final report

Project code: A.MIS.1002

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Date submitted: Oct 2014

PUBLISHED BY
Meat & Livestock Australia Limited

Meat Industry Services

Shelf-life: Improving Beef Colour

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

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Government and contributions from the Austra

Abstract

Meat colour has important implications for consumer acceptability and eating quality, with dark-cutting carcasses incurring economic penalties, and thus negatively impacting the beef industry. Meat colour non-compliance can cost $400 per carcass, and this is estimated to impact the industry up to $177 million per annum. These carcasses can also have problems with shelf-life and eating quality.

The project objectives were to (1) Investigate slaughter factors that contribute to variations in beef colour, (2) Investigate the influence of beef meat colour at grading on shelf-life, eating quality and water-holding capacity and (3) Initiate development of capability in meat science at CSIRO.

Firstly, a statistical model was developed to determine animal and carcass characteristics which would impact on the beef meat colour at grading. The time from slaughter to grading was particularly important, with carcasses graded later (~31h post mortem) showing nearly a 3 fold reduction in dark cutting compared to those graded earlier (~14h post mortem). The reduction in dark cutting was most pronounced between 14-19h post mortem. These are the first findings in Australia to highlight the importance of time at grading to the colour and hence the value of the carcass. We suggest a possible mechanism behind the colour change and also possible solutions to improve meat colour at grading.

Secondly, beef striploins from ‘light’, ‘medium’ and ‘dark’ carcasses were stored for up to 20 weeks to evaluate the impact of shelf-life on eating quality and water-holding capacity. Regardless of meat colour at grading, all striploins had a “satisfactory everyday eating quality”. Consumers indicated beef aged for 20 weeks was still acceptable for tenderness, juiciness, flavour, satisfaction and overall liking, using the MSA eating quality scores. In addition, purge (as a measure of water-holding capacity), indicated no large variations between colour groups and showed a maximum value of 3.5% over the whole storage period. Thus, beef aged for 20 weeks was still acceptable to consumers, regardless of meat colour at carcass grading.

Lastly, the developments of capability in meat science at CSIRO are highlighted by the two papers published/ in press from the project (see appendices). Also, these results have been given to the MSA pathways committee and have been influential in decisions with the MSA pathway committee to; (1) no longer include colour as a criteria for consumer acceptability and (2) reconsider the importance of ultimate pH in the MSA grading. So the project has had significant scientific outcomes as well as significant industry impacts.

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Executive Summary

Meat colour has important implications for consumer acceptability and eating quality. Sensory studies have correlated dark colours with a low desirability score. The highest level of desirability is associated with beef loins that are a pink or pale red in colour where consumers have described such steaks as “meatier”. Darker steaks from various muscles have been described as having an “off- flavour” and can be “peanutty”, “sour” and “bitter” in comparison to normal steaks.

In addition, if meat colour specifications are not met, carcasses can incur economic penalties, and thus negatively impact both processors and producers financially. Nationally, this figure is estimated to be in the region of $177 million per annum, with some industry sources describing the penalty for a dark cutting to be approximately $400 per carcass. Hence, optimising the colour of beef muscle is an extremely important topic to consider for both the consumer and the economic impact on the processor and producer.

The objectives of this project were to (1) Investigate slaughter factors that contribute to variations in beef colour, (2) Investigate the influence of beef meat colour at grading on shelf-life, eating quality and water-holding capacity and (3) Initiate development of capability in meat science at CSIRO. The findings are summarised below.

1. Slaughter factors that contribute to beef colour

Unacceptable meat colour scores at the time of carcass grading are associated with reduced meat quality and consumer rejection. We hypothesised that the meat colour at carcass grading would be influenced by the pH and temperature decline post slaughter, as these would be determined by animal and processing factors. Beef carcasses (n = 1512) at seven Australian processing plants were assessed, at grading, for the meat colour of the *M. longissimus thoracis* (cube roll). Statistical modelling determined the animal, carcass and processing factors contributing to the meat colour score at carcass grading. The occurrence of unacceptably dark meat dropped from 8 to 3% when the time of grading was increased from 14 to 31 hour post slaughter (P<0.01). A high temperature at pH 6 (rigor temperature), high final pH (pH_F), pasture feeding and older animals were all associated with dark cube roll at carcass grading (P<0.05 for all). Less than 30% of carcasses with non-compliant pH_F displayed a dark non-compliant meat colour >3, indicative of an opportunity to determine the mechanism behind this pH-induced colour development and thus reduce the incidence of non-compliance. Structural changes in the muscle lattice are thought to impact the lightness and incidence of dark meat, the extent of which are pH and time post-mortem dependent. It is recommended that when there is a high occurrence of carcasses with a dark meat colour >3 that the time from slaughter to grading is checked to ensure carcasses are in full rigor at the grading point. This will assist in minimising economic penalties due to dark-coloured carcasses, increasing the profitability for the processor and producer and consequently the competitiveness of the industry.

