Improving beef colour at grading - Final report

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1.0   EXECUTIVE SUMMARY

This project focused on improving colour of non-compliant dark cutting beef. The project re-examined the role of muscle structure in determining meat colour and investigated whether possible interventions could be used to manipulate meat structure to improve meat colour. The project consisted of four main stages: 1) the development of a reflectance scanning laser electron microscopy (rCLSM) method to study muscle microstructural changes and light scattering, 2) an industry survey on the incidence of dark cutting and identification of potential interventions for prevention of dark cutting, 3) a cost benefit analysis (CBA) and 4) design and optimisation of a proof-of-concept heating process for improving colour of dark cutting beef.

The newly developed rCLSM method is a world first technique for the combined visualisation and quantification of light scattering in light, medium and dark muscles. This technique allows the assessment of one of the main achromatic reasons for dark meat, where larger and more swollen muscle fibres in dark meat reduces light scattering and hence the meat appears darker in colour. This new technique provides a tool to study muscle microstructure and light scattering in a new dimension and test opportunities for reducing the size of the muscle fibres, and consequently, improving the meat colour. This new technique can be used in future projects by the Australian meat industry to improve the understanding of the relationship between muscle structure and meat quality, particularly meat colour.

The industry survey of 15 processing plants highlighted a higher incidence of dark cutting carcasses (an average of 12.0 %) compared to that reported by Meat Standard Australia (5.3 % in 2017). There was a wide variability in dark cutting incidences between plants, which ranged from 0.6 to 41.3 %. Processing plants with the highest incidence would benefit more from interventions which reduce dark cutting, and thus, must be the primary target for future research. The financial loss for downgrading of dark carcasses ranged from 10c/kg to $2/kg or $50/head to $1000/head depending on the category and grade of animal. Hence there is opportunity for capturing more revenue by limiting downgrading of carcasses.

In conjunction with Greenleaf Enterprises Pty Ltd, an Excel spreadsheet was developed as a cost benefit analysis (CBA) tool, to assist the industry to estimate the costs associated with implementing an intervention, the payback time and related benefits. This CBA model is a very useful decision making tool for processors when considering implementing different intervention strategies to improve the colour, and hence, the value of the carcass at grading. This model is available to the industry through AMPC and CSIRO.

A proof-of-concept intervention based on a mild heating step was developed to manipulate the structure of non-complaint dark primals. This intervention involved heating dark beef cube rolls at 45 °C for 5 hours. This heating process induced muscle lightening and increased both lightness (L^*) and redness (a^*) compared to control (untreated). This colour change led to a higher consumer acceptability of the steaks compared to the control samples on day 0 of retail display. However, the heat-treated samples showed a lower colour stability during display, and thus, control samples had a higher acceptability than heated samples on day 5. The heating step also resulted in additional 0.5 and 1.0 % drip loss during vacuum storage for 0 and 24 days, respectively. Furthermore, heating of the

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primals resulted in an accelerated tenderisation of meat (a reduction in shear force); however, the ability of meat to age was greatly impaired by the heat treatment (i.e. heat-treated meat did not age). Microbial counts were not impacted by the heat treatment, hence, meat colour can be improved with this intervention without jeopardising microbial shelf life.

Overall, this intervention can provide a competitive method for improving the colour of dark primals. It is possible to apply the heat-treatment for up to 96 hours post-mortem without compromising the benefits. This enables the collection and accumulation of dark primals during a working period, and then subjecting them to a batch heat-treatment later on. The heat-treated primals need to be dispatched for retail sale within 1-3 days after treatment and sliced into steaks, to maximise the benefits from this intervention. This intervention would only require a recirculating water bath with temperature control. It is recommended that commercial-based trials are undertaken to test this heating intervention on a number of different dark primals of economical relevance, preferably in those processing plants with high incidence of dark cutting.

Dark cutting is still a significant economic problem for the Australian red meat industry. The industry would greatly benefit from the development and implementation of interventions that can tackle this quality defect. This will require a multi-disciplinary approach encompassing pre-slaughter to post-slaughter factors and the development of methods and techniques for detection, remediation and valorisation of dark carcasses and cuts.

2.0 INTRODUCTION

The colour of beef at grading is one of a number of criteria that determines the value of a carcass. Beef carcasses can be downgraded if the loin is too dark, usually as a result of high ultimate pH. Electrical stimulation (ES) can be used to increase the number of carcasses with 'ideal' colour scores (colour score <4) but application of ES can also result in deterioration in other quality traits such as sensory appeal. Research on meat colour has focused on reducing ultimate muscle pH, through increased muscle glycogen (less stress prior to slaughter), and also, optimising the effectiveness of electrical stimulation. Although early research showed that muscle structure has a role in determining beef colour at grading, there has been little recent research.

In this project, a novel microscopy technique was developed to elucidate the role of muscle structure in determining beef colour. Strategies to manipulate muscle structure to improve muscle colour, through pre- or post-rigor interventions were also investigated. These findings were then utilised to develop expertise and capability to induce lightening of dark meat and improve beef meat colour.

2.1   Fundamentals of beef colour - The role of muscle structure

Colour is the most important pre-purchase quality trait of beef because consumers use colour as an indicator of quality and freshness at the point of sale. The Australian processing plants assess colour at grading and is one of the criteria that determines the value of a carcass. Beef carcasses can be downgraded when the loin is either too dark, has a high ultimate pH ($pH_u > 5.7$) or the associated AUS-Meat colour score > 3 (Figure 1).

1A	1B	1C	2	3	4	5	6	7
								Colour darker than the 6 chip

Figure 1. AUS-MEAT meat colour reference standards.

The colour of fresh meat primarily depends on the concentration, and the chemical and physical state, of myoglobin and the attached ligand (e.g. O_2, CO, NO) and, to some extent, on the structure of the meat (Mancini & Hunt, 2005; Suman & Joseph, 2013). The role of structure in determining meat colour relates to how the light travels and interacts with the meat as it traverses. When light comes in contact with the surface of the meat, it can either be reflected, absorbed or scattered. It is a combination of these three behaviours which determine the meat colour, but primarily it is the reflected light which determines consumer perception, and hence, acceptability. Thus, the light reflected from the surface of the meat to the eye is often compared with quantitative assessment made using either a spectrophotometer or colorimeter, and distinguished into chromatic colour attributes (redness and yellowness) and achromatic colour attributes brightness (lightness) (Figure 2).
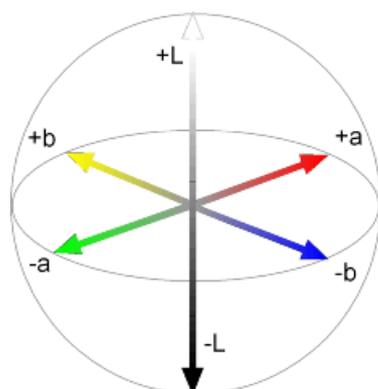
Figure 2. Model of CIELAB colour solid, illustrating tristimulus colours in 3 orientations (a-redness/ greenness; b –yellowness/ blueness; L -lightness/darkness) (JISC Digitalmedia, 2014).

The colour attributes for redness ($a*$) and yellowness ($b*$) tend to be strongly associated with the chromatic attributes of the muscle. The main chromatic determinant is the pigment myoglobin, which can influence the meat colour based upon the oxidative state of the iron atom embedded in the porphyrin ring structure of the heme group. In comparison, the lightness or $L*$ value is related to structural attributes of the muscle. Consequently, light is absorbed primarily by myoglobin, while the non-absorbed light that is either transmitted or scattered around the structural elements. The latter behaviours are in turn related to the reflected light that is visualised.

Light scattering is the process in which light is diffused or deflected by collisions with particles of the medium that it traverses (Figure 3). In the muscle, the medium can either be the connective tissue, muscle fibres, myofibrils or any fluid (extracellular fluid or sarcoplasm) within or surrounding these structures. Other proteins, lipids or macro molecules could contribute to the refraction of this fluid, which could also contribute to the light scattering properties of the meat. The more light that is scattered within the microstructure, the less the light will be transmitted into the depth of the muscle and the more that can be reflected. In short, the extent of light scattering is influenced by the structural attributes of the muscle, and thus, can contribute to the perceived lightness or colour as viewed by the eye (Hughes et al., 2014).
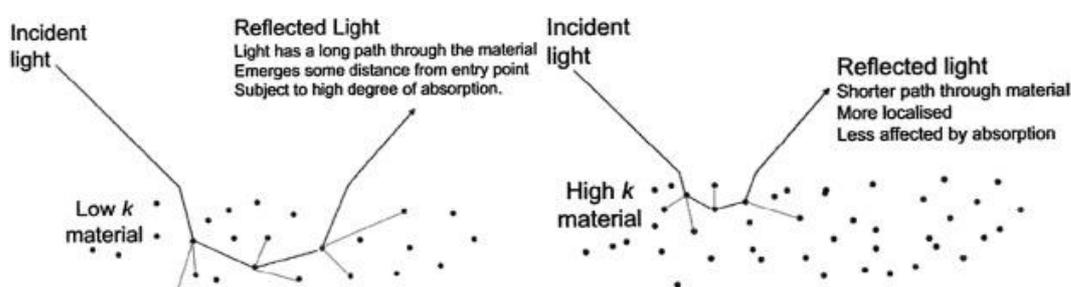
Figure 3. Light propagation through a material with low scattering (left) or high scattering (right). Where the parameter k is a function of both the ability of a particle to scatter and its concentration in a medium (McGunnigle, 2012).

2.2   Light scattering and reflection confocal scanning laser microscopy (rCLSM)

The fibre bundle of the muscle creates a light scattering speckle pattern that can be visualised longitudinally using rCSLM (Offer et al., 1989). Lighter muscles from pale meat have more speckles, which tend to be larger, indicative of a looser, more open packing of structural elements. This speckle pattern is possibly an indicator of the muscle structure responsible for light scattering. This scattering on fresh muscle can be observed, and further, quantified using rCSLM. The advantage of rCSLM is that it can be used to characterise the structure, without the use of fixation or cryo-sectioning, which may introduce artefacts into images. This technique can be used both in the *x-* and *y-* orientation, but also allows for the visualization into the depth (*z*-plane). This allows for a 3D projection of the muscle tissue and provides very useful tool for the determination of structural attributes of the muscle which may contribute to light scattering.

2.3   Post-mortem changes affecting muscle structure

During the transformation of muscle to meat, a major change is the decrease in pH as a consequence of muscle metabolism of glycogen into lactic acid. The pH decreases from a physiological pH of about 7, down to an ultimate pH (pH_u) about 5.4-5.7 within 24 hours. If there is insufficient muscle glycogen concentration at slaughter, there is limited formation of lactic acid, resulting in a high pH_u (pH > 5.7) resulting in dark meat. It is known that the pH condition of the muscle is highly related to the amount of light scattering, with a lower pH being associated with increase in scattering (Winkler, 1939). With higher pH values, the light scattering decreases, which may result from changes in the structure of the meat. This increase in light scattering has also been observed during the early post-mortem period, concurrent with the pH decline (MacDougall, 1982). Furthermore, during the pH decline the meat approaches the isoelectric point of the proteins and the structural spacing of the myofilament lattice undergoes changes, which results in less filament separation (Diesbourg, Swatland, & Millman, 1988). In addition, there is a reduction in fibre diameter and an increase in extracellular space (Heffron & Hegarty, 1974; Offer & Cousins, 1992) (Figure 4). In parallel, the translucency of the meat is reduced and the scattering coefficient increases, indicative of the increase in light scattering (Bate-Smith, 1948; MacDougall, 1982).
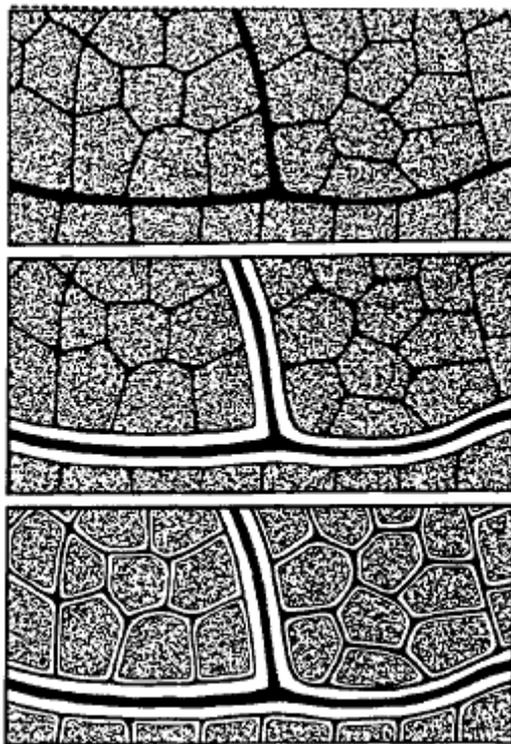
(a) Living muscle ~pH 7
"Muscle fibres fill the endomysial network and fibre bundles fill the perimysial network".

(b) 4-6 hours PM ~pH 6.6 – 6.8
"Large, presumably fluid filled, gaps (typically 20-50 μm apart) open between fibre bundles".

(c) Post rigor (24-48 hours PM) ~pH 5.6- 6.0
"Gaps appear because of separation between muscle fibres and endomysial network". Gaps between fibres are typically 20 μm apart.

Figure 4. Diagram representing structural changes occurring in beef muscle post-mortem (PM). Transverse sections of 3 muscle fibre bundles illustrating the extracellular space generation and the reduction in muscle fibre diameter size during the early PM period ((Offer & Cousins, 1992).

The magnitude of these structural changes during the early post-mortem period is a time and pH dependent process. For example, if carcasses are graded ~14 h post-mortem compared to ~31 h, there is a 3 fold increase in dark cutting (Hughes, Kearney, & Warner, 2014). This mostly likely due to carcasses being graded before onset of rigor and ultimate pH. Thus, the extent of the pH decline and the structural changes that occur in the lattice early post mortem, need to be further explored, taking into account differences in translucency and light scattering which may contribute to the incidence of dark cutting beef.

The rate of the early PM pH-temperature decline is known to be important for many meat quality parameters including meat colour. Meat Standards Australia (MSA) provides recommendations for the optimal temperature "window" when a muscle passes through pH 6 (Temp@pH6) to be between 15 and 35 °C (Meat and Livestock Australia Limited 2011). For the best meat colour, the optimum temperature within this window is about 25 °C which has been shown to deliver the highest rate of optimal meat colours 2 and 3; the incidence of dark cutting (DC) increasing as the temperature becomes colder. It has also been shown that increasing the rate of glycolysis PM induced by using electrical stimulation of the carcass early PM is associated with an increase in lightness of meat. Similarly, early studies on pork *longissimus* indicated that some properties of pale "watery" fibres can be induced in normal muscle, by "heating a minced sample of normal muscle at 37 °C for 1½ h or by

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allowing a sample of muscle cut straight from animal after death to pass into rigor at 37-41 °C" (Bendall and Wismer-Pedersen 1962). Solubility of muscle proteins and muscle shrinkage and their dependence on pH and temperature conditions are the driving factors affecting both lightness and water holding capacity of the muscle.

Overall, the present project has used standard meat colour and quality measurements in combination with the newly developed rCLSM visualisation method to characterize light, medium and dark muscles in order to better-understand the impact of different conditions on muscle structure, and further, their effect on determining beef colour, with a focus on improving the colour of dark cut beef. Furthermore, the theories of meat science and muscle structure have been considered from a practical application for beef processors and potential intervention technologies were identified. The project undertook an industry survey to quantify the incidence of dark cutting and cross-check the potential feasibility of these interventions and associated cost benefit analysis. Finally, the project has explored targeted interventions to change muscle structure to induce a colour change, and to develop a proof-of-concept intervention based on a heating step to manipulate the structure of non-complaint dark cuts, whereby the colour is more acceptable and valuable under commercial conditions.

3.0 PROJECT OBJECTIVES

3.1 Overall objectives

The overall objectives of the project – as per the contract were as follows:

1. Understand the role of muscle structure in determining beef meat colour

2. Develop strategies to manipulate muscle structure to improve muscle colour

3. Develop scientific expertise and industry capability in beef meat colour

4. Incorporate new disciplines into meat quality (Confocal microscopy, mathematics, process engineering) and undertake training of a PhD student.

3.2 Specific objectives

The specific objectives for the various stages of the project were as follows:

1. Conduct experiments to investigate the influence of muscle pH on muscle structure and light scattering.
   (i) Develop techniques to measure light scattering using scanning confocal microscopy.
   (ii) Collect samples ranging in ultimate pH from low to high and investigate the influence of pH on light scatter and relate to surface colour.

2. Monitor the changes in light scattering in response to changes in pH and relate to changes in the myofibrillar diameter and myofilament lattice spacing.
   (i) Collect samples ranging in rate of pH fall (slow to fast). Measure light scattering and relate to surface colour.

3. Undertake literature review of factors influencing the incidence of dark cutting, in Australia and overseas, and conduct a survey of processors on the incidence and impact of dark cutting in beef carcasses.

4. Develop a cost-benefit analysis tool with a sub-contractor (Greenleaf) to assist industry to determine:
   (i) What is the value /cost to industry of dark cutting meat i.e. cost of non-compliance?
   (ii) If R&D was successful in developing a procedure/technology to reduce the incidence of dark meat, what is the cost of implementation/installation that would be viable?

5. Investigate the influence of a thermal treatment on muscle structure and light scattering using the confocal microscope to determine the temperature/time required to achieve a change in light scattering/ colour.

6. Investigate the influence of a thermal treatment on light scattering properties of pre- and post-rigor loin steaks and on microbial counts.
   (i) Determine if a mild heat treatment can induce lightening of dark non-compliant beef cube rolls.
   (ii) Assess the effect of a heat treatment on meat quality (colour and tenderness) and meat structure (sarcomere length and drip loss).

