could be causative of a lower activity of the reducing enzyme system during storage.

Table 7. Comparison of treatments for myoglobin forms (DMb- deoxymyoglobin; OMb- oxymyoglobin; MMb- metmyoglobin) of striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. REML analyses displays the standard error of differences (sed) and approximate F-test probability (FPr) values, with 5, 2 and 10 degrees of freedom (df) for storage week, colour group and colour group.storage week interaction, respectively.

	storage week	colour group	colour group. storage week
DMb	<0.001	<0.001	0.049
OMb	<0.001	<0.001	0.037
MMb	<0.001	0.002	<0.001

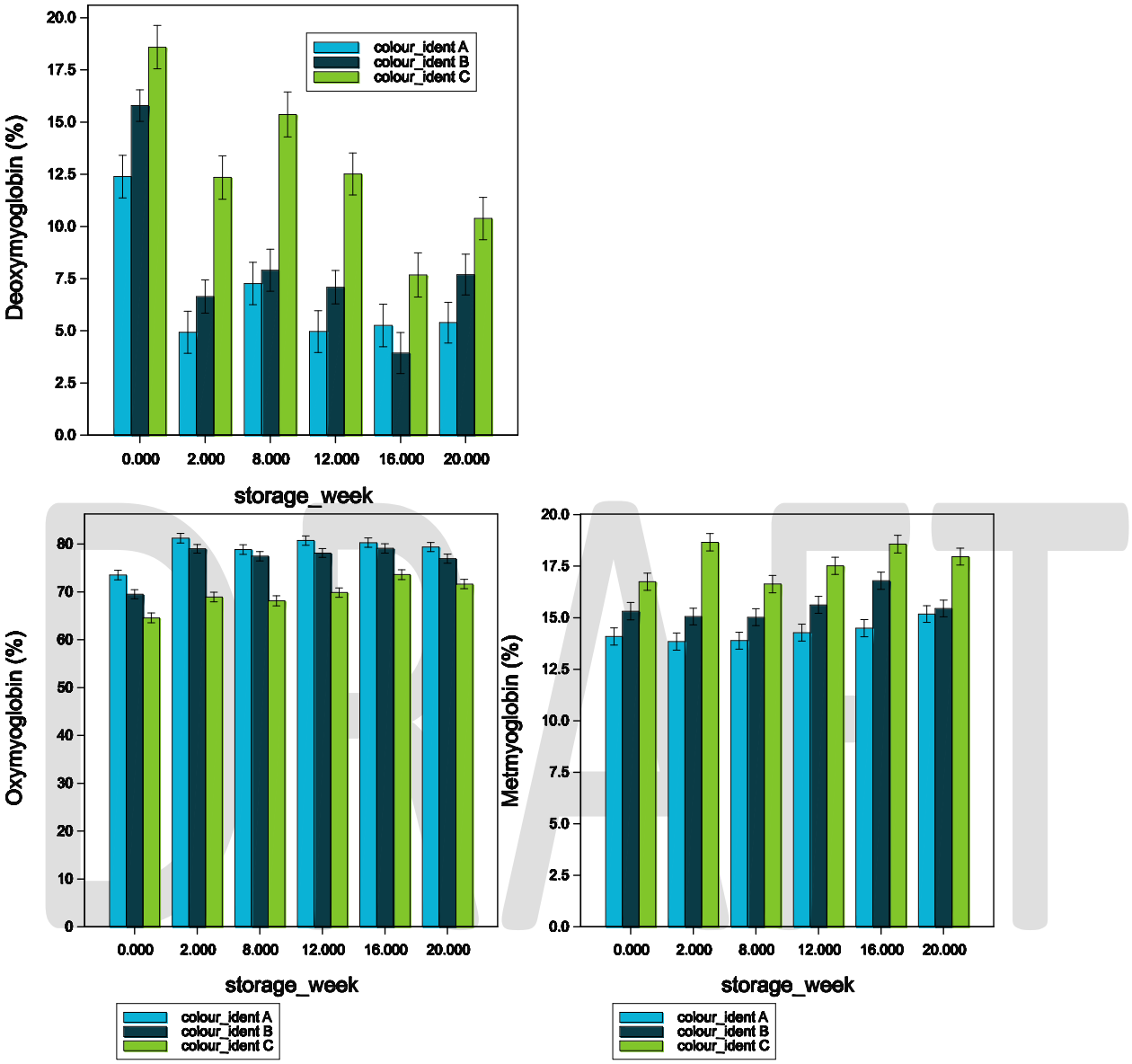


Figure 7: Predicted means for percentage of 3 myoglobin forms (+/-) s.e.d. plotted against storage week from striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for between 0 to 20 weeks. At each time point, steaks were cut and bloomed for 60 minutes at 5°C prior to colour measurement. Values generated from 60 minute bloomed steaks using a Hunterlab Miniscan EZ.