2. Literature Review

Unfavourable meat colours are associated with a reduced value of the carcass, with dark cutting carcasses costing processors or producers up to $400 per carcass. In addition, consumer acceptability is reduced with either very pale or dark meat colours. Excess in surface wetness or weep can also be viewed with reduced quality. Dark cutting beef can also have variable tenderness and a “bitter”, “sour” or “off” flavour characteristics. Therefore, the industry has tried to minimise the occurrence of non-compliant meat through animal interventions such as improved handling and better feeding regimes prior to slaughter.

Meat colour is determined by both the pigment myoglobin and the structural aspects of the muscle. Myoglobin reacts with oxygen in the air and is either oxygenated to a bright red colour or oxidated to a brown colour. The extent of this reaction is dependent upon the oxygen consumption rate and the structure of the muscle. Techniques to optimise the red oxymyoglobin

have been employed, and include improved packaging and addition of antioxidants. The structure of the muscle can also determine the light scattering properties of the muscle and are much less researched. The extent of light scattering is determined by the extent of myofibril shrinkage, anisotropy of the sarcomere and the refractive index of the sarcoplasm and extracellular space. The integrity of myofibrillar and cytoskeletal proteins is believed to be involved in structural behaviours, especially during the early post-mortem period. In addition, sarcoplasmic binding to myofibrillar proteins may also play a role. The mechanisms and techniques to optimise the structural characteristics are yet to be substantiated and provide an interesting area for future research into optimisation of meat colour.

3. The influence of beef meat colour at grading on eating quality and water holding capacity

Optimal beef meat colour is associated with increased consumer acceptance, whereas dark or pale meat has a reduced desirability. Dark beef also has a variable eating quality and reduced shelf-life. We hypothesised that a poor meat colour at carcass grading would generate an unacceptable eating quality after vacuum packed chilled storage for up to 20 weeks, due to the unfavourable pH conditions commonly associated with light and dark muscles. At three beef processing plants, beef *longissimus thoracis* (cube roll) muscles from 81 pasture and grain fed cattle (mix of *Bos taurus* and *Bos indicus* x *Bos taurus*) were graded at ~24 hours post-slaughter for meat colour. The carcasses were allocated to light, medium and dark colour groups with n=27 carcasses per colour group. From the 81 carcasses, a total of 162 *longissimus lumborum* (*LL*) or striploin muscles were collected and half striploins were randomly allocated to six ageing times (0, 2, 8, 12, 16 and 20 weeks) within colour group and six half striploins were used per colour group within storage period and plant. Vacuum packed muscles were stored at -1.0±0.5°C for the designated period and sampled for biochemical and sensory assessments.

The effects of colour group, storage week and carcass traits were analysed. Dark muscles had higher pH compared to the lighter colour group ($P<0.05$). Contrary to our hypothesis, after 2, 12 and 20 weeks of vacuum packed chilled storage; eating quality was similar for all 3 meat colour groups ($P>0.05$). With increasing storage time, all eating quality attributes improved ($P<0.001$ for all). The carcass traits dentition, feed type and fat depth did not influence the eating quality ($P>0.05$). Lipid oxidation increased with storage time and although values at 20 weeks were slightly above accepted levels for rancidity detection, MQ4 scores indicated the product would still be categorised as a 3 star product, indicative of the opportunity to store striploins for this length of time, whilst maintaining an acceptable eating quality, regardless of meat colour at carcass grading. Between colour groups, purge (as an indicator of water-holding capacity) was similar, with no significant differences observed. With aging, purge values increased, especially after 12 weeks storage, but all muscles had less than 3.5% purge. This suggests muscles could be stored with no major impact to the colour or quality of the final product. A parallel study (A.MIS.1004) confirmed acceptable microbiology. These were the first results to show striploins could be stored for 2, 12 and 20 weeks, and subsequently achieve 6, 4 and 3 days retail display respectively. Thus, beef striploin eating quality improved up to 20 weeks storage and was unrelated to meat colour at carcass grading. This provides a competitive advantage for the Australian export market, by extending the shelf-life of the product.