Determine the effect of a heat treatment on microbiological and retail display life of cube rolls.

4.0 METHODOLOGY

4.1 Light scattering and reflection confocal scanning laser microscopy (rCLSM)

In this project a novel microscopy technique was developed to determine the role of muscle structure in determining beef surface colour. A light scattering technique based on reflection confocal scanning laser microscopy (rCLSM) was developed as this technique allowed visualisation of fresh muscle without the need for fixation or cryo-sectioning. The detail of the method is outlined in Section 4.5.3.

This light scattering method was used to determine the structure of:

1. Muscle fibre fragments

2. Muscles from high and low pH meat

3. Muscles from light, medium and dark meat

4. Muscles ranging in rate of pH fall

4.2 Industry survey on dark-cutting beef carcasses

A literature review was undertaken to identify post mortem factors that influence meat colour and establish strategies for optimising meat colour at grading. The findings from this literature review formed the basis for the potential interventions that were discussed with the processors.

Seven processors were interviewed on the incidences and impact of dark cutting in beef carcasses at their plants. Data was obtained from a total of fifteen plants over five states on the eastern seaboard of Australia and comprised ~42% of all Australian cattle slaughtered in 2014 (ABARES 2015). Data was not able to be obtained from processors in the Northern Territory and Western Australia. The processors interviewed ranged from single plants to large processors that owned more than six plants.

The processors were asked questions about their slaughter procedures, post slaughter treatments and carcass handling, time post slaughter for MSA grading, meat colour scores in various carcass categories, incidences of dark cutting and related cost. Their opinion was also sought on some intervention strategies which were reviewed and developed to reduce dark cutting. The questionnaire used for the interviews is included in Appendix 1.

4.3 Cost Benefit Analysis (CBA)

In order to highlight and understand the financial cost of dark cutting to processors and how possible interventions could impact on their business, CSIRO contracted Greenleaf Enterprises Pty Ltd to develop a cost-benefit analysis (CBA) model on dark colour for beef carcasses. Greenleaf have experience and expertise in developing and implementing business strategies and operational processes that promote insight, innovation and company growth for the meat industry.

4.4 Mild heat treatment trials

4.4.1 Preliminary trials

Mild heat treatment of dark cuts was selected as an intervention to induce lightening in dark meat as initial trials proved the lightening effect and the lower technical requirements compared to other processing methods, and thus, higher feasibility for further implementation in the industry. A range of trials were undertaken during various project milestones to verify and optimise the format of the meat (i.e. steaks or primals), optimal time of application post-mortem (14 hours to 4 days) and the heating conditions (temperature and time required), which is summarised in Table 1.

Initially, for optimising the treatment conditions, trials were conducted on steaks cut from dark primals. Then, whole cube rolls were used to determine the most effective method of inducing lightening in dark muscles.

Table 1. Summary of experimental conditions used for the different trials during the course of the project.

Milestone	Primal/ steak	Time post mortem (h)	Grader pH	Grader colour AMC	Temp water bath °C	Duration (h)	Internal Temp (°C)
3 (a)	steak	14	5.99	6	40	1	--
3 (b)	steak	70	5.92	5	40	1	39.5
6 (a)	primal	70	5.92	5	40	1	23
6 (b)	primal	22	5.60	4	40	3.5-4	35.0-37.1
6 (c)	steak	22	5.60	4	30	1	29.8
6 (c)	steak	22	5.60	4	35	1	34.7
6 (c)	steak	22	5.60	4	40	1	40.1
8 (a)	steak	24	6.29	≥5	40	1	~40.0
8 (b)	steak	24	6.29	≥5	45	1	~45.0
8 (c)	steak	96	6.03	≥5	40	1	~40.0
8 (d)	steak	96	6.03	≥5	45	1	~45.0
9 (a)	Primal	--	6.59	6	45	4	45.0
9 (b)	Primal	--	5.83	5	45	16	45.0

4.4.2 Mild heat treatment method to induce lightening in dark primals

Based on the findings from the various preliminary trials, a mild heat treatment method was developed to induce lightening of dark (Australian meat colour (AMC) ≥5) non-complaint beef cube rolls.

For this trial, twenty beef cube rolls (primals), from ten animals (left and right cube rolls) were collected and accumulated over a working week in order to minimise application of a single heat treatment every day, and thus, facilitate future industry adoption. Once primals arrived at CSIRO Agriculture & Food (Coopers Plains), left and right primals were randomized into heated or non-heated groups, and each

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pair was kept together in a 4 °C chiller until the heat treatment. The primal pairs were further randomised into storage times of 1 or 24 days. The heat treatment was applied to the cube rolls within 49-96 hours post-mortem and all primals collected during the week were treated as a unique batch. The experimental design and treatment is shown in Figure 5.

At the specified time, primals were removed from vacuum packaging, weighed, and i-buttons were inserted into the centre of the primals to monitor the internal temperature. Control primals were stored at 4 °C in a chiller. The heat treated samples containing the i-buttons were placed in a circulating water bath at 45 °C for 5 hours. Once heating was completed, the primals were immediately cooled in an ice slurry for 60 min, and subsequently, held at 4 °C overnight. On the following day, day 1 primals were cut into steaks from caudal to cranial end (Figure 5 below). The first two steaks were cut to 20 mm thickness, followed by a 40 mm thick steak for texture analysis, and the subsequent three steaks were cut to 20 mm in thickness (Figure 5). All steaks were placed into black polystyrene trays, overwrapped and placed in a display cabinet at 4 °C for monitoring colour stability and shelf life over chilled display. Day 24 primals were held in a 4 °C chiller for 24 days and then cut into steaks and subjected to the same evaluations as for day 1 samples.
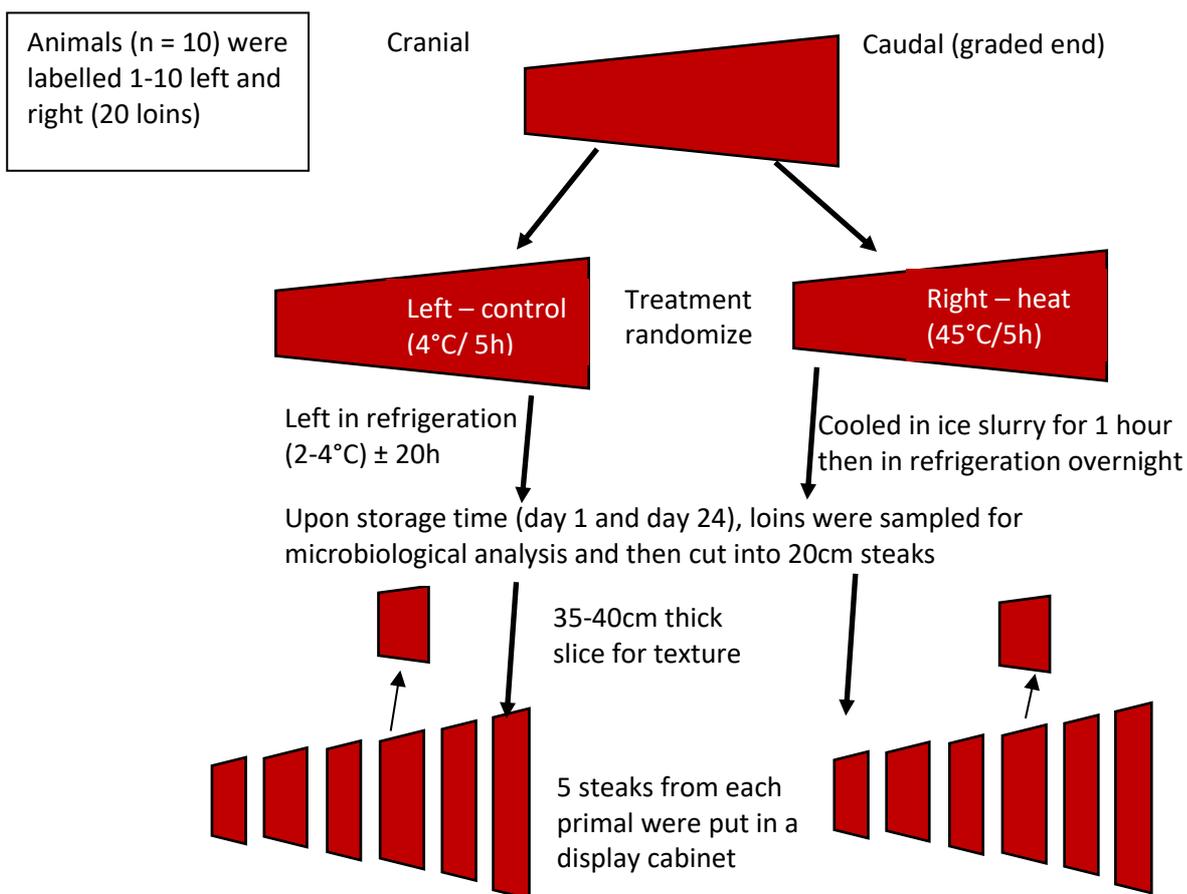

Figure 5. Flow chart of experimental design.

4.5 Analytical methods

4.5.1 Collection of muscles, grading and processing

Beef cube rolls were collected from a local abattoir (Queensland) from animals that were handled, slaughtered and processed according to standard industrial practices. The carcasses were chilled, and the left and right sides quartered between the 13th and 14th vertebrae. After 30 min blooming, the exposed cube roll or *M. longissimus thoracis* (LT), was assessed by a qualified AUS-MEAT grader for meat colour (AMC, Figure 1), pH and rib fat (mm) as per the chiller assessment recommendations (AUS-MEAT, 2005). Dentition scores and hot carcass weight (HCWT) were also obtained. Carcasses were selected based on having an AMC ≥5 (dark non-compliant meat) and pH ~ 6.00 or higher. After grading, beef cube rolls were removed from the carcass, vacuum packed, and transported in an insulated container to the laboratory where mild heat treatment was applied. For the trial where samples were collected from animals ranging having different rates of pH fall (slow to fast), primals were collected from electrically simulated and non-stimulated carcasses.

4.5.2 Preparation of muscle fibre fragments for rCLSM

Muscle fibre fragments were extracted according to the method of Warner, Kauffman, and Greaser (1997) with modifications. Samples 1±0.05 g were homogenised on ice (Ultraturrax, 11,000 rpm, 3* 4 second pulses) in 10 mL of 380 mM mannitol and 50 mM potassium acetate buffer, pH 5.6, to minimise osmotic modification of fibre fragments (Winger & Pope, 1981). Homogenates were centrifuged (4,000 g, 10 minutes, 4 °C) and the supernatant was discarded. Pellets were resuspended in 5 mL of homogenising buffer, stirred rigorously and were filtered through flyscreen (1x2 mm dimensions) to remove the connective tissue. The residue was washed using another 5 mL of homogenising buffer and subsequently discarded. The combined filtrate was centrifuged as previously described. After discarding the supernatant, the pellet was washed twice using 5 mL of homogenising buffer and was finally resuspended in 5 mL homogenising buffer. A drop containing extracted muscle fibre fragments was placed onto a circular 30 mm diameter coverslip (ProSciTech, G430, Thuringowa, QLD, Australia) and covered with a square 24x24 mm coverglass (ProSciTech, G410). The fibre fragment width, measured as a straight line perpendicular to the fibre axis (μm), was recorded. For each sample, a total of six replicates were measured and the mean of these results was considered the width of the fibres for that sample.

4.5.3 Method for reflection confocal scanning laser microscopy (rCLSM)

An Olympus Fluoview™ 1000 confocal laser-scanning microscope at Griffith University (Eskitis centre), was used in reflectance mode (473 nm blue diode laser applied at 15%, bandwidth of 470-545 nm). Therefore, <u>no fluorescence</u> was used. The microscope had an inverted objective lens, a numerical aperture (N.A.) 1.35, 60x magnification and aperture of 105 μm. Signal amplification was achieved using a photomultiplier tube (PMT) at 400 V (8% offset) and 2 X gain. For image acquisition, high pixel resolution at 1024x1024 and a slow speed (20 μs/pixel) was used. Muscles (4 images per sample) were viewed longitudinally (20 μm depth) and global brightness and fibre fragment widths (μm) were made using Image J software (Rasband 2014).

4.5.4 Global brightness determination from image analysis

Global brightness was used as the quantification measure of light scattering and was calculated from the intensity of the image generated. It was determined using Image J software program (Rasband, 2014). Briefly, images were opened in TIFF format and calibrated using the scale bar (10 μm length) on

the image. Polygon sections of the fibre fragment area were selected (μm^2) and the software generated a global brightness or mean grey intensity (average brightness of the pixels) within the selected area (i.e. the muscle fibre). For each sample, a total of six replicates were measured and the mean of these results was considered the global brightness for that sample.

4.5.5 Colour measurements

Two different types of colourimetric equipment were used to determine the objective colour measurements of steaks and primals.

<u>Hunterlab Colorimeter</u>

Objective colour measurements were made on steaks post-heating and after blooming at cold temperatures (5-10 °C), for 60 ± 10 min. A Hunterlab Miniscan EZ 45/0 LAV (light source A, observer angle 10°, 25 mm viewed area) was used to measure *L** (lightness), *a** (redness) and *b** (yellowness) attributes in triplicate. The instrument was calibrated, using white and black calibration tiles, as supplied with the instrument (Novasys group Pty Ltd, Ferntree Gully, Vic, Australia) at the same temperature. Colour parameters were calculated as follows:

$$hue = arctac\left(\frac{b^*}{a^*}\right)$$

$$chroma = \sqrt{a^{*2} + b^{*2}}$$

A spectral scan from 400 to 700 nm was also completed and used to calculate the three forms of myoglobin; deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) using the isobestic points (Krzywicki 1979; Hunt 2012).

<u>Minolta Chroma Meter</u>

The Minolta had a different illuminant (D65) and smaller aperture (8 mm diameter measuring area) than the Hunterlab colorimeter. The instrument was calibrated using a standard white calibration tile and triplicate colour measurements were taken through the plastic overwrap film covering the steaks (as done for calibration). Colorimetric measurements *L**, *a** and *b** with calculations for hue and chroma were made as previously described, but no reflectance scans or myoglobin forms could be calculated.

4.5.6 Drip loss in heated primals and steaks

All samples were weighed before and after heating to determine the impact of heating on drip loss. Drip was calculated as a percentage of the difference between the original weight and the post-process/storage weight. The samples were weighed before treatment and the following day after treatment to record any weep during storage overnight at 4 ˚C. The total drip loss due to the heat treatment was calculated using the weights before heating and after overnight storage in the chiller.

4.5.7 Conductivity

The conductivity of the steaks was measured using a LF-Star (Matthaus GmbH and Co, Nobitz, Germany) conductivity meter for meat. The conductivity meter was checked against a 10 µS/ cm reference supplied with the instrument according to the manufacturer's instructions. The conductivity is an indicator of the state of the meat structure as it reflects an increased cell-liquid and ion flows between the intercellular spaces of muscle (i.e. PSE-like meat will have a higher conductivity).

4.5.8 pH measurements

The pH of the primals was measured using a TPS WP-80 pH meter with a polypropylene spear-type gel electrode (IJ 44) and temperature probe (all obtained from TPS Pty Ltd, Brisbane, QLD, Australia). It was calibrated at pH 4.00 and 7.00 at a similar temperature to measurement (~10 °C).

4.5.9 Texture measurements

Texture measurements were carried out on cooked samples using a Lloyd LS 2.5 with a 500 N load cell (Lloyd Instruments, West Sussex, United Kingdom) and a modified Warner-Bratzler shear device (Bouton, 1971 and 1972). The samples were cut into rectangular shapes with dimensions of 15 mm width, 6.7 mm height, giving a cross-sectional area of 1.00 cm^2, and at least 25 mm long to enable secure clamping of the sample into the holder. A triangular shaped blade with a thickness of 0.64 mm was attached to an overhead clamp and was pulled up through the muscle fibres, perpendicular to the fibre direction, at a speed of 100 mm/min. The maximum peak force (PF) and initial yield (IY) were objectively determined using Nexygen Plus V3.0 software (Lloyd Instruments, West Sussex, United Kingdom). The difference between these measurements (PF-IY) was also reported. At least, six determinations were made on each sample and the mean recorded.

4.5.10 Sarcomere lengths

Sarcomere length was measured using a helium-neon gas laser diffraction technique on unfixed, previously frozen (-20 ˚C) samples. A laser wavelength of 635 nm was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides. Sarcomere length was determined from the diffraction pattern displayed on a frosted screen (Bouton et al. 1973). Sarcomere length (μm) was calculated from the average distance (mm) of the inner and outer diffraction bands from the centre of the screen. The mean of eight readings was taken per sample.

4.5.11 Microbiological analysis

Microbiological testing was conducted on primals upon opening from vacuum with a composite sample comprising 20 cm^2 of adipose and 20 cm^2 of lean tissue collected from each primal for analysis. Samples were placed into a stomacher bag containing 100 mL 0.85 % saline, then stomached for one minute and decimal dilutions prepared in 0.85 % saline (for total viable count (TVC) and E. coli/ coliforms) or MRS broth (for lactic acid bacteria (LAB)).

All samples were microbiologically assessed for total viable counts (TVC), E. coli, coliforms and (lactic acid bacteria (LAB) using Petrifilm Aerobic count plates, Petrifilm E. coli/ coliform plates and the Petrifilm Aerobic count and MRS plates, respectively. Petrifilm Aerobic count plates were incubated at 25 ± 1°C for 72 ± 3 h; Petrifilm E. coli/ coliform plates were incubated at 35 ± 1 °C for 24 ± 2 h; LAB plates were incubated anaerobically at 25 ± 1°C for 120 ± 3 h.