4.4.4 Purge

The percentage purge from each meat colour group, as segregated by the 6 storage time points, are displayed in figure 8. The data was observed to be heteroscedastic and so was transformed using a natural log, so the back transformed values are those within the figure. At week 0, purge scores were the lowest, with values being observed less than 0.5% for all colour groups. As time progressed, the purge values increased upto around 3 to 3.5% at 12 weeks, and remained fairly stable thereafter. There was not a significant difference between the 3 meat colour groups (P=0.921), however the difference between storage weeks was highly significant (P<0.001). Also, there was no interaction between treatments (P=0.77). This indicates the maximum purge loss was reached around the 12 week mark and stabilised.

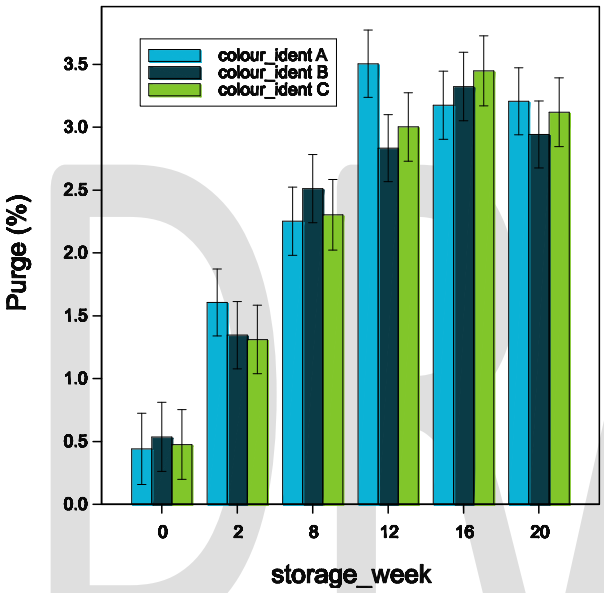


Figure 8: Purge (%) back transformed means (+/-) s.e.d's from striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for between 0 to 20 weeks.

4.4.5 pH

The pH of the meat appeared to be confounded with some of the other variates and for this reason, further analysis must still be completed in order to investigate the possibility of this variable being used as a covariate within the REML model. However, for the purposes of reporting at this stage, the pH means are displayed in figure 9. As would be expected, the darker meat group C displayed higher pH values for all storage weeks. In comparison, the lighter groups had a lower pH at all the storage weeks. Further analysis will need to be completed to verify the effect of pH on other attributes measured.

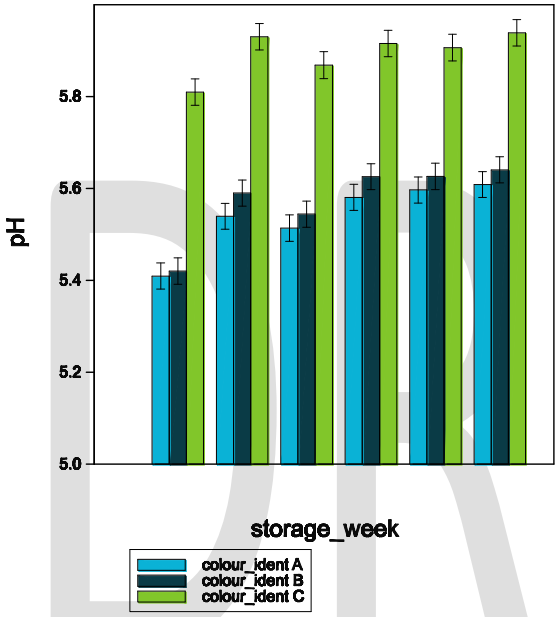


Figure 9: pH (%) means (+/-) s.e.d's from striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for between 0 to 20 weeks.

4.4.6 Thiobarbituric acid species (TBARS)

The lipid oxidation values (as measured by TBARS) for each colour group over the storage period are displayed in figure 10. Results from week 0 appeared unusually high, especially for meat colour group B and should be repeated to check for consistency. However, in general lipid oxidation appeared to increase with storage time, reaching a maximum at week 20. Between colour groups, there appeared to be no distinct difference or trend indicative of similar quantities of lipid oxidation in all samples. Although, there was a significantly negative correlation of TBARS with pH of the samples (table 5), which would be interesting to investigate further.

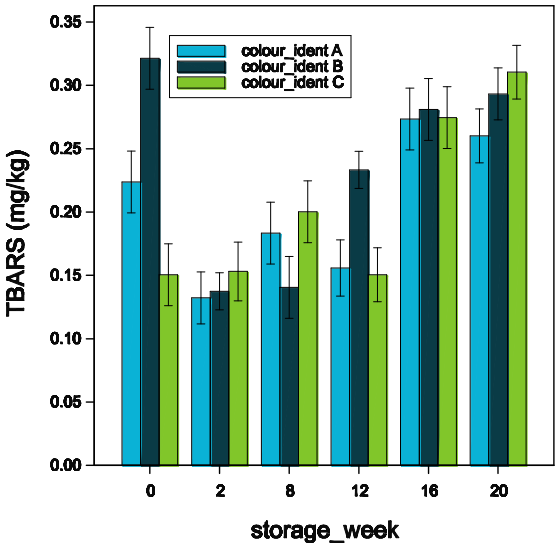


Figure 10: Predicted means for TBARS (+/-) s.e.d. plotted against storage week from striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for between 0 to 20 weeks and subsequently frozen at -80°C prior to analysis.

4.4.7 Glucosyl and lactate content

A summary of the lactate and glucosyl content within the muscle for each colour group and storage week are shown in figure 11 and table 8. The darker colour group C had a significantly lower glucosyl ($P=0.065$) and lactate ($P<0.001$) content. In order to prevent dark cutting, the recommended muscle glycogen content is 1-1.5% and at values below 0.6% the likelihood of dark cutting is largely increased (Ferguson, Bruce et al. 2001). The mean scores for glucosyl content are all less than 0.6%, whereas the lighter groups A and B show means above this value (data not shown). Correlations reveal both the glucosyl and lactate contents are negatively correlated with pH value ($P<0.001$). Thus, from the data, the darker meat colour group C displays a glucosyl and lactate content which is characteristic of dark cutting meat.

Over the storage period, the glucosyl content seemed to increase in colour groups B and C up to week 8, which is similar to the pattern observed for lactic aerobic and aerobic bacterial counts (data not shown). For lactate, the same trend was not as apparent, but did appear to decline with time. Further relationships between the carbohydrate content and the microbiology can be found in the complimentary report (A.MIS.1004).

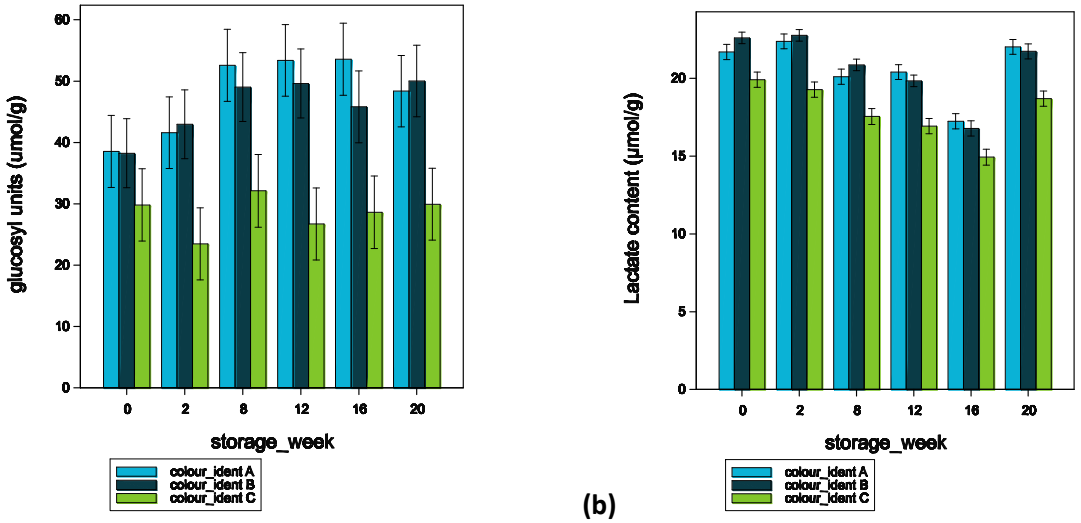


Figure 11: Predicted means for glucosyl (a) and lactate (b) (+/-) s.e.d. plotted against storage week from striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for between 0 to 20 weeks.