4.5.12 Simulated retail display

Thick steaks (20 mm) were cut from control and heated cube rolls and placed in black foam trays (205 x 130 x 12 mm) overwrapped and stored in a 4 °C display cabinet to determine the visual colour shelf life of the steaks. The light source in the cabinet consisted of 3 light tubes (Osram Natura L 36W/76) located on the top and sides of the interior of the cabinet which was illuminated for up to 10 h a day. The light intensities on the top, middle and bottom shelves were 1081 lux, 350 - 721 lux and 290 – 474 lux, respectively. Steaks were rotated between top, middle and bottom shelves daily.

Steaks were displayed for a total of 5 days and photographs and Minolta colour measurements were

taken on every second day.

4.5.13 Visual assessment

Visual assessment of meat was conducted on day 0 (initial), day 3 and day 5 (final day). A panel of 9-10 consumers (3 female, 6-7 male) assessed the steaks and scored the samples on a scale of 1-10 for meat colour, fat colour and overall acceptability, and were asked whether they would purchase these steaks from a store. The questionnaire used is in Appendix 1.

4.6   Statistical Analysis

ANOVA statistical analysis was completed using Genstat 18th edition (GenStat 2008).

5.0 PROJECT OUTCOMES & DISCUSSION

5.1   Light scattering and reflection confocal scanning laser microscopy (rCLSM)

The present section describes the development of a new rCLSM technique to visualise muscle microstructure.

5.1.1   Sample viewing

The depth through the muscle was visualized between 0 and 40 µm and sample images are illustrated in Figure 6 (x/y planes) and in Figure 7 (z plane). An incredibly light and over-exposed image was generated at the surface of the muscle and there was a subsequent darkening effect as the depth of imaging was increased. Difficulty was experienced in determining the depth of one fibre alone for 2 main reasons. Firstly, it was hard to determine where one fibre finished and the adjacent fibre started, and secondly, the increase in darkness observed through the depth of the fibre made it difficult. This made it hard to standardize observations at the same depth of the muscle fibre. For this reason, the decision was made to extract fibre fragments for viewing. These were then viewed in the longitudinal orientation.

(a)   (b)   (c)
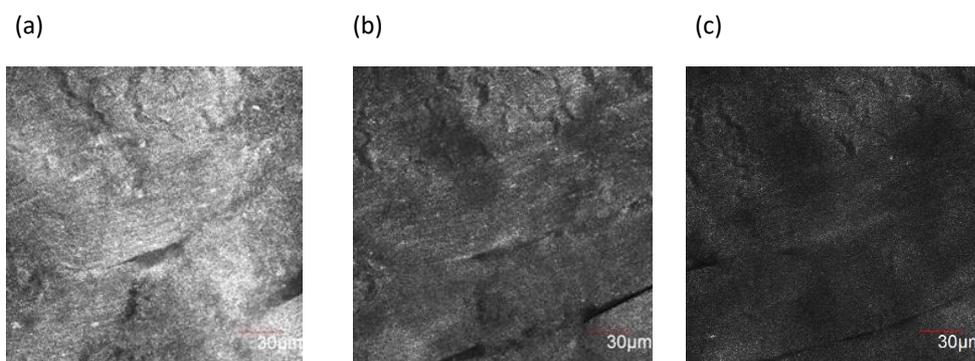
Figure 6. Light scattering through the z plane of a longissimus muscle, displaying; (a) a high level of scattering at the surface (~10 µm deep); (b) intermediate scattering in the middle (~20 µm deep); and (c) low scattering at the bottom (~40 µm deep). Images are in the x/y orientation, with red scale bar is for 30 µm and all settings were standardised.

Figure 7. Light scattering through the z plane of a longissimus muscle, displaying a high level of scattering at the surface (top) and low scattering at the bottom. Images were taken at 0.5 µm intervals from 0 to 40 µm into the depth of the tissue.

5.1.2   Specificity/ interferences

When viewing the extracted individual fibres, there were some similarities to the muscle. The top surface of the extracted fibre often showed a high level of scattering as was observed in with muscle. As the viewing window was moved down into the z plane of the fibre, the intensity of scatter

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decreased. Thus, the optimal image depth was determined to be at ⅓ fibre depth, where connective tissue interference was avoided and underexposed images were minimised.

5.1.3 Bias/ trueness:

For one muscle extract, 2 slides were prepared and a total of 6 images were made from these slides (3 images/ slide). Bias was minimised by completing analysis using a blind sampling technique. Prior to analysis, samples were allocated a random 3 letter code, which was unknown to the microscopist during image capture. In addition, when viewing a slide, the first fibre observed was always captured and included in the data analysis and was considered to minimise microscopist discretion.

5.1.4 Laser and bandwidth

A 473 nm diode laser (blue) was applied at 15%. The extent of light scattering is known to be wavelength dependent, with shorter blue wavelengths being scattered more than longer red wavelengths (S Jacques, 1996; S. Jacques, 2013). Thus, a laser wavelength was chosen in the region of the visible spectrum with most scattering. The largest bandwidth at the top of the viewing window was also selected, which spanned over both blue and green wavelengths. Therefore, the bandwidth of the variable band filter (VBF) was adjusted to 470-545 nm for both excitation and emission spectra, in order to obtain the reflectance data.

5.1.5 Optimised detector & magnification settings

To view extremes in light scattering, the photomultiplier tube (PMT) detector settings (voltage, gain, offset) was optimised to achieve suitable images for both light and dark muscle extremes. These settings were:

1. high voltage (HV) of the photomultiplier tube (PMT) was set to 400 V (8% offset) post-PMT amplification (gain) was 2x
2. a numerical aperture (N.A.) 1.35 and 60x magnification was used. The scatter unit confocal aperture (SU-CA) was set to 105 µm.

For image acquisition, high pixel resolution at 1024x1024 and a slow speed (20.0 µs/pixel) was used.

These settings were kept constant for all the image analysis, regardless of level of scattering of the sample. Consequently, some images did appear over exposed, but this allowed visualization of fibres that had a lower amount of scattering.

5.1.6 Image analysis/ Global brightness determination:

Global brightness was used as the quantification measure of light scattering and was calculated from the intensity of the image generated. The method used for image analysis is described in Section 4.5.4. Example images of the longitudinal light and dark muscle fibre fragments and their relevant individual lightness and pH_u values are displayed in Figure 8, with the group means summarised in Table 2. The 'dark' muscles with a lower colorimetric lightness (P<0.001) displayed a lower global brightness (P<0.001) compared to the 'light' muscles. Interestingly, 'dark' muscles also displayed a significantly higher (P<0.001) fibre fragment width compared to those of 'light' or 'medium' muscles. Thus, there appears to be a swelling effect, or lack of shrinkage, occurring in the fibres from the 'dark' group. This change in the structure of the fibre may be contributing to the reduction in global brightness and the colorimetric lightness values observed. These associations are described further below.

Between 'light' and 'medium' coloured muscles, there was no significant difference in global brightness or pH_u values. The pH_u data was observed to be heteroscedastic (larger variation with 'dark' muscles) and was unable to be rectified with transformation, hence considered to be non-normal data. The large variation observed in the 'dark' muscle group, illustrates inconsistencies that occur within the beef industry; best illustrated by the observation that only approximately 28% of muscles with a pH=5.80 (high), had a dark meat colour score >3 (Hughes et al., 2014). So, high pH muscles are not necessarily dark and *vice versa*. In comparison, 'light' and 'medium' muscles showed a consistently low pH_u, with no difference being observed between these groups. Similarly, the 'light' and 'medium' groups also displayed a similar lightness, global brightness and fibre width, whereas the darker muscles were significantly different.

(a) 'Light' muscle

L*= 37.4

pH_u = 5.43
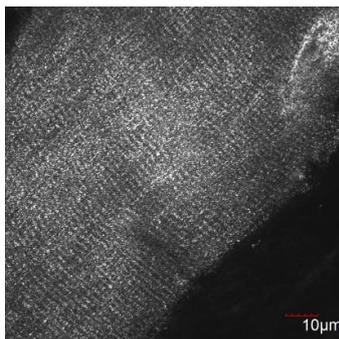
(b) 'Light' muscle

L*= 35.5

pH_u = 5.43
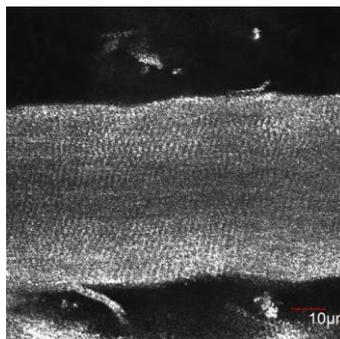
(c) 'Light' muscle

L*= 35.7

pH_u = 5.38
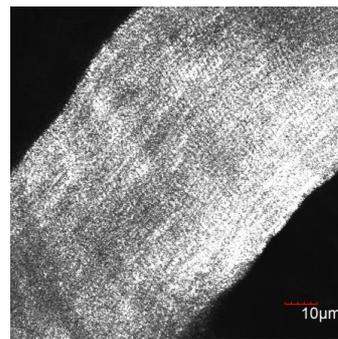
(d) 'Dark' muscle:

L*= 24.8

pH_u = 6.41
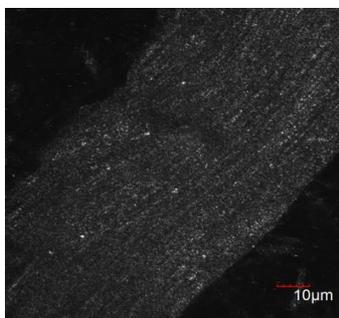
(e) 'Dark' muscle:

L* = 23.7

pH_u = 6.31
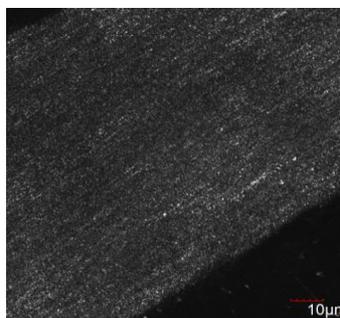
(f) 'Dark' muscle:

L*= 27.2

pH_u = 6.53
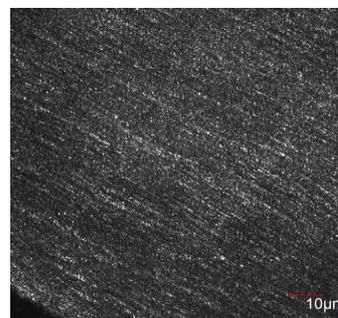
Figure 8. Effect of colour group on confocal reflection microscopy images of beef *longissimus lumborum* muscle fibre fragments obtained from light (a, b and c) or dark (d, e and f) muscles. The different meat colour groups 'light' or 'dark' are defined by AUS-MEAT Colour (AMC) scores: 1B or 1C; >3 respectively. Red scale bar is for 10 μm.

Table 2. Effect of colour group (light, medium, dark) on variates of beef *longissimus lumborum* muscles (n=6). The three different meat colour groups light, medium or dark are defined by AUS-MEAT Colour (AMC) scores: 1B or 1C; 2 or 3; >3 respectively. Values are ANOVA means and the least significant differences (LSD) and *P*-values from comparison tests are shown.

	Light	Medium	Dark	LSD	*P* value
pH_u[1]	5.41	5.42	5.84	0.132	<0.001
Global brightness (grey value)	98.5	112.9	77.9	16.10	<0.001
Fibre fragment width (µm)	61.4	60.8	72.3	5.90	<0.001
Lightness (*L**)	37.5	35.0	29.7	1.80	<0.001
Redness (*a**)	29.4	28.5	26.6	1.22	<0.001
Yellowness (*b**)	21.6	20.4	18.6	1.42	<0.001
Chroma	36.5	35.1	32.5	1.82	<0.001
Hue	36.4	35.5	34.7	0.80	<0.001
Myoglobin concentration (mg/g)	4.8	5.2	6.6	0.68	<0.001
Oxymyoglobin (%)	74.3	70.1	65.0	3.77	<0.001
Deoxymyoglobin (%)	12.1	15.4	18.6	3.83	0.005
Metmyoglobin (%)	13.7	14.6	16.4	1.37	<0.001
Myofibrillar protein concentration (mg/g)	75.1	74.5	68.7	3.58	0.001

[1]Not normal data-heteroscedastic data and unable to be transformed, statistical output from ANOVA only.

Significant correlations (*P*<0.05) were found with global brightness and lightness (R^2=0.27), pH_u (R^2= -0.47) and fibre fragment width (R^2= -0.27) (see Appendix 2). Overall, there were three main findings. Firstly, the lightness as measured colorimetrically has a positive correlation with the global brightness as measured using the newly developed rCLSM method. As colorimetric lightness values increased, so too did the global brightness, with the 'light' meat colour group having the higher values for both variates and vice versa for 'dark' muscles (Figure 9a). This illustrates that the global brightness of the fibres, as generated from the images, provides an indication of the overall lightness of the muscle surface. Hence, these results confirm the early observations, which suggest pale, soft, exudative muscles have an rCLSM speckle pattern which is different to normal muscles (Offer et al., 1989). Together, these results highlight the importance of the structural elements to the achromatic colour component of the muscle.

Secondly, the global brightness was negatively correlated with the pH_u (R^2= -0.47, *P*<0.05), again confirming pH dependence with the light scattering properties of the muscle (Figure 9b). The separation of the 'dark' colour group was evident and showed less variation, especially above pH_u 5.8, whereas between pH_u 5.4 to 5.8, the variation was much larger. The similarity in pH_u values for 'light' and 'medium' groups was less than favourable, but the large variation in global brightness was expected. Thus, from the data it appears that muscles passing through pH_u 5.8 have different structural properties, compared to those muscles that remain above this value. Dark cutting beef muscle, with a high pH has been described as having more of a 'close' structure, (Lawrie, 1958) which results in higher transmission, more absorption and reduced light scattering (Bate-Smith, 1948; H.J Swatland, 2008). As illustrated in Figure 10, this could be related to the fibre diameter or width within the muscle.
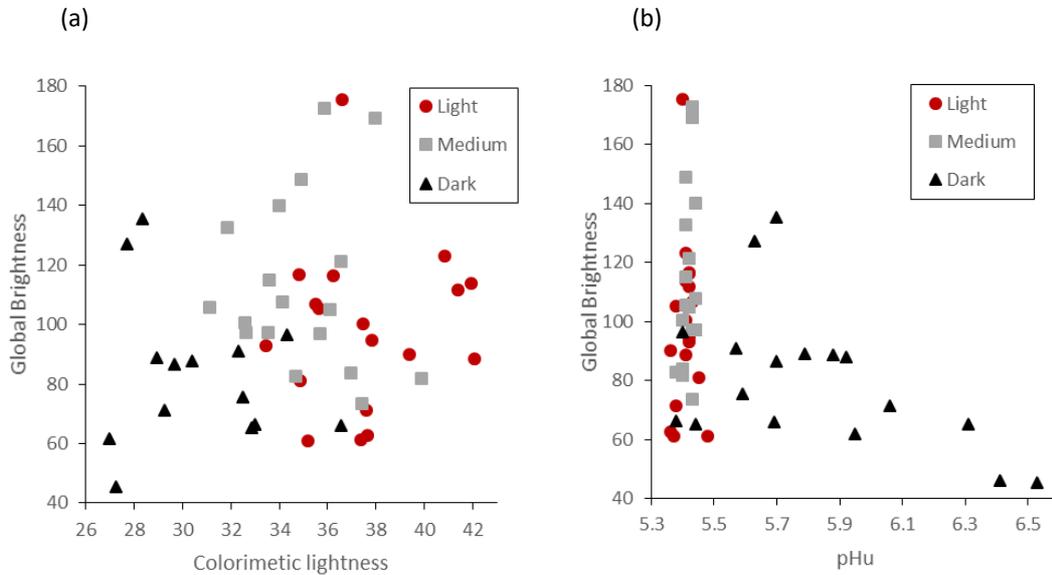
Figure 9. Scatter plot of (a) colorimetric lightness values and (b) pH_u on global brightness values of beef *longissimus lumborum* muscle fibre fragments from light, medium or dark muscles as defined by AUS-MEAT Colour (AMC) scores: 1B or 1C; 2 or 3; >3 respectively.
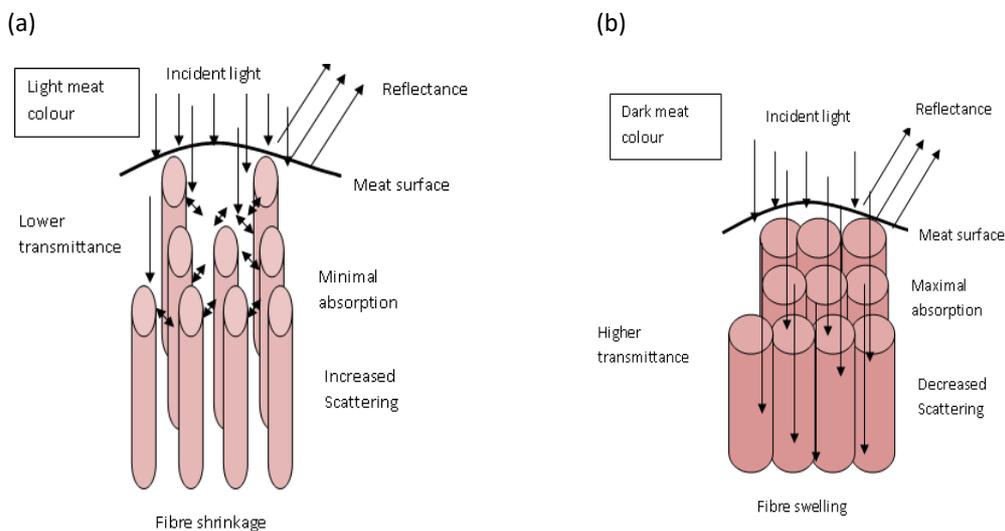
Figure 10. Diagrammatic representation of muscle fibres in (a) 'light' or (b) 'dark' muscles. As incident light hits the surface of 'light' muscles, the shrunken fibres allow for lower transmittance, minimal absorption and increased scattering. In comparison, 'dark' muscles with more swollen fibres, display higher transmittance, maximal absorption and decreased scattering of light. Adapted from (H. J. Swatland, 2004).

Finally, these structural attributes are confirmed with the negative correlation that exists between the fibre fragment width and the global brightness (R^2= -0.27, *P*<0.05). Thus, the wider fibres are scattering less light, as determined by this new rCLSM method and clearly illustrate the swelling (or lack of shrinkage) that occurs in the high pH, 'dark' group (Figure 10b). In comparison, fibres from 'light'

muscles have a smaller width and appear to scatter more light (Figure 9a). These are the first results that clearly show the negative correlation that exists between fibre shrinkage and light scattering, highlighting the importance of structural contributions to colour.

In addition, the colorimetric lightness is also negatively correlated with fibre fragment width (R^2= -0.61, P<0.001). This again confirms that wider muscle fibres give a darker appearance to muscle. This leads to the consideration of the swelling mechanism driving the visual appearance of the meat and therefore provides opportunities for modifying the fibre diameter to impact the colour and hence the value of the carcass.

Table 2 also shows that 'dark' muscles were less red and yellow and had lower values of chroma and hue. There was also a reduced level of oxymyoglobin (OMb) and increased amount of deoxymyoglobin (DMb) at the surface of the meat which is consistent with other findings and has been reported as being due to the "unusual structural features" in dark-cutting beef (Lawrie, 1958). Thus, 'dark' beef has only a thin OMb layer and confirms the present observations. There was also a higher concentration of myoglobin in the 'dark' group, which could have been additive to the darker colour observed.

Lastly, the myofibrillar protein concentration was lower in the 'dark' muscle group. These results illustrate the difference that exists in protein solubility with this particular buffer system. The lower protein concentration of the 'dark' group was unexpected and difficult to explain, as previous observations in other species have indicated this group would have a higher concentration; however other studies typically use a phosphate buffered system, which may contribute to the difference observed here (Warner et al., 1997).

5.1.7   Effect of rate of pH fall on light scattering

Figure 11 shows that the pH decline for stimulated carcasses was much faster compared to non-stimulated carcasses and no differences were observed in the pH_u at 24 h PM, which is similar to previous findings (Hopkins et al. 2014). Mean values for pH_u at 24 h PM for non-stimulated and stimulated muscles were 5.61±0.06 and 5.54±0.16 respectively, which are well below the MSA dark-cutting non-compliance value of pH 5.71. The stimulated carcasses passed through pH 6 at 30°C, and so would be within the "window" as recommended by MSA (Meat and Livestock Australia Limited 2011), whereas non-stimulated carcasses passed through pH 6 at <5°C and can be considered to be cold shortened, as confirmed with the shorter sarcomere lengths (P<0.05) as documented in Table 3.

The faster glycolytic rate in the stimulated carcasses yielded a different meat colour, as shown in Table 3. Electrically stimulated *longissimus* muscles were lighter, redder, yellower and consequently had higher hue and chroma values. The muscles had significantly (P<0.05) higher levels of red oxymyoglobin and lower levels of purple deoxymyoglobin, whilst brown metmyoglobin levels were similar. Similar colour changes have been observed in other studies, with electrical stimulation promoting the oxygenation of myoglobin and hence resulting in a redder appearance (Ledward et al. 1986; Li et al. 2011). Lightness values from non-stimulated muscles were similar to those reported for AMC 2 or 3, whereas redness and yellowness were lower than those reported for dark AMC. These findings are indicative of the large impact that the lack of myoglobin oxygenation had on non-stimulated muscles. This highlights the importance of effective electrical stimulation for promoting myoglobin oxygenation and hence redness of the meat within this type of carcass.
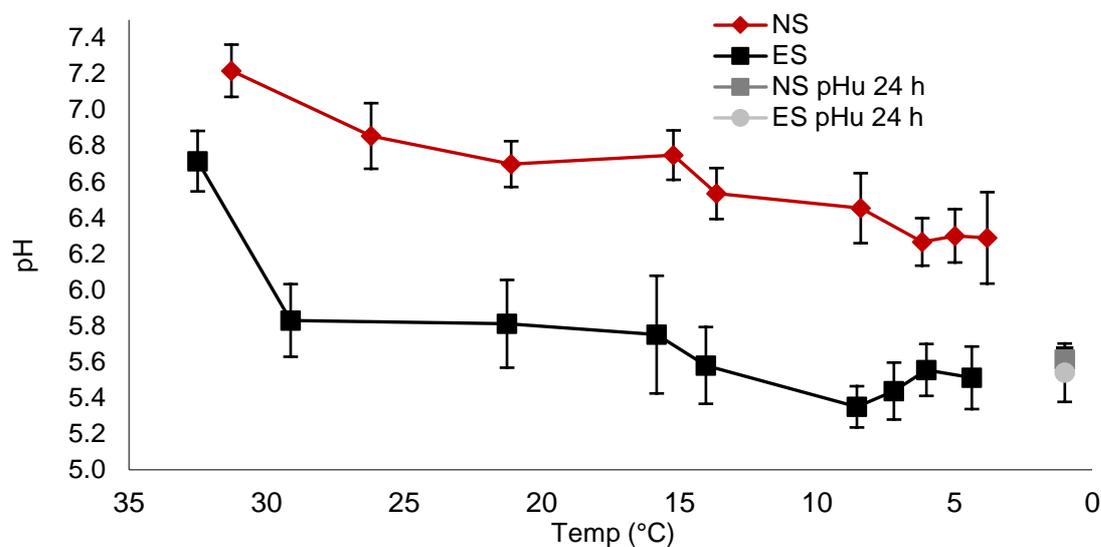
Figure 11. Effect of stimulation (ES, electrically stimulated; NS non-stimulated) on the pH-temperature decline of beef *longissimus* muscles (n=8). Each point represents the mean and error bars represent standard deviation.

Table 3. Effect of stimulation (ES, electrically stimulated; NS non-stimulated) on variates of beef *longissimus lumborum* muscles (n=8). Colour measurements made using Hunterlab Miniscan EZ on the transverse cut of the muscle between the 12th and 13th vertebrae. Values are ANOVA means and the least significant differences (LSD) and *P*-values from comparison tests are shown.

	ES	NS	*P*-value	s.e.d
Lightness (*L**)	39.9	33.6	0.002	1.58
Redness (*a**)	28.0	24.4	0.006	1.09
Yellowness (*b**)	19.6	15.8	0.006	1.16
Hue	34.9	32.9	0.016	0.72
Chroma	34.2	29.1	0.006	1.55
Oxymyoglobin (%)	73.1	63.4	0.006	2.93
Deoxymyoglobin (%)	12.5	20.2	0.013	0.16
Metmyoglobin (%)	14.5	16.3	N.S.	1.19
Sarcomere length (µm)	1.82	1.59	0.003	0.065
Global brightness	114	139	0.036	23.4
Fibre width (µm)	58	62	N.S.	8.1
Myofibrillar protein concentration (mg/g)	74	72	N.S.	5.2
Sarcoplasmic protein concentration (mg/g)	32	31	N.S.	1.2

At the time of colour measurements, no differences in oxidation of myoglobin were observed, as there were similar brown metmyoglobin levels between treatments. In short, these findings support the widely published findings that the faster glycolytic rate, induced by electrical stimulation, increases

myoglobin oxygenation and promotes redness. Lightness is also improved by the treatment, but it is believed the mechanism behind this lightening is not solely due to myoglobin but also due to changes in sarcomere length.

Light scattering measurements, using the newly developed rCLSM revealed differences between treatments (Table 3 and Figure 12), however these differences were opposite to those hypothesized earlier and maybe a confounding effect due to cold shortening of the non-stimulated muscles. In the previous trial colorimetric lightness values were positively correlated to global brightness. However, within this latter experiment, the lighter electrically stimulated muscles displayed lower global brightness, indicative of another mechanism involved. The higher global brightness values in the non-stimulated muscles occurred due to the sarcomere shortening, demonstrating an inverse relationship between sarcomere length and light scattering which has also been observed elsewhere (Swatland 2003, 2004). The short sarcomeres induced by cold shortening may have decreased the isotropic region (I-band), through increased overlapping between myofilaments and thereby increased the anisotropic properties along the muscle. In turn, the reduced I-band region and increased level of birefringence created by the A-band may have increased the global brightness within the muscle fibre. This may have resulted in a confounding of the colour differences as measured using the colorimeter. In short, electrical stimulation promoted lightness, but the scattering effect was negated by the longer sarcomeres within the myofibrils, indicative of the complexity of the microstructure and its impact on dark cutting.

Figure 12. Reflectance confocal laser scanning microscopy (rCLSM) of beef *longissimus* muscles (n=8), without stimulation (a-c) or with electrical stimulation (d-f). Scale bar 10μm.

5.2 Industry survey on dark-cutting in beef carcasses

A literature review on potential intervention technologies to optimize meat structure and reduce dark cutting was undertaken and some of these interventions were discussed with processors durin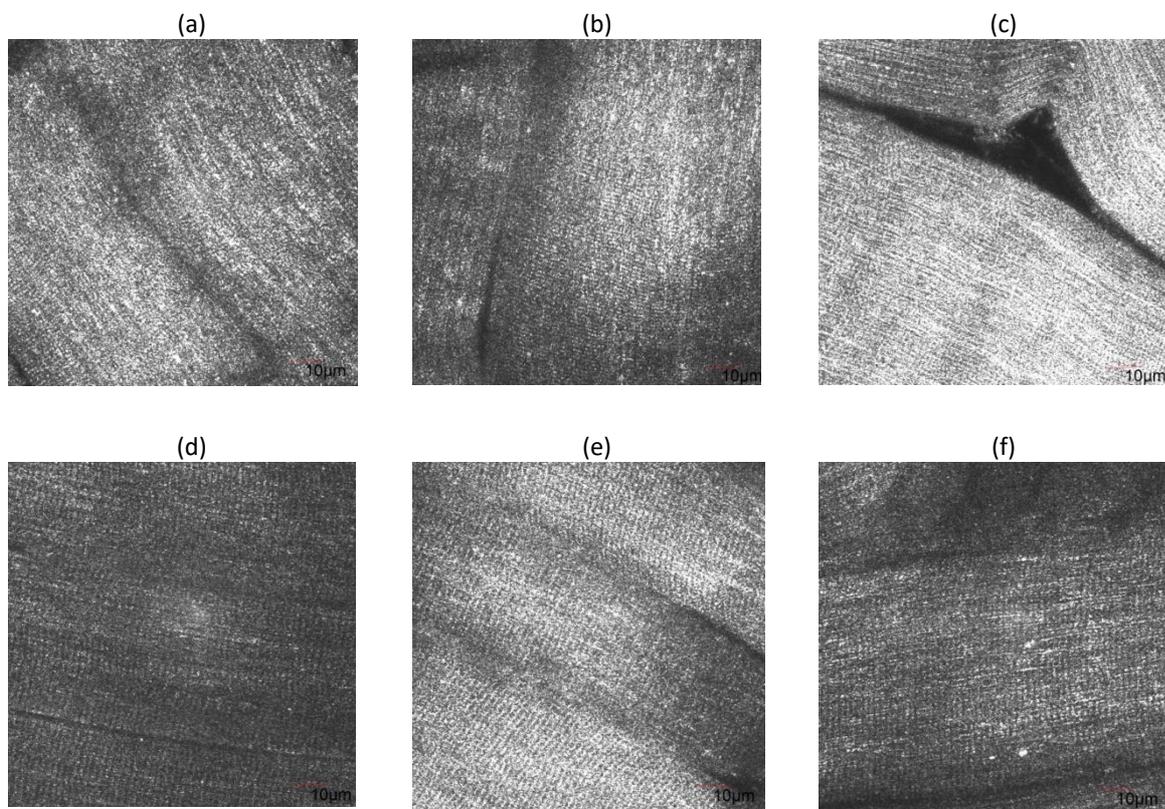g the survey. The number of cattle slaughtered annually by the seven processors and 15 processing plants ranged from 60 000 to 820 000. This included cattle for export and domestic markets, grain and grass fed animals, a range of categories of animals and plants that operated from 7.6 to 20 hours per day, on average 5 days a week.

5.2.1 Literature review on optimising meat colour during post mortem handling

Increase time from slaughter to grading

Increased time from slaughter to grading will allow for adequate full rigor development and shrinkage of the lattice, which will ensure maximum lightness of the meat. The time from slaughter to grading is influential on the colour of the meat graded, with shorter times associated with a higher incidence of dark cutting (DC) in beef (Murray 1989; Hughes et al. 2014a). Meat graded 14 h PM was 2 to 3 times more likely to be graded as DC, compared to those graded later, after 31 h PM (8% vs. 3% respectively). Initially, gaps start to form between muscle fibre bundles at around 4-6 h PM, but the gaps between muscle fibres are still forming 24-48 h PM and are partially responsible for the development of light scattering in the muscle. The effects of this longer storage prior to grading is believed to be most evident where electrical stimulation is not being applied or if it is not functioning properly, as unstimulated carcasses will have a slower pH decline and hence slower glycolytic rate, which is believed to impact on the colour to a greater magnitude between 24-48 h PM (Orcutt et al. 1984).

Considering that grading of beef loins occurs normally between 15 to 30 h PM, structural changes are still occurring in the muscle and that extending the time of grading for those carcasses that reach a pH of <5.70, but do not meet Australian meat colour (AMC) compliance are most likely to benefit from this intervention. The decline in pH is believed to drive these structural changes, which gives rise to enhanced light scattering but is likely to lag behind the time that the meat reaches the target meat colour, so that the benefit of the pH decline is not observed immediately. Therefore, if the pH is compliant, and AMC 4 is allocated to the carcass, perhaps if given a few more hours, the AMC would drop to a compliant level of 3.

The limitation of this intervention is the amount of chiller space available to meat processors, which is at a premium. Often chillers have limitations in capacity and once empty are required for the next day's kill. However, if space is available, lengthening the time from slaughter to grading would be an option to improve meat colour compliance, especially for those carcasses that are compliant for pH, but are downgraded due to an AMC 4.

During quartering or refacing ensure a smooth, flat surface is achieved and beware of weep on the surface prior to grading.

Meat containing the same pigment content (myoglobin) can appear to be lighter in colour, due to the influence of changes in surface texture and its impact on light scattering (Bate-Smith 1948). Reflectivity is more significantly related to scatter rather than absorption properties of the meat, indicative of the importance of meat microstructure to subjective colour perception by the naked eye (Macdougall 1970). Variations in glossiness, smoothness or roughness of the surface can influence the scatter (Zijp

and Bosch 1998), which could thus be impacted by the quantity of weep on the exposed loin prior to grading. Roughening of the surface is known to reduce iridescence (rainbow appearance) in cooked meat (Lawrence et al. 2002), thus illustrating the importance of an even surface at grading on visual attributes of the meat. A convex surface has a higher reflection compared to a plane or flat surface (Elliott 1967), so sometimes when the cut surface of the loin is sunken, or concave, this could impact on the light scattering which the grader visualises. Thus, during quartering a consistently smooth, flat surface should be achieved and graders should have a consistent level of moisture on the surface of the meat, whilst still maintaining the appropriate viewing angles (AUS-MEAT 2014).

Narrow blooming window and standardise if possible
Currently, the standard blooming procedure specifies that the grading procedure should be conducted between 20 min and 3 h after refacing (AUS-MEAT 2014). There is evidence of variations of this magnitude in bloom time of loin muscles showing significant differences in meat colour redness and yellowness values up to 75 min bloom (Wulf and Wise 1999). The most dramatic changes in colour are observed in the first 10-20 min of refacing (Wulf and Wise 1999; Lee et al. 2008), but can still be apparent after 75 min, although this does reduce in difference with longer periods of time.

It is suggested that bloom times should be standardised to between 90 and 120 min after refacing, to minimise variations in lightness, redness, yellowness and consequently overall colour as perceived by graders. Thus, the level of oxygenation of the muscle would be more similar between carcasses, which would aid in a more consistent, less variable colour score. It is recommended to consider the installation of chiller timers which would aid in a coordinated approach to timing and keep a consistent time for grading after quartering or refacing. The practicalities of a tighter time window may be difficult to approach, and therefore another option would be the use disposable plastic bags/ cards to provide a barrier to oxygenation at the meat surface.

Ensure adequate chiller temperatures & ensure consistent capacities are maintained
The temperature within the chiller may impact on the rate of muscle fibre shrinkage PM and also the rate of oxygenation at the surface of the meat. As discussed above, lower temperatures result in a longer time for rigor and resolution of rigor to occur. Small temperature differentials in the muscle may cause fibres to shrink more slowly and thus, take longer for light scattering to develop. In addition, chillers at lower capacities or only half-full may result in colder temperatures for individual carcasses, which could slow the rate of muscle fibre shrinkage and increase the incidence of DC. Further research is required to investigate the effect of chiller temperatures and chiller capacity and their impact on rate of glycolysis, muscle fibre shrinkage and the development of lightness.

Early detection methods as prediction tools
The ability for early detection of dark cutters, especially during the early PM period has the potential for beef processors to modify processing conditions to optimize the meat quality. Modification of processing conditions may involve optimization of temperature and chilling, hanging methods, stimulation or other processing technologies such as Rinse & Chill® or high pressure processing. These methods could be optimized to reduce incidence of DC, and this review will highlight the potential early detection methods and their limitations.