Table 8. Comparison of treatments for glucosyl and lactate content from striploin muscles with 3 colour groups (A, B or C), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -0.5°C +/- 0.5°C for between 0 to 20 weeks and frozen at -80°C prior to analysis.

week	A	B	C	storage week		colour group		storage week. Colour group	
	mean			sed	F Pr	sed	F Pr	sed	F Pr
glucosyl units (umol/g)									
0	38.54	38.25	29.82	2.009	<0.001	9.138	0.065	9.669	0.007
2	41.59	42.97	23.48						
8	52.58	49.03	32.13						
12	53.38	49.62	26.72						
16	53.57	45.81	28.62						
20	48.37	50.02	29.94						
lactate (umol/g)									
0	21.69	22.59	19.91	0.6871	<0.001	0.5682	<0.001	0.6521	0.291
2	22.37	22.75	19.28						
8	20.1	20.86	17.54						
12	20.4	19.84	16.93						
16	17.24	16.78	14.93						
20	22.02	21.73	18.69						

5 Conclusions and recommendations - Section

5.1 Conclusions

5.1.1 Week 0 analysis

- Allow for adequate blooming time prior to grading, as differences in the side (with different bloom times) of the carcase were observed
- Dentition score must be considered as influential on measured myoglobin levels in the longissimus muscle
- Tocopherol analysis should be conducted when considering oxidation, as this anti-oxidant was negatively correlated to lipid oxidation values
- Meat colour at grading appeared to have no difference to protein solubility and oxidation within fresh longissimus muscles.

5.1.2 Retail display

- Colour stability declined with storage week, with week 2, 12 and 20 generating steaks suitable for the consumer at days 6, 4 and 3 respectively.
- No differences in colour stability were observed between all meat colour groups.
- For all colour groups, lipid oxidation (TBARS) increased with day of retail display.

5.1.3 All weeks biochemical analysis

- With storage, all colour groups were lighter and less red. Purge increased and reached a plateau around week 12.The glucosyl units increased and reached a plateau around week 8, whereas lactate values started to decline around the same time.
- No difference in lipid oxidation was observed between colour groups, but did increase with storage time. No appearance of significant volatiles were apparent
- Between colour groups, spectral scan data exposed differences in myoglobin forms. The lighter colour group A had higher levels of red oxymyoglobin, whereas the darker colour group C showed higher levels of the purple deoxymyoglobin.

5.2 Recommendations

There are still many unknowns in relation to colour at grading, both from a pre- and post-grading perspective. This report has illustrated the importance of bloom time on the colour generated at grading, however there are many other aspects surrounding the grading practice which should be further investigated (e.g. temperature of the chiller, pH-temperature decline, presence of purge, cutting technique, effect of spray-chilling, etc). Generating a more thorough understanding of early factors which determine the meat colour at grading could provide new insights into the industry in order to minimise economic losses and maximise consumer satisfaction.

This study has generated some interesting findings, which suggest that the striploin muscle may have the potential to be stored for up to 20 weeks, whilst still having a retail display of approximately 3 days. However, this value is provided as a guide on a small selection (n= 6) of steaks and further consideration should be given in order to confirm the findings of this study on a larger scale, perhaps with a more objective consumer panel.

This report has also provided some new insight as to the biochemical aspects of the meat from different colour groups. The relationships between protein and lipid oxidation are still not known, with the mechanistic basis still lacking clarity (Faustman, Sun et al. 2010). This report has generated some conflicting data, with some aspects of lipid oxidation being apparent without correlation of protein oxidation measures such as sulphydryl content. This is an intriguing finding of the study, which would be interesting to explore further in order to understand the role of this functional group on such mechanisms.

Further from this, a relationship is definitely evident with oxidation measures and protein solubility. The effect of protein biochemistry on the properties of the structural elements of the meat could elucidate the measures required to optimise meat colour. This is especially important for interpreting the effect of pH of meat in relation to colour development. Such information would be extremely useful for optimising colour for both the producer, the processor and consequently the consumer.

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