Hot pH measurement of all carcasses
The importance of obtaining an optimal pH decline whilst avoiding the cold and heat shortening windows is highlighted as a key tool that processors can use to obtain ideal eating quality with the

associated meat colour of the carcass (Meat and Livestock Australia Limited 2011). This is a procedure used in the industry whereby carcasses are measured for pH and temperature at the loin muscle on an hourly basis for a pH decline slope to be generated. Furthermore, some research has suggested using tpH_{45} (pH at 45 min PM), can be used to assess the progress of pH decline and predict the rate of glycolysis in relation to pork meat quality attributes (Warriss 1982). There is a relationship of pH_{45} with drip and light scattering in rigor muscle, with a higher pH_{45} being associated with less drip and lower reflectance (Offer and Knight 1988). So, although this has been used as a predictor of pale, soft, exudative (PSE) meat in pork, it has the potential to be used as a predictor of both heat shortening in beef and dark cutting.

Hot reflectance, near infrared (NIR) or Raman measurement of all carcasses
Similar to the measurement of pH_{45}, other techniques may be employed to detect structural and chemical aspects of the meat early PM and include, but are not limited to colorimetric and spectroscopic measurements. Colorimetric probes or instruments could provide reflectance data about the muscle structure and the extent of dark cutting. Fibre optic probes have been used to assess the incidence of DC meat using lightness coordinates and have the potential to discriminate carcasses one day PM (Gariépy et al. 1994). Using a probe 35 min PM, has not been able to distinguish DC, high ultimate pH beef loins (Chrystall 1987), but perhaps other measures could be explored.

Preliminary trials on pork *semimembranosus* muscle illustrate that Raman spectroscopy is a useful tool for early PM metabolites within the meat, especially during the first 10 h PM (Scheier et al. 2014). Pre- and post-rigor meat is able to be distinguished and the potential for PSE detection, especially on pre-rigor pork is possible. In future, the method should be investigated as a potential early detection tool for beef as a predictor of DC.

Using a hand-held device, scanning of post-rigor beef *longissimus* steaks ~48 h PM in the visible and near infrared (NIR) regions, revealed dark cutters can be identified 95 % of the time (Prieto et al. 2014). This is mainly due to the variations in myoglobin oxidation and the water retained in the structure which allow for differentiation. Other researchers have measured NIR spectrum at early (35 min) PM of beef and found low correlations with pH_u and glycogen contents (Lomiwes et al. 2010), but have highlighted the importance of inclusion of carcass data such as gender and category. So perhaps, the initial development of a predictive model would need to take into account carcass attributes such as ossification, carcass weight, and marbling and also incorporate colour measurements or water-holding capacity of the tissue. In addition, perhaps inclusion of the visible spectrum may also aid in the prediction of carcass attributes. Although this model development could take some time to establish, the development of an early prediction model would enable processors more control for reducing the incidence of DC.

Rinse & Chill® to avoid dark cutting
Rinse & Chill® is a pre-rigor, pre-evisceration red meat slaughter technology (Crane 2009; MPSC.INC. 2014) which reduces the temperature (4-5 °C) in the carcass and provides the muscle with extra substrates to promote optimal conditions in the muscle, including more consistent pH response. It leaves no detectable residues and advances rigor mortis. Immediately after sticking, a vascular rinse of a very dilute, super chilled, isotonic glucose electrolyte solution is pumped around the circulatory system and promotes blood removal. The process has been approved for commercial use in export processing plants in Australia, U.S.A., Canada and New Zealand, and requires no additional labelling.

There is evidence to indicate that this improves colour and tenderness (Farouk et al. 1992; Hunt et al. 2003) and reduces microbiological counts for coliforms and *E. Coli* (Feirtag and Pullen 2003). Using this process, the increase in objective lightness values is thought to be due to an increase in scattering within the microstructure of the meat (Hunt et al. 2003). The process has been reported to improve hide removal too.

High pressure processing (HPP) of valuable primals
HPP treatment is known to induce paleness and lightness, sometimes referred to as "whitening" (Carlez *et al.* 1995) in post-rigor meat and is dependent on the level of applied pressure. At higher pressures (≥350-400 MPa), there is a reduction in redness and increase in yellowness (Jung et al. 2003; Marcos et al. 2010). This is caused through the oxidation of oxymyoglobin at or above 300 MPa (20°C, 30 min), (Carlez et al. 1995; Goutefongea et al. 1995) and by HPP-induced protein denaturation of muscle proteins that increase reflectance across the visible wavelengths of a spectral scan (Jung et al. 2003). Therefore, if processors want to improve lightness, pressures below 300 MPa should be used. Also, in pre-rigor treatments, there can also be improvements in eating and microbiological quality, but this is dependent upon the temperature and level of pressure applied (Macfarlane 1973).

HPP is normally conducted within a processing fluid normally water. Whole carcass treatments would be very difficult to achieve since the large size of the whole carcass will not fit the vessel. However, HPP is a technique that could be used to value-add to specific cuts of the carcass in either post-rigor or hot-boned pre-rigor meat. Primals of highest value could be vacuum packed and pressure treated at low temperatures for short times to improve the lightness of the cut. In terms of limitations, initial installation and maintenance could be expensive. However, this is a new emerging technology in the food industry and has potential for value adding.

5.2.2 Industry Survey

Slaughter and post slaughter treatments
Animals were stunned using pneumatic stun in seven plants, mushroom head stun in four plants and captive bolt stun in two plants. Halal sticking was used by ten plants while the other five performed thoracic stick. Immobilisation stimulation was used by most of the plants except two; however the intensities and time of stimulations varied from plant to plant. Ten plants used bleed rail stimulation, six being low voltage (LV) between 8 and 90 seconds, three plants used high voltage, only one plant used SmartStim™ and four plants did not stimulate at all. All plants used a rigidity probe for 5 to 15 seconds. Only half the plants used hot water decontamination at ~80 °C for 10 to 25 seconds and one was in the process of installation. No plants used Rinse & Chill® on their carcasses. Two plants used tenderstretching, while the majority were Achilles hung to maximise chiller space. Carcasses were loaded into the chillers at temperatures ranging from 0 to 15 °C at different fan speeds with the final boning temperature ranging from 4 to 9 °C. All plants, except two, used interval spray chilling.

MSA Grading
All plants except one conducted MSA grading of their carcasses. The one plant that did not grade for MSA considered their product was of a high enough quality and thought there was no additional benefit for MSA grading. The percentage of carcasses graded varied from 8.5% to 100% of annual production. The percentage of non-compliant carcasses (AMC ≥4) graded varied from 0.6% to 41%

(average ~12%). There were similar percentages of non-compliance for both pH and meat colour. The MSA standard for pH decline is a minimum of 20 carcasses per month and all plants met this requirement. Six plants conducted pH declines on a weekly basis and a few completed 20 extra declines for each chiller/ feed type/ or category as well.

Meat Colour

Figure 1 shows the AUS MEAT colour charts used by the graders during AUS MEAT chiller assessment. Table 4 shows that of the 15 plants surveyed, an overall average of 12% were non-compliant for meat colour, which is substantially higher than MSA value for 2015-16 at 5.26% (Meat Standards Australia 2017). Thus, 87% of graded carcasses were within the compliant meat colour score of 1B to 3. Only one plant had a minor issue with colour score of 1A. Of the non-compliant carcasses, the highest average percentage had a colour score of 4 (6.6%) and that percentage decreased with increasing colour scores to 7. There was also a wide distribution of colour scores within some plants and percent of non-compliance ranged from 0.6% to 41.3%, indicating that for some of the plants dark cutting was a major issue.

Table 4. AUS-MEAT colour score percentages for graded carcasses from each of the fifteen plants surveyed.

Plant	AUS-MEAT Colour Score								
	1A	1B	1C	2	3	4	5	6	7
1	0	0	5	15	80	0	0	0	0
2	0	0	19.1	38.7	24.5	10.0	4.2	2.1	0.1
3	0	0	3	26.6	41.4	14.3	9.4	2.9	0.3
4	0	0	13.1	37.5	22.7	13.4	7.5	3.3	0.4
5	0	0	6.3	14.2	33.2	21.5	9.0	10.7	0.1
6	0	0	5.9	63.0	25.4	2.3	1.3	1.2	0.1
7	0	0	0	95	0	3	0	0	0
8	0	3.9	35	46	8.7	3.5	1.6	0.9	0
9	0	79.5	15.3	2.8	1.9	0.6	0.3	0.1	0
10	0	5	24	44	17	4	2	3	1
11	0	3	23	45	19	5	2	2	1
12	0	5	10	10	60	13	2	0	0
13	1	4	30	38	11	8	4	3	1
14	0	24.4	43.3	29.4	2.3	0.4	0.1	0.0	0
15	0	0	85	0	15	0	0	0	0
Average (%)	0.07	8.32	21.20	33.67	24.13	6.60	2.89	1.94	0.26

Plants that processed very specific categories of animals with consistent weights and feeding regime tended to have lower incidences of dark cutting. Consistency in size and weight allowed these processers to operate optimal post slaughter treatments, including chilling regimes, to maximise their product quality. In general processors who slaughtered a wide range of categories of animals using standard post slaughter treatments had higher incidences of dark cutters as they were unable to adjust for the large variability of their product. For example, large and small carcasses were placed in the same chiller hence their chilling rates and pH decline rates were different. In order to reduce this variability, some of the processors separated the large and small carcasses and placed them in separate

chillers based on their size. Carcasses from less mature animals had a lower colour score than those from more mature animals, as observed in (Hughes et al. 2014). Most plants also indicated that animals killed on Friday and graded on Monday had a higher percentage of carcasses in the compliant colour range, confirming that an increased time from slaughter to grading can improve meat colour (Murray 1989; Hughes et al. 2014).

Cost of dark cutting

The majority of the processors were cautious about providing economic information on the impact of dark cutting within their business. A few of the processors indicated that the cost was from 10c/kg to $2/kg or $50/head to $1000/head financial loss depending on the category/grade of animal. However, all processors indicated that if the colour scores were in the non-compliant range then the entire carcass was downgraded to the next grade or to economy/budget beef or trim/mince.

Some of the plants did not know the financial impact of dark cutting or non-compliant carcasses on their business. It is recommended that these processors would benefit by using the cost benefit model developed as part of this project to gain a better understanding of the economic impact of dark cutting and the economic analysis of implementation of a range of interventions to their business, which is discussed in Section 5.3.

Interventions

Seven potential interventions (as listed below) were proposed to processors and asked their view on the intervention and whether they would consider implementation:

1. **Secondary grading of non-compliant carcasses i.e. holding the carcasses in the chillers for longer and re-grading them**.
   (i) Most plants viewed this unfavourably due to the requirement for additional chiller space and indicated that if more chiller space was available they would kill more animals instead. However, most plants had observed that animals killed on a Friday and graded on a Monday had better colour scores. Hence, there is awareness in the industry that holding a carcass for longer prior to chiller assessment results in improved meat colour score, but that benefit does not outweigh the attractiveness of higher throughput.
   (ii) Only one plant had the capacity to divert non-compliant carcasses into a space chiller and said that re-grading of primals would be beneficial due to the colour improving with time from boning

2. **Standardising bloom time to 90-120 mins after refacing i.e. allowing additional time and a narrower window for the surface to bloom prior to grading, as the MSA standard bloom time is 20 to 180 mins**.
   (i) Most plants would consider this but timing would be a factor that would need to be taken into consideration. Boning rooms often started at 5 am, so if the bloom time is increased from 20 to 90 mins then the plants would need to start quartering earlier to allow sufficient time for blooming and grading prior to boning.

3. **Hot pH measurement of carcasses at end of slaughter floor. This would enable processors to decide if the carcass should be held at room temperature for longer for the pH to fall further or to put the carcass in a chiller**.
   (i) Half of the processors were interested in this method but the time and personnel required for measuring the pH were of concern. Additional staff would be required to measure the pH and redirect carcasses to appropriate holding areas. This could potentially slow down the chain as well.
   (ii) One plant enquired about a hot colour measurement as well.

4. **Rinse & Chill® i.e. pumping a very dilute, super chilled, isotonic glucose electrolyte solution through the carcass after slaughter. This is a commercially approved system in USA, Canada, Japan and Australia (MPSC INC., 2014).**
   (i) Majority of the processors were unaware of the Rinse & Chill® system.
   (ii) Some of the processors requested more information on the Rinse and Chill system which CSIRO sent to these processors
   (iii) One plant said that they would need to discuss this system with their customers to see if they had any concerns about this process.

5. **Aitch bone hanging i.e. Tenderstretch**
   (i) Two plants interviewed already used this process
   (ii) One plant stated they had used the process previously and saw no improvement in tenderness hence they no longer use this technique.
   (iii) All the other plants said they had insufficient chiller space.

6. **Heating at 37 °C to assist with pH decline**
   (i) None of the processors were interested in heating a chilled carcass or primal back to 37°C after chilling as it would be energy inefficient and have potential food safety concerns.
   (ii) A possible application of this intervention would be in combination with the hot pH measurement to determine where the carcass was located in relation to the pH/temperature decline window.

7. **Treatment of primals by high pressure processing (HPP)**
   (i) A few plants said that would require MSA and customer feedback before they would consider this technology
   (ii) Some said that they would consider this technology if it was profitable and sustainable.
   (iii) There was some concern about the browning of the red colour of the meat using this technology.
   (iv) Half the plants said no due to their perception of the time and the costs associated with this technology

Areas of research interest for the processors

All of the processors were interested in a predictive model based on measuring certain attributes of the animal just prior to kill and/or at slaughter (e.g. blood analysis, pH, glycogen content or hot colour) that could be used to predict the final colour score at grading. This would allow early interventions (while the carcass is still hot) to ensure that the carcass colour and pH are compliant at grading. As all of the processors were interested in a predictive model, CSIRO and AMPC need to have further

discussions with processors to develop a project on a predictive model for dark cutting, which would be beneficial for the entire meat industry.

5.3   Cost Benefit Analysis (CBA) Model

The document – "Milestone 2C: Value opportunity for improving beef colour at grading" outlines objectives and methodology behind the CBA model and provides detailed instructions on how to use the model. The CBA model is provided as an Excel spreadsheet, hence, it is a standalone document and is not incorporated into this report. The CBA model can include all the costs associated with implementing an intervention (e.g. extra chillers, labour etc.), the payback time and the benefit of that intervention to the processors. We believe that the CBA model will be a very useful decision making tool for processors when considering implementing different intervention strategies to improve the colour, and hence, the value of the carcass at grading. This CBA is available to industry through AMPC and CSIRO.

5.4   Mild heat treatment trials

5.4.1   Preliminary trial results and discussion

The hypothesis was formed that exposing dark muscles to a mild heat treatment (40°C for 1 h), similar to the conditions used by Bendall (1962), will induce muscle fibre shrinkage s and increase the light scattering properties of the muscle.

This theory was tested on post-mortem dark meat (14 to 96 hours post-mortem) obtained from a commercial meat processor. Initially, heat was applied to steaks to facilitate the optimization of the heating conditions for the development of an appropriate heating method so as to induce lightening and improve meat colour. Table 5 summarises the various heating conditions used in different trials. Even though this post-mortem temperature intervention was appealing to beef processors, as it improves value, appearance and meat quality properties of the higher value dark primals, there were concerns regarding microbiological safety and shelf-life stability. Once the mild heating parameters were optimised, microbiological safety and display shelf life of the heated steaks and primals were also investigated.

Table 5. Summary of the effect of the mild heat treatments applied on the meat colour properties of dark cut beef.

Milestone	Primal/ steak	Time post mortem (h)	Grader pH	Grader colour AMC	Temp water bath °C	Duration (h)	Internal Temp °C	Objective pre-heat (lightness/ L^*)	Objective post-heat (lightness/ L^*)	Significantly different* lightness/ L^*	Objective pre-heat (redness/ a^*)	Objective post-heat (redness/ a^*)	Significantly different* redness	Subjective consumer visual change
3 (a)	steak	14	5.99	6	40	1	--	26.8	32.8	S	18.5	23.9	S	n/a
3 (b)	steak	70	5.92	5	40	1	39.5	31.5	34.3	S	23.5	21.2	N.S	n/a
6 (a)	primal	70	5.92	5	40	1	23	28.8	31.1	N.S.	21.3	19.9	N.S.	n.d.
6 (b)	primal	22	5.60	4	40	3.5-4	35.0-37.1	34.9	34.4	N.S.	26.0	27.6	N.S.	n.d.
6 (c)	steak	22	5.60	4	30	1	29.8	36.4	32.4	N.S.	26.1	26.1	N.S.	n.d.
6 (c)	steak	22	5.60	4	35	1	34.7	36.4	32.1	N.S.	26.1	26.0	N.S.	n.d.
6 (c)	steak	22	5.60	4	40	1	40.1	36.4	33.8	N.S.	26.1	26.1	N.S.	n.d.
8 (a)	steak	24	6.29	≥5	40	1	~40.0	29.0	30.6	N.S.	22.9	20.2	N.S.	n/a
8 (a)	steak	24	6.29	≥5	45	1	~45.0	29.0	32.6	N.S.	22.9	22.8	N.S.	n/a
8 (a)	steak	96	6.29	≥5	40	1	~40.0	31.9	34.1	S	23.0	22.0	N.S	Yes
8 (a)	steak	96	6.29	≥5	45	1	~45.0	31.9	36.2	S	23.0	27.5	S	Yes
8 (b)	steak	n/a	6.03	≥5	40	1	~40.0	33.3	34.0	N.S.	23.5	23.7	N.S	n.d.
8(b)	steak	n/a	6.03	≥5	45	1	~45.0	33.3	37.5	N.S.	23.5	25.8	N.S	Yes

n/a = not applicable or not available; n.d. = non detected; S = significantly different (*P*<0.05); N.S. = not significantly different (*P*>0.05)

Heating of steaks early post mortem (14 hours)

Table 6 and Figure 13 show the results from the initial trials where dark steaks were subjected to heat treatment at 40°C for approximately 1 hour, 14 hours post slaughter. As can be seen from Table 6 most of the meat colour parameters - lightness, redness, yellowness, chroma and global brightness, showed an increase, which was confirmed by visual observation (Figure 13). Although colour differences were observed, no significant differences were found for any form of myoglobin or hue ($P > 0.05$) as shown in Table 6. These results indicated that colour differences were caused by an entity other than the oxidative state of the pigment, suggesting that the differences in scatter resulted from the structural elements. As displayed in Table 6, after heat treatment, muscle fibres had a significantly smaller fibre width and a larger global brightness value ($P < 0.001$) indicative of muscle fibre shrinkage and a concomitant increase in light scattering. The images in Figure 14 illustrate heat-induced shrinkage, distortions and twisting of muscle fibres. Previously, similar structural characteristics have been observed in muscle fibres of pork watery muscles (Bendall and Wismer-Pedersen 1962) indicating that the heat treatment induced a similar structural reconfiguration. These structural changes were associated with increased light scattering.

Table 6. Effect of heating (40 °C, 1 hour) on loin steaks after collecting at ~14 h post mortem (n=7).

	Pre-heat	Post-heat	LSD	*P*-value
pH	5.81	5.69	0.089	0.007
Lightness (L*)	26.8	32.8	3.97	0.006
Redness (a*)	18.5	23.9	3.09	0.002
Yellowness (b*)	12.0	16.7	3.63	0.016
Chroma	22.1	29.3	4.38	0.004
Hue	33.0	34.6	3.88	N.S.
Deoxymyoglobin (%)	9.2	5.3	4.95	N.S.
Oxymyoglobin (%)	67.5	71.0	5.95	N.S.
Metmyoglobin (%)	23.4	23.8	3.52	N.S.
Global brightness	58.7	73.6	5.26	<0.001
Fibre width (μm)	75.7	62.8	5.33	<0.001

Sample number	Pre-heat	Post-heat
1	1 Dark	1 Dark
2	2 Dark	2 Dark
7	7 Dark	7 Dark
8	8 Dark	8 Dark
9	9 Dark	9 Dark

Figure 13. Effect of heat treatment on the colour of steaks from five different cube rolls. After heating, steaks were significantly lighter and redder in colour.

Sample number	Pre-heat
7	
8	
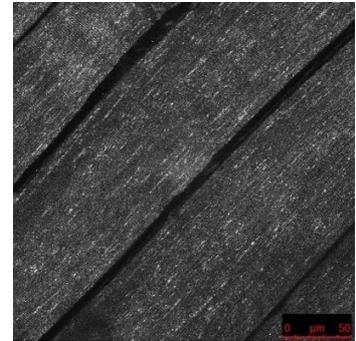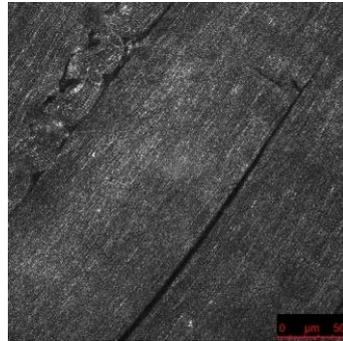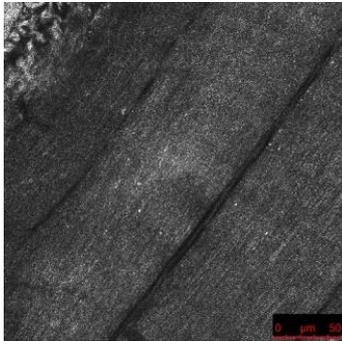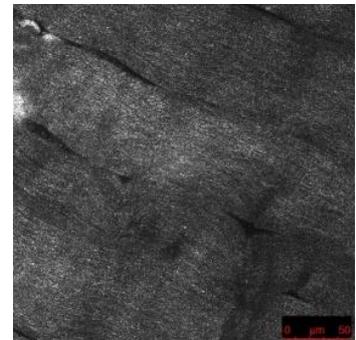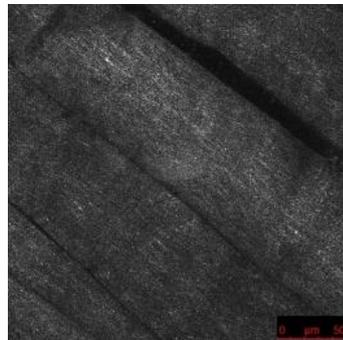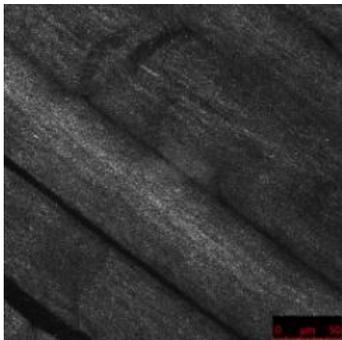
Post-heat
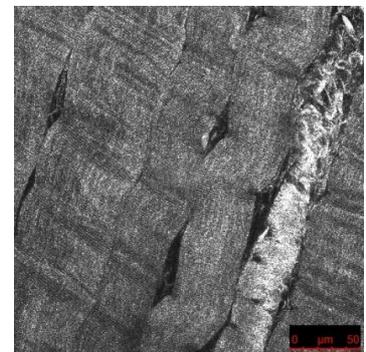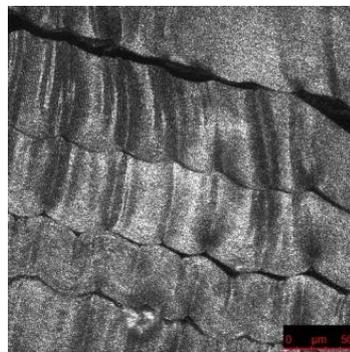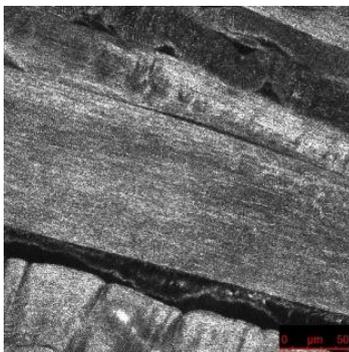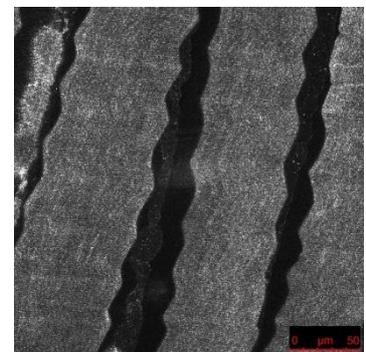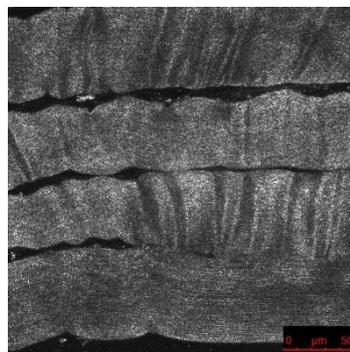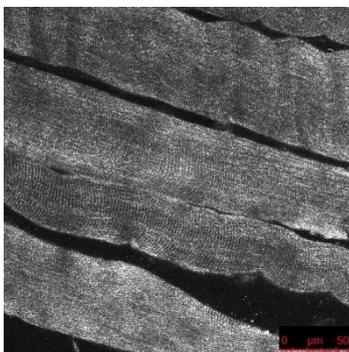
Sample number	
7	
8	

Figure 14. Beef loin muscle appearance pre- and post- heating (40 °C, 60 mins) for sample numbers 7 and 8. Images were made using a Leica TCS SP5 confocal laser scanning microscope in the reflectance mode ~30μm depth into the muscle fibres (scale bar 50μm).

Heating of steaks post rigor (>70 hours)

Dark steaks were subjected to heat treatment at 40°C for 1 h approximately 70 h post slaughter. Unlike the steak treated ~14 h post slaughter, ~70 h post slaughter steaks did not show any significant changes in the meat colour parameters except for a significant increase in lightness. Therefore, exposing the muscle to 40°C for 60 mins increased the lightness of the muscle, regardless of time post mortem when treatment had been applied.

Optimisation of heat treatment and dark muscle parameters

Heat treatment at 30, 35 and 40°C

As heat has an impact on the microbial growth and colour stability of meat, trials were conducted to determine the minimum temperature required to induce structural changes, and hence, lightening in dark muscles. Dark beef steaks (collected at 22 h PM) were heated at 30, 35 and 40 °C for 1 h and evaluated for colour stability in simulated retail display for 6 days.

No significant differences were found for any colour parameters between the control and any heat treatment (30, 35 or 40 °C), indicating that conditions were not met for heat-induced lightening.

In previous trials, it was observed that the most dramatic increases in the colorimetric lightness value were observed where lightness values of the meat prior to heat treatment were less than 30. In this study, lightness values before heat treatment ranged from 30 to 39 units which were too high initially to induce significant changes. Muscles that already have a lightness score >30 are unlikely to increase lightness scores dramatically, unless a more rigorous heating regime is used. As an outcome of this project, this intervention method, should focus on mild heat treatments to primals from carcasses with a graded meat colour of 5 and above (colorimetric lightness value <30) and non-compliant for pH >6.

Heat treatment at 40 and 45°C

In this experiment, beef loin primals with AMC ≥5 and high pH (>6) were cut into steaks and heated at 40 and 45 °C for 1 h, either early PM (<24 hours) or after 4 days (>70 hours). The results from early PM steaks (Table 7) did not show any difference in colour attributes or reflectance properties in comparison to the control (P>0.05), however the lightening of the colour could be seen visually. Contrary to previous results, there was a significant increase in lightness when steaks were heated after 4 days (Table 7) rather than 24 hours PM (P<0.05) (Table 7). The application of heat resulted in an increase in total viable microbial counts of approximately 1.00 $\log_{10}$ CFU/cm^2 during the first day of storage, which was maintained throughout storage (28 days).

Based on the results from these trials the following parameters were selected to develop a mild heat treatment method to induce lightening and improve the colour of dark meat post mortem:

- Meat colour at grading had to be >5 (downgraded primal for non-compliant for colour)
- Graded pH to be >6 (lower lightness value (<30) to induce lightening of the muscle during heating)
- Time post slaughter up to 4-7 days (processors can collect dark muscle over a week and heat treat them in a batch at a suitable time)
- Heating temperature of 45 °C (to induce muscle fibre shrinkage)
- Meat format– whole primals (as this would facilitate the adoption for processors)

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Table 7. Colorimetric, pH and conductivity data of beef loin steak before and after heat treatments (40 or 45 °C for 1 h), where blooming was completed both at 30 and 100 min (~10 °C).

(a)

Day 0	30 min bloom					100 min bloom				
	Control	Heat (40°C/1h)	Heat (45°C/1h)	LSD	*P*-value	Control	Heat (40°C/1h)	Heat (45°C/1h)	LSD	*P*-value
Lightness	29.0	30.6	32.6	3.09	N.S.[B]	29.8	30.7	33.0	2.95	N.S.
Redness	22.9	20.2	22.8	3.07	N.S.	23.3	22.7	24.9	3.39	N.S.
Yellowness	14.8	12.3	14.8	3.25	N.S.	15.5	14.8	17.0	3.57	N.S.
Hue	32.7	31.1	32.4	2.35	N.S.	33.6	32.9	33.9	2.06	N.S.
Chroma	27.3	23.6	27.2	4.33	N.S.	28.0	27.1	30.2	4.81	N.S.
Metmyoglobin (%)[A]	16.9	20.3	21.3	1.79	<0.001	17.7	21.8	23.6	1.59	<0.001
Deoxymyoglobin(%)[A]	21.3	25.2	21.2	7.57	N.S.	16.8	13.5	9.0	6.86	N.S.
Oxymyoglobin (%)[A]	61.8	54.6	57.6	7.34	N.S.	65.5	64.7	67.4	6.45	N.S.
pH						6.29	6.31	6.21	0.44	N.S.
Conductivity (mS/cm)						5.9	8.6	10.5	2.73	0.008

(b)

Day 4	30 min bloom					100 min bloom				
	Control	Heat (40°C/1h)	Heat (45°C/1h)	LSD	*P*-value	Control	Heat (40°C/1h)	Heat (45°C/1h)	LSD	*P*-value
Lightness	31.9	34.1	36.2	1.70	<0.001	32.5	34.2	36.1	1.80	0.002
Redness	23.0	22.0	27.5	2.16	<0.001	23.8	23.9	29.7	2.44	<0.001
Yellowness	15.4	13.4	19.5	2.22	<0.001	16.1	15.4	21.3	2.52	<0.001
Hue	33.6	31.2	35.3	1.64	<0.001	33.9	32.6	35.5	1.53	0.002
Chroma	27.7	25.7	33.7	2.99	<0.001	28.8	28.4	36.5	3.40	<0.001
Metmyoglobin (%)[A]	19.8	25.3	26.6	2.25	<0.001	21.7	26.4	28.3	2.04	<0.001
Deoxymyoglobin (%)[A]	12.8	14.1	5.7	5.40	0.008	8.9	6.5	1.3	3.98	0.002
Oxymyoglobin (%)[A]	67.4	60.6	67.7	4.06	0.002	69.4	67.2	70.4	2.91	N.S.
pH						5.87	5.77	5.75	0.26	N.S.
Conductivity (mS/cm)						4.3	5.5	8.6	1.53	<0.001

Heating of whole primals - beef cube rolls

A preliminary trial was undertaken to apply the heat treatment (45 °C) to two whole primals to determine the time required for the internal temperature to reach 45 °C and induce lightness/colour change in the entire primal. Figure 15 shows the heating profiles of the two primals, recorded using i-buttons placed in each primal. The primal that was heated for 4 h reached an internal temperature of 44.6 °C while that heated for 16 h reached an internal temperature of 45 °C after 8 h. Thus, based on this experiment, it was decided to heat primals for 5 h to reach an internal temperature of 45 °C. This led to the development of the method to apply a mild heat treatment to dark primals in order to induce lightness of the colour and thereby adding value to dark primals.
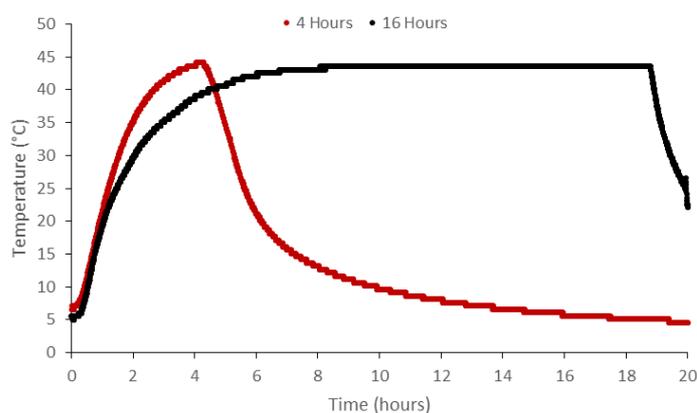
Figure 15. Internal temperature of beef cube rolls during heating in a water bath at 45 °C for either 4 or 16 h.

5.4.2 Mild heat treatment of primals

The results from various mild heat treatment trials led to the optimisation and scale up of this heat treatment intervention for dark beef primals.

Carcass and grader data

A total of 20 cube rolls, (right and left loins from 10 animals) were evaluated in this trial. The carcass data is summarised in Table 8. Ten cube rolls were subjected to a mild heat treatment whereas other 10 cube rolls were kept as unheated controls. The ten heated cube rolls were allocated to two storage times, 0 and 24 days (5 loin each). Animals had dentition scores of 0-4 teeth with low hot carcass weight (HCWT). The rib fat for some of the carcasses was also low, indicative of pasture-feeding regime to which the animals had been exposed to prior to slaughter. The time from slaughter until the application of heat intervention ranged from 49 to 96 hours as the primals were collected on different days. This was also done for industrial relevance since, from a practical viewpoint, a meat processor could then accumulate dark primals over a production week and apply heat treatment to primals at one time point rather than having meat treatments staggered over the week. Both the meat colour and the pH of the carcass at the point of grading was high and non-compliant that assuring that all the cube rolls were dark cutting.

Table 8. Descriptive statistics of beef cube roll carcasses after grading (n = 10).

	Mean	Median	Minimum	Maximum	Standard Deviation
Meat Colour (AMC)	5.8	6.0	5.0	6.0	0.4
Grader pH	6.2	6.1	6.0	6.7	0.2
MSA Marbling (MB)	335	340	220	550	96
Eye muscle area (EMA, cm^2)	77	76	72	86	4
Ossification	154	150	130.0	190.0	18.2
Dentition	2.4	2.0	0.0	4.0	1.3
Rib Fat (RF)	8.1	8.0	3.0	15.0	4.4
Hump	88	75	65	135	26
HCWT (kg)	160	160	145	183	11
Time from slaughter till heat applied (hours)	70.6	74.2	49.8	96.6	17.2

Heating of primal

The heating profile of five cube rolls is shown in Figure 16. The water bath temperature was set at 45.0 °C and the cube rolls were removed after 5 hours. The internal temperature of the cube rolls ranged from 43.5 to 45.6 °C (Figure 16).
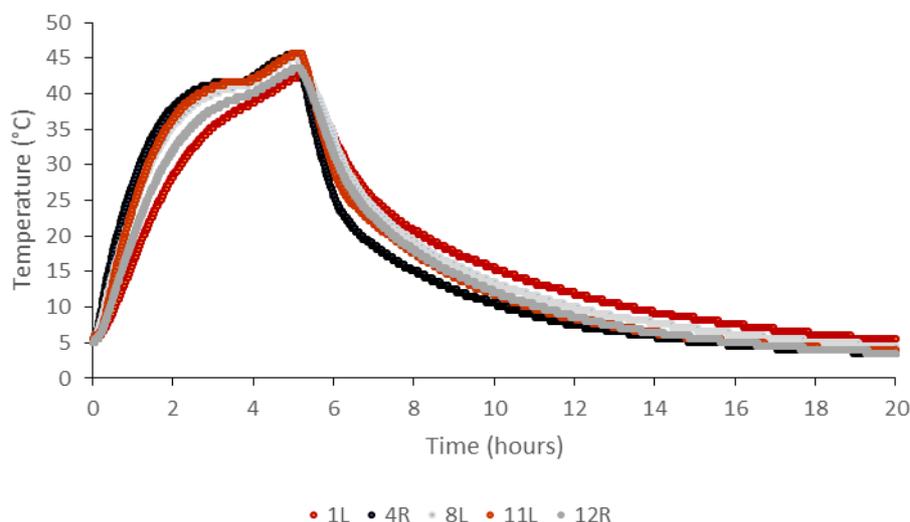
Figure 16. Internal temperature of beef cube rolls during heating in a water bath (45 °C for 5 h) and subsequent cooling in an ice slurry (0 °C for 1 h) and then in a chiller (4 °C overnight).

pH, conductivity and treatment drip loss of primals before and after treatment

The pH, conductivity and treatment drip loss of the primals are shown in Table 9. The pH range for control and heat-treated samples was 6.10 to 6.25. The change in the pH after heating and storage was not statistically significant indicating that there was no impact from the heat intervention nor storage time on pH. The conductivity of the primal increased from 7.5-8.0 mS/cm before heating (preheat) to 9.6-12.7 mS/cm (postheat). The conductivity of the control (untreated) sample was 9.62 mS/cm which increased to 12.86 mS/cm ($P = 0.055$) after heating. This indicated that heat treatment had a significant impact on the muscle structure and the flow of the cell fluids in the cube rolls between intercellular spaces of the muscle. At 24 days of storage, the conductivity was nearly the same for both control and treated samples, 12.46 and 12.68 mS/cm, respectively. Thus indicating that ageing also contributed to change of the muscle structure resulting to the increase in conductivity.

The ANOVA analysis revealed that heat treatment had a significant effect on the conductivity of heated samples ($P = 0.018$), while there was no effect from the storage time under vacuum for the conductivity. There is a significant interaction between heat treatment and storage day for the conductivity. Thus, the conductivity of control and heated samples was different when heat was applied at day 0 of storage ($P = 0.055$) but tended to be the same for control and heat-treated samples at 24 days of storage ($P = 0.078$). In general, the conductivity measurement can be an indicator to predict drip loss, as such high values of conductivity corresponded to high values of drip loss (Table 9). The drip loss for the control sample at day 0 was 0.14 % which increased to 0.65 % after heating ($P = 0.009$). At 24 days of storage under vacuum-packaging, the drip loss was 0.91 and 1.89 % for the control and the heated samples, respectively. It was evident that the drip loss was greater for the heated

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samples that were stored longer (increasing from 0.14 - 0.65 % up to 0.91 - 1.89 %), and that both heat treatment and storage day had a significant effect in this case (Table 9, P=0.037).

Table 9. pH, conductivity and treatment drip loss of control and heated (45°C for 5 hr) primals stored after heating in vacuum packaging for 0 and 24 days.

		Storage timepoint	Mean Values			Heat Treatment		Storage Day		Treatment x Day	
	Treatment		Control	Heat (45°C/5h)	*P*-value	LSD	*P*-value	LSD	*P*-value	LSD	*P*-value
pH	Preheat	0	6.10	6.15	0.386	0.246	N.S.	0.246	N.S.	0.347	N.S.
		24	6.25	6.21	0.469						
	Postheat	0	6.19	6.25	0.216	0.239	N.S.	0.239	N.S.	0.338	N.S.
		24	6.19	6.17	0.804						
Conductivity (mS/cm)	Preheat	0	7.54	7.52	0.976	2.916	N.S.	2.916	N.S.	4.124	N.S.
		24	7.64	8.04	0.543						
	Postheat	0	9.62	12.86	0.055	1.403	0.018	1.403	N.S.	2.045	0.037
		24	12.46	12.68	0.078						
Treatment Drip Loss (%)		0	0.14	0.65	0.009	0.556	0.012	0.556	0.001	0.786	N.S.
		24	0.91	1.89	0.109						

Colour of steaks during display

The application of a mild heat treatment (45 °C for 5 hr) had a significant impact on the objective colour measurements (Figure 17) and the visual appearance of steaks during display (Figure 18 and 19). The purpose of the heat treatment was to induce lightening in dark muscles. Figure 17 shows that the heated samples were lighter (higher L^*-value, $P < 0.001$) than the controls. Dark cutting meat usually has low L-values, so consequently an increase in L^*-value is positive since it means that the colour of the treated meat is that of "normal" meat.

The difference in L^* was maintained over the period of retail display for 4 to 5 days (Figure 17). The heat treatment also had an impact on the redness of the steaks. Redness (a^*) was higher for the heated samples ($P < 0.001$) compared to the controls but the redness value of the steaks decreased faster in heated samples. This decline in the redness values is associated with conversion of oxymyoglobin into metmyoglobin. The heated samples thus had less colour stability upon display than the control (untreated) samples. Yellowness (b^*) was also higher for heated samples ($P < 0.001$) which was maintained over the display period for 4 to 5 days.
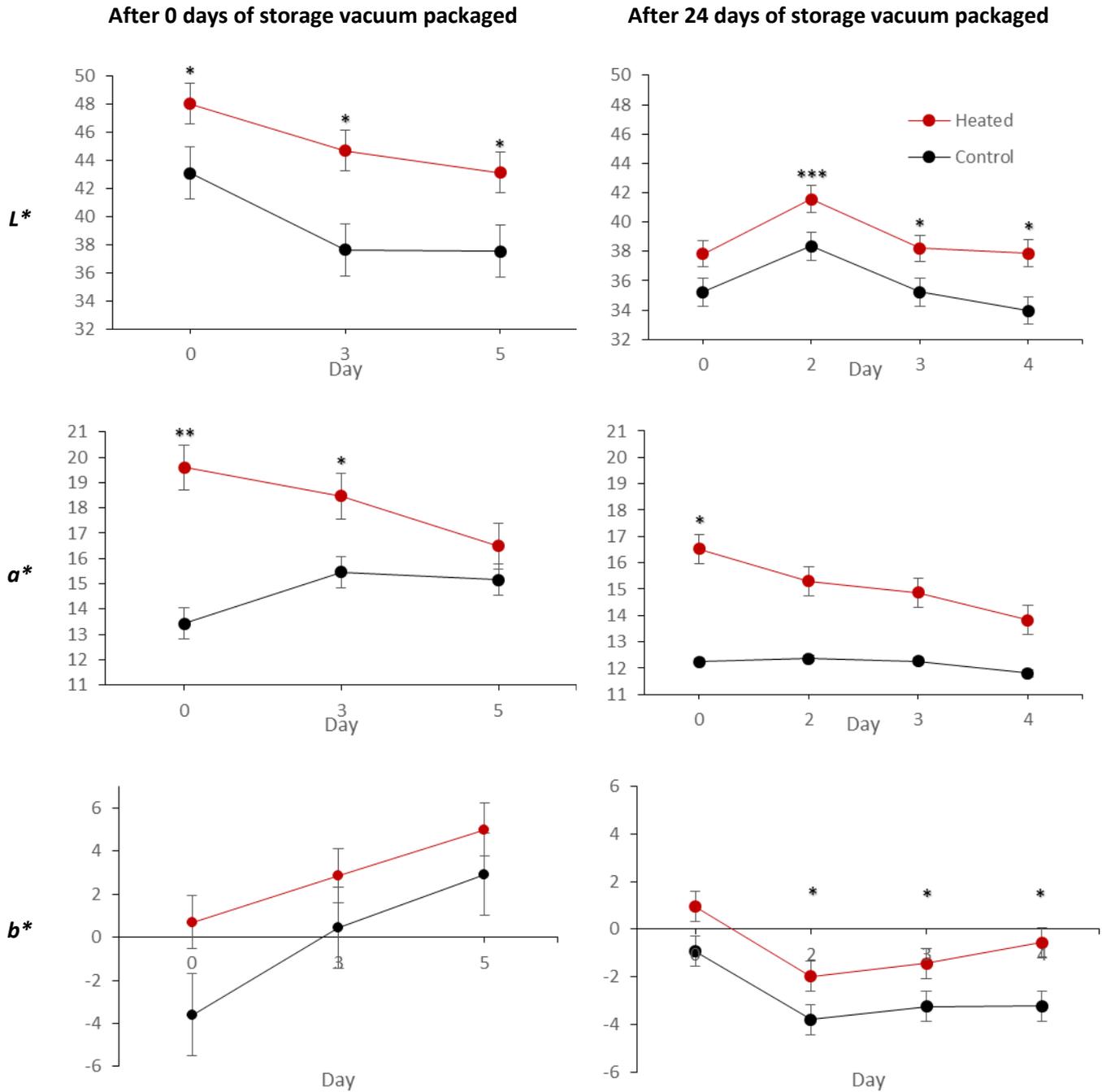
Figure 17. Colour parameters (*L**, *a**, *b**) of control (untreated) and heated (45 °C for 5 hr) beef cube roll steaks at retail display (0, 3 and 4-5 days) after storage under vacuum packaging (0 and 24 days after heating).

Control and heat (45°C/5h) parameters were compared at same storage time under vacuum (0 or 24 days) using a *t*-test and the significance is indicated by ⁺*P*< 0.1 * *P* < 0.05 ** *P* < 0.01 *** *P* < 0.001

	Day 0 - Control	**Day 0 - Heated**

Display Day

Figure 18. Representative images of control (untreated) and heated (45 °C for 5 h) beef cube roll steaks at retail display (0, 3 and 5 days) after 0 days of storage under vacuum packaging after heating.

Display Day	Day 24 - Control	Day 24 - Heated
0		
2		
4		
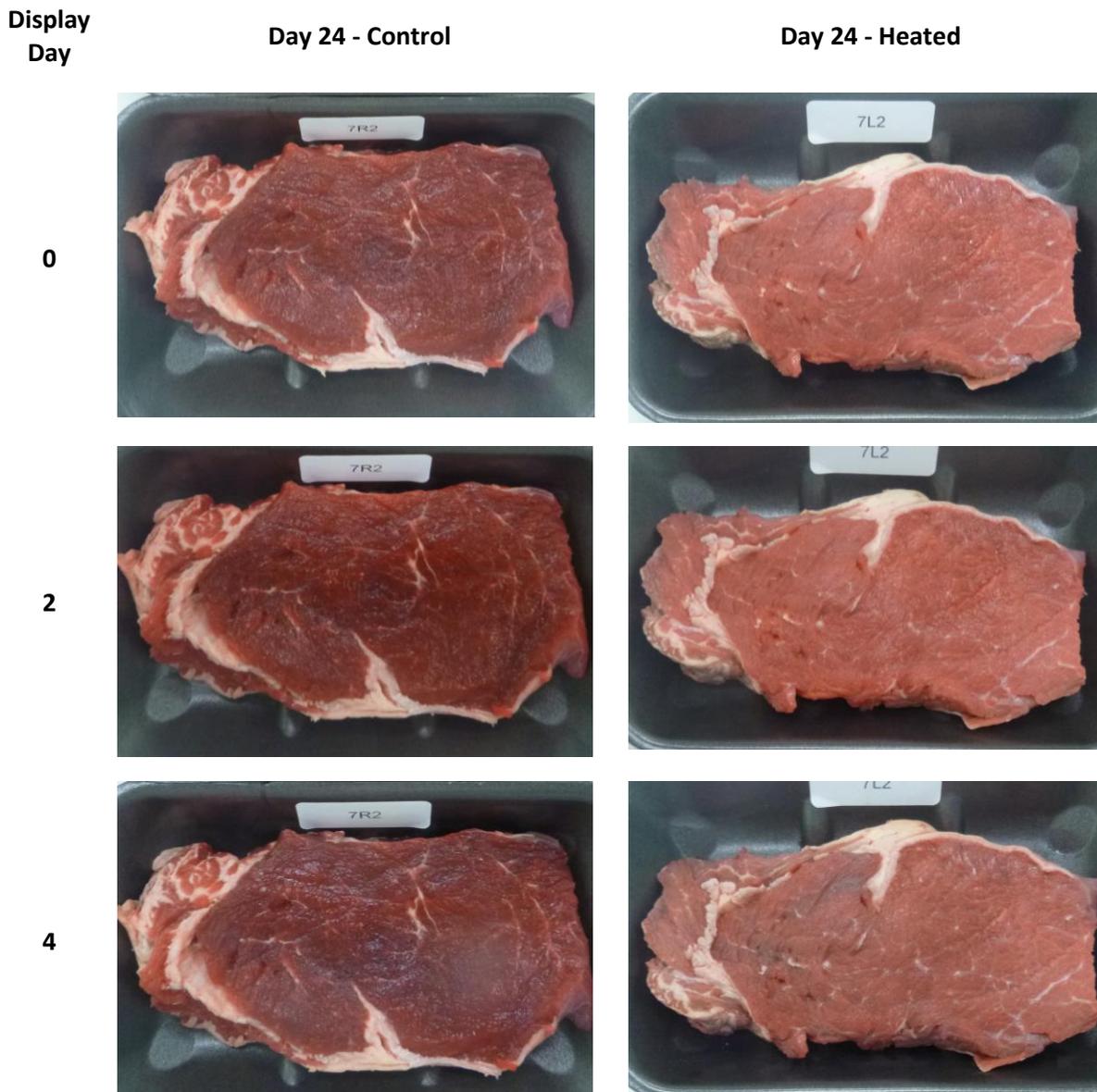
Figure 19. Representative images of control (untreated) and heated (45 °C for 5 hr) beef cube roll steaks at retail display (0, 3 and 5 days) after 24 days of storage under vacuum packaging after heating.

Consumer acceptance during retail display

Five steaks were cut from each of the heated and control primals, overwrapped and placed in a display cabinet at 4 °C for 5 days. The visual evaluation by consumers provided supportive evidence regarding the colour changes observed objectively from the Minolta colorimeter data (Figure 17). On day 0, the panellists preferred the colour of the heated steaks, but as storage time at retail display progressed, the panellist's preferences moved towards the control (untreated) sample (Figure 20). This was likely due to the inferior colour stability of the heat-treated meat. At each time point, fat colour was better in the control sample (Figure 20). Acceptability followed the same pattern as meat colour where there was a shift in preference. Whether consumers would purchase this as a retail product was not conclusive as acceptance was different following different chilled storage periods, 0 or 24 days, in vacuum packaging. On day 0, about 90% of the panellists stated that they preferred the heat-treated

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samples. In contrast, the purchasability of the samples stored for 24 days under vacuum prior to display decreased to 40 %, and in this case, the control (untreated) had a higher acceptability (60 %) than heat-treated samples (Figure 20).

Figure 20. Visual evaluation of control (untreated) and heated (45 °C for 5 hr) beef cube rolls steaks at retail display (0, 3 and 5 days) after storage under vacuum packaging (0 and 24 days after heating).
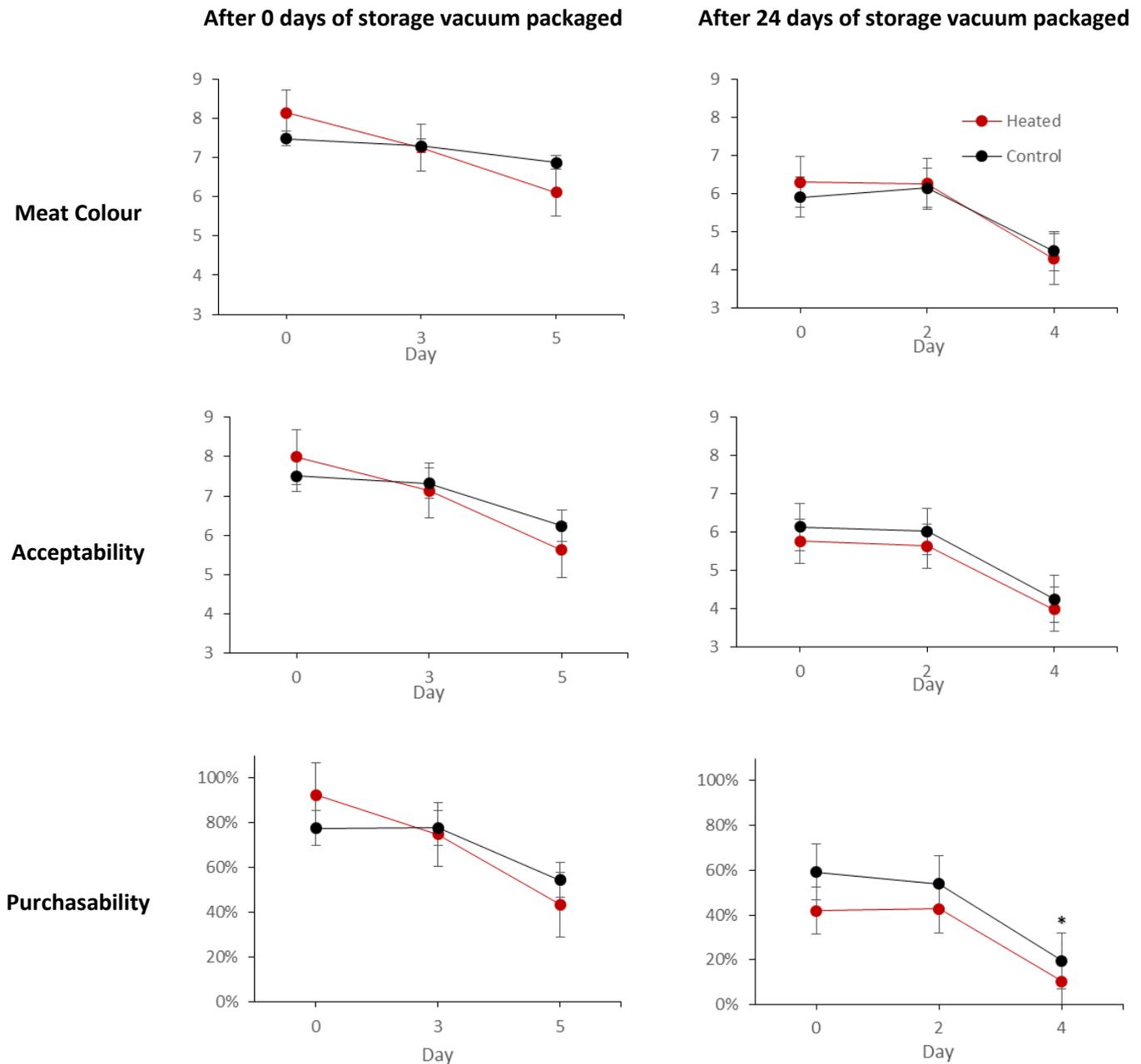
Control and heat (45°C/5h) parameters were compared at same storage time under vacuum (0 or 24 days) using a t-test and the significance is indicated by $^+P < 0.1$ * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Texture

The texture data showed that heating of the primal significantly improved tenderness ($P = 0.03$), with a reduction in peak force of 10.8 N on day 0 (Table 10). At day 0, the initial yield (IY), an indicator of the contribution of the myofibrillar component to tenderness, was also significantly lower ($P = 0.03$). This supports the hypothesis of the effect that the heat-activation of muscle proteolytic enzymes has on the myofibrillar structure. As expected, with increasing storage time the meat became more tender (Table 10). It is known that when meat is aged, meat tenderness improves due to the breakdown of muscle myofibrillar structure as a result of the ageing progress. The control samples which had been aged for 24 days showed a significant reduction in peak force, from 63.8 N on day 0 to 37.4N on day 24. However, the tenderness of heated samples did not improve significantly (from 53.0 to 48.3 N), indicating that the heating intervention caused the meat to lose its ability to age. It is interesting to note though that there was a significant interaction between the effects of heat and storage day ($P = 0.045$) indicating the interplay of these two factors together play a significant role in meat tenderisation. Overall, a mild heat treatment applied to fresh meat could be a method to accelerate ageing. However the use of heat also seems to reduce meat's ability to age. The control, non-heated meat, was more tender after 24 days of ageing compared to the heated meat (Table 10).

Table 10. Warner-Braztler (WB) Shear Force parameters (peak force (PF), initial yield (IY) and PF-IY) of control (untreated) and heated (45 °C for 5 hr) beef cube roll steaks stored under vacuum packaging (0 and 24 days after heating).

WB measurement	Storage Day	Control	Heat (45°C/5h)	*P*-value	LSD	*P*-value
			Treatment		Treatment x Day	
PF (N)	0	63.8	53.0	0.03	12.32	0.010
	24	37.4	48.3	0.36		
IY (N)	0	54.8	46.6	0.03	12.97	0.048
	24	25.6	34.9	0.42		
PF-IY (N)	0	9.1	6.4	0.19	4.11	N.S.[A]
	24	11.8	13.4	0.24		

[A]N.S. = not significant

It is known that ultimate tenderness is dependent on the extent of proteolysis of key structural proteins within muscle fibres such as costamere, titin and desmin (Koohmaraie & Geesink, 2006; Kemp, 2010). Proteolytic calpain activity also contributes to meat tenderisation (Sentandreu et al., 2002) with optimal calpain activity occurring at neutral pH, therefore calpains are *per se* more active in dark cut meats with high pH (> 6.0). Hence, it would be expected that calpains could have been activated during heating, resulting in tenderisation. At the same time, the exposure to heat treatment for long periods (45 °C for 5 hours) would have resulted in the inactivation of the proteolytic calpain system, and consequently, the meat lost its ability to age further during storage. Differential scanning calorimetry (DSC) analysis has shown that heat-induced denaturation of myosin begins at about 45 °C (Wright, 1977). Therefore, the partial denaturation of myosin at the temperatures between 40 to 45 °C for prolonged times could contribute to increased light scattering resulting in a lighter meat colour. The extent of denaturation of muscle proteins can be elucidated by DSC analysis, thus further work in this area using this technique would assist in improving the understanding of the relationship between meat colour and structural protein modifications occurring with heat at 45 °C.

Sarcomere lengths

Table 11 shows that the sarcomere length became significantly shorter in the heated samples on day 0 compared to the control (P = 0.0036). Sarcomere shortening is expected when heat is applied to meat since the fibre length decreases as a result of muscle protein denaturation and shrinkage. However when considering texture, the data shows that on day 0 of storage, the larger the peak force the larger the sarcomere. This is contrary to the expected results since larger sarcomere lengths are associated with improved tenderness. These expected results are usually found in meat which has not undergone heat treatment. Despite the sarcomere shortening observed in the heated samples, this meat was more tender (i.e. lower shear force value, Table 10) than the untreated control which had longer sarcomeres. This was most likely due to the proteolytic enzymes activity in the muscle.

In contrast, the sarcomere lengths of control and heat-treated samples kept in vacuum packaging storage for 24 days were similar (1.61 and 1.59 µm, respectively, Table 11). Control samples had lower peak force (PF) than heat-treated (Table 10). This may indicate that the enzymes responsible for meat tenderisation have disrupted the muscle structure in control samples.

Table 11. Sarcomere length from beef cube rolls heated (45 °C for 5 h) and cold stored under vacuum packaging for 1 and 24 days after heating before slicing and put it on display

	Storage	Treatment			Treatment x Day	
	Day	Control	Heat (45°C/5h)	*P*-value	LSD	*P*-value
Sarcomere Length (µm)	0	1.80	1.53	0.0036	2.36	N.S.[A]
	24	1.61	1.59	0.8741		

[A]N.S. = not significant

Microbiological quality

Microbial counts; *E. coli*, coliforms, total viable count (TVC) and lactic acid bacteria (LAB), were analysed in heat-treated and control (untreated) samples after vacuum-packaged storage for 0 and 24 days. At 24 days of storage, *E. coli* and coliforms were not detected (limit of detection = 0.4 $\log_{10}$ cfu/cm^2). At day 0, coliforms were present in 1 of the 5 control samples and 2 of the 5 heat-treated samples, but the small e differences between treatments was irrelevant in practice (Table 12). Average TVC on day 0 was 2.57 $\log_{10}$ cfu/cm^2 for control samples and 2.48 $\log_{10}$ cfu/cm^2 for heat treated, which was also not statistically significantly different. The same occurred at 24 days of storage, the average TVC was 6.82 $\log_{10}$ cfu/cm^2 for control samples and 6.30 $\log_{10}$ cfu/cm^2 for heat treated, again not statistically significantly different (Table 12).

The microbial population at 24 days were dominated by LAB with 6.47 and 5.91 $\log_{10}$ cfu/cm^2 for control and heated samples, which is typical of cold storage vacuum packaged beef. These results confirmed that there was no impact of the heat treatment in the microbial populations studied. In contrast and as expected, storage day did impact on TVC and LAB counts (P < 0.001) as counts significantly increased with storage from 2.5-3 $\log_{10}$ cfu/cm^2 to 6.0-6.5 $\log_{10}$ cfu/cm^2 (Table 12) from day 0 to day 24 of storage. *E. coli* and coliforms counts were not significantly higher at 24 days of vacuum packaging storage, most likely due to the growth restriction imposed on them namely by the LAB growth under vacuum. The higher counts of TVC and LAB after storage for 24 days can potentially reduce shelf life though results have to be considered taking into account colour stability.

Table 12. Total viable count (TVC) and lactic acid bacteria (LAB) counts of meat heated at 45 °C for 5 hours and stored vacuum-packaged at 4 °C for 0 and 24 days.

	Storage Day	Treatment			Storage Day		Treatment x Day	
		Control	Heat (45°C/5h)	*P-value*	LSD	*P*-value	LSD	*P*-value
TVC ($\log_{10}$ cfu/cm^2)	0	2.57	2.48	0.72	0.42	<0.001	0.59	N.S.[A]
	24	6.82	6.30	0.27				
LAB ($\log_{10}$ cfu/cm^2)	0	2.43	2.95	0.22	0.68	<0.001	0.96	N.S.[A]
	24	6.47	5.91	0.45				

[A]N.S. = not significant

6.0   CONCLUSIONS & RECOMMENDATIONS

Conclusions

- The developed reflectance scanning laser electron microscopy (rCLSM) method is a world first technique for the combined visualisation and quantification of light scattering in muscle fibres. This technique enables the assessment of dark meat, where the larger and more swollen muscle fibres present, reduce light scattering, which leads to darker meat colour. This new technique provides a tool to study muscle microstructure and light scattering in a new dimension and test opportunities for reducing the size of the muscle fibres, and consequently, improving meat colour. This new technique can be used in future projects by the Australian meat industry to better understand the relationship between muscle structure and meat quality, particularly colour of light, medium and dark muscles.

- The industry survey of 15 processing plants highlighted a higher incidence of dark cutting carcasses (average of 12.0 %) compared to that reported by Meat Standards Australia (5.3 % in 2017). There was a wide variability of dark cutting incidence between plants, which ranged from 0.6 to 41.3 %. The processing plants with the high incidence of dark cutting would greatly benefit from interventions which would reduce this, and thus, must be the primary target of future research.

- The survey determined a financial loss for downgrading of dark carcasses to range from 10c/kg to $2/kg or $50/head to $1000/head depending on the category and grade of animal.

- A cost benefit analysis (CBA) tool has been developed as an excel spreadsheet to estimate the costs associated with implementing an intervention, the payback time and the estimated benefits. This CBA model is a very useful decision making tool for processors when considering implementing different intervention strategies to improve the colour, and hence, the carcass value at grading.

- A proof-of-concept intervention based on a heating step to manipulate the structure of non-complaint dark cuts has been developed. This heat intervention (45 °C for 5 hours) induced lightening in dark muscle and increased both lightness ($L*$) and redness ($a*$) compared to the control (untreated). Based on visual appearance, this colour change led to a higher consumer acceptability of the heated sample compared to the control at day 0 of retail display. However, the heat-treated samples showed a lower colour stability at display, and thus, the control samples at the end of retail display (5 days) were more acceptable than the heated samples. The heating step also resulted in additional 0.5 and 1.0 % drip loss for vacuum packaged storage for 0 and 24 days, respectively.

- The heating of primals (45 °C for 5 hours) resulted in an accelerated tenderisation of meat (reduction in shear force), however, the ability of meat to age was greatly impaired by the heat treatment (i.e. heat-treated meat did not age).

- Microbial counts were not impacted by the heat treatment; hence, meat colour can be improved with this intervention without jeopardising microbial shelf life.

- This heating intervention (45 °C for 5 hours) can provide a competitive method for improving the colour acceptability of dark cuts that are intended for retail sale within a short time frame of 1-3 days after heating and sliced into steaks. It is possible to apply this procedure on primals for up to

96 hours post-mortem without compromising the benefits. This enables the collection and accumulation of dark primals during a working period, and at a later time, subjecting them to a batch heat treatment process. In addition, this intervention would only require a recirculating water bath with thermo-regulation.

Recommendations

- Conduct commercial-based trials using the mild heating intervention technique on a number of different dark primals of economical relevance, preferably in those processing plants with high incidence of dark cutting (>10 %).

- Dark cutting is still a significant economic problem for the Australian red meat industry. The industry would benefit from the development and implementation of interventions that reduce this quality defect. A multi-disciplinary approach is recommended to ensure that factors, from pre-slaughter to post-slaughter, are considered and that methods and techniques are developed for the detection, remediation and valorisation of dark carcasses and primals.

- Further research on the use of mild heat treatments as a method for tenderisation of high and low value cuts is recommended. Ideally, this research should investigate the impact of mild heat treatments on the microstructure, protein denaturation and the activity of the endogenous muscle proteolytic system to gain a better understanding of the basic principles related to this intervention which will assist in the development of targeted heat interventions for the accelerated tenderisation of beef cuts.

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8.0   APPENDICES

8.1   Appendix 1 – Questionnaire for the dark cutting industry survey

Survey of Australian beef processors

AMPC and CSIRO are trying to improve beef colour at grading, so the maximum value of the carcass is achieved for the processor and producers, whilst improving the appearance and eating quality of the meat. We would like your help to allow us to gauge the impact of dark cutting and/ or heat toughened meat and your opinion about how best to tackle this problem.

Section A: Overall numbers

1. How many processing plants do you have nationally...
2. Where are they located ………………………………………………………………………………………….
3. How many head of cattle are you slaughtering annually (per plant)?
   ....
4. What percentage is Export ………………………………… Domestic ……………………………………………
5. What percentage is Grainfed ……………………………………. Grassfed ……………………………………………
6. Main Categories of animals killed ……………………………………………………………………………………….
7. Days/Hours of operation Kill floor ………………………………… Boning room ………………………………….

Section B: Treatment post slaughter

I am now going to ask some questions of what process you use to improve meat quality post slaughter.

Treatment	Application				
	plant 1	plant 2	plant 3	plant4	plant 5
Location					
Stunning method					
Sticking method					
Bleed rail stimulation					
Immobilisation					
Rigidity probe (length of time applied)					
Electrical stimulation (specify HV or LV)Voltage					
SmartStim™					
Hot water decontamination					
Rinse & chill					
How carcasses are hung					
Chiller conditions (Temp)					
Spray chill conditions					
Other (please provide details)					

Section C: MSA grading

I am now going to ask questions about MSA grading of carcasses.

1. Do you MSA grade your carcasses   **YES   NO**
2. How many beef carcasses are MSA graded **annually**?
    ………………………………………………………..
3. What percentage of the MSA graded carcasses are non-compliant?
    Meat colour ≥4.0 ………………………………… pH ≥5.71 …………………………………………………………………
4. Can you provide reasons for non-compliance and what proportions are failing for these
    reasons?………
    ………
    ……
5. How often do you measure a pH declines and how many carcasses do you measure? …..
    ………

Section D: Meat colour

I would like to go through the data of the different meat colour scores you provided. How many carcasses (%) would you allocate to each meat colour?

meat colour score	Location					Pricing ($/kg) per colour score
	plant 1	plant 2	plant 3	plant4	plant 5	
1A						
1B						
1C						
2						
3						
4						
5						
6						
7						

If unable to answer, what is the pricing ($/kg) of **beef whole carcass** between meat colour 3 and 4 or between 1A and 1B?
………
If unable to answer, what is the pricing ($/kg) of **most expensive cuts** between meat colour 3 and 4 or between 1A and 1B?
………
What happens to non-compliant carcasses that are failing due to high colour scores (equal to or above 4 and 1A)?

………

………

……..

Section E: Dark cutting in your business

1. Have you done any research internally to minimise the occurrence of dark cutters?

   ……

   ……

   ……

2. Would you support research into this area?

   ……

   ……

   ………..

3. Would you consider implementing systems to minimise dark cutting.

   YES   NO

Section F: Possible intervention strategies:

Based on literature and our research we have developed some interventions/methods and would like your thoughts/opinions on them.

1. Which of the following procedures would you consider implementing to improve meat colour at grading:

Intervention technology	YES	NO	Reasoning
Secondary grading of non-compliant carcasses, extra holding/ storage			
Standardising bloom time to 90-120mins after refacing			
Hot pH measurement of carcasses @ end of slaughter floor			
"rinse & chill" or vascular perfusion			
Aitch bone hanging			
Heating @ 37˚C			

2. Would you consider treatment of valuable primals, by HPP or stretching to improve meat quality?

   ……

   ……

   ……

Section G: Heat toughening in your business

The following questions are about Heat Toughening.

1. Have you seen or heard of heat toughening & is it a problem for your business?

   ………
   ………

2. Do you ever observe/ get any feedback about too pale meat or excessive drip?

   ………
   ………
   ………

8.2 Appendix 2 – Correlation coefficients for muscle colour variates and protein concentrations

Table depicting correlation coefficients (*R*) for muscle colour variates and protein concentrations. If the |r| is > 0.26, the probability value is *P*<0.05 and if |r| is >0.44, the probability value is *P*<0.001. Number of observations = 54.

		1	2	3	4	5	6	7	8	9	10	11	12	13
pH_u	1	-												
Global brightness (grey value)	2	-0.47	-											
Fibre fragment width (μm)	3	0.68	-0.27	-										
Lightness (L*)	4	-0.75	0.27	-0.61	-									
Redness (a*)	5	-0.67	0.21	-0.49	0.46	-								
Yellowness (b*)	6	-0.60	0.15	-0.45	0.44	0.99	-							
Chroma	7	-0.64	0.19	-0.47	0.46	1.00	1.00	-						
Hue	8	-0.49	0.08	-0.39	0.41	0.94	0.97	0.96	-					
Myoglobin concentration (mg/g)	9	0.77	-0.28	0.67	-0.84	-0.46	-0.43	-0.45	-0.35	-				
Oxymyoglobin (%)	10	-0.68	0.21	-0.55	0.63	0.81	0.82	0.82	0.83	-0.51	-			
Deoxymyoglobin (%)	11	0.59	-0.18	0.49	-0.51	-0.64	-0.66	-0.65	-0.68	0.38	-0.94	-		
Metmyoglobin (%)	12	0.37	-0.10	0.27	-0.45	-0.61	-0.61	-0.61	-0.57	0.47	-0.36	0.02	-	
Myofibrillar protein concentration (mg/g)	13	-0.41	0.27	-0.19	0.21	0.46	0.44	0.45	0.42	-0.06	0.41	-0.37	-0.21	-
		1	2	3	4	5	6	7	8	9	10	11	12	13