4. Development of capability in meat science at CSIRO

As is evident from the research described above, undertaking research on meat colour has increased the capability of meat science team at CSIRO-Coopers Plains. New methods for meat colour analysis and biochemistry techniques to identify proteins and extend knowledge in the meat space have been developed. In addition, Joanne Hughes has published 2 papers as an outcome of this project (listed below) and has developed excellent skills in research and development for the meat industry. She has communicated these results to the meat industry in several forums and has started a PhD, under the supervision of Professors Robyn Warner (now at Melbourne University) and Frank Clarke (Griffith University).

(i) Hughes, J. M., Kearney, G., & Warner, R. D. (2014). Improving beef meat colour scores at carcass grading. *Animal Production Science, 54,* 422-429. http://dx.doi.org/10.1071/AN13454

(ii) Hughes, J.M., McPhail, N.G., Kearney, G., Clarke, F. & Warner, R.D. (2014). Beef *longissimus* eating quality increases up to 20 weeks storage and is unrelated to meat colour at carcass grading. *Animal Production Science* – in press.

5. Recommendations & limitations

The report highlights animal, carcass and processing factors contributing to meat colour scores at grading and provides recommendations to reduce the incidence of dark meat colour at grading for beef carcasses. The report also indicates the beef industry can age striploins for up to 20 weeks whilst still achieving acceptable eating quality for the consumer. Unfortunately, consumer acceptability and eating quality was not conducted on retail display steaks at the end of display period, so this would need to be considered for future studies.

1 Background

Problems with the colour of red meat have important implications for consumer acceptability, eating quality and economics of the industry. Consumers are seeking an attractive red colour, and show less preference for beef steaks that are either extremely dark or extremely pale, with dark colours in particular showing a low desirability score (Jeremiah, Carpenter et al. 1972). Dark meat has also been associated with variable tenderness and a reduced meat flavour and increase in "sour" or "bitter" flavours (Viljoen, de Kock et al. 2002). In addition, a dark meat coloured carcass is estimated to cost approximately $50-400 per carcass (industry sources). Thus, the national impact of downgrading carcasses (from industry source values) is estimated to be in the region of $15 to 177 million annually. This has a detrimental impact on one of Australia's largest domestic and export industries.

Meat Standards Australia (MSA) uses a grading system, a portion of which relies on the AUSMEAT chiller assessment language (AUS-MEAT 2005) where qualified graders assess the carcass for traits such as meat colour, fat colour, marbling and ossification. For assessing meat colour, carcasses are quartered and allowed to bloom for at least 20 minutes at a low temperature (8°C). The *longissimus thoracis* (cube roll) at the $10^{th}/11^{th}$ rib or at the $12^{th}/13^{th}$ rib is graded using colour chips on a colour scale (Figure 1). The scale ranges from 1A to 7 and is scored by the most predominant colour present in the muscle surface. Carcasses are considered non-compliant and unacceptable by MSA if the colour of the striploin is not in the range of 1B to 3. Colour score 1A is considered to be pale, soft, exudative (PSE) or heat toughened and scores ≥4 are known as "dark cutters" (DC). Dark cutting meat is a considerable difficulty for processors due to unacceptability of colour.

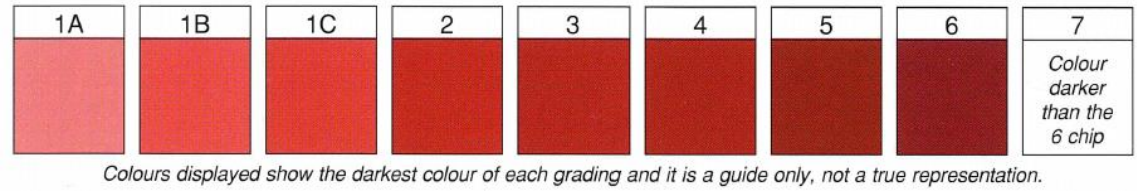


Figure 1: The AUS-MEAT colour scale (AUS-MEAT 2005) used as part of the chiller assessment language for meat colour assessment on beef cube roll muscle by qualified graders.

Early post mortem, biochemical mechanisms in the muscle contribute to the differences observed in the meat quality and overall colour. It is proposed that these differences could influence the shelf life of vacuum packs, especially when stored for export markets, which could be long periods of time (>12 weeks). A previous report (A.MFS.0166) demonstrated that vacuum packed primal could be stored confidently for 26 weeks or more, provided the appropriate conditions were obtained (Small, Jenson et al. 2012). Thus, this project aimed to determine animal, carcass and processing factors impacting beef meat colour at grading and the influence of beef meat colour at grading on the shelf-life of vacuum packed meat.

2 Project objectives

The project objectives were as follows:

1. To investigate slaughter factors that contribute to variations in beef colour
2. Investigate the influence of beef meat colour at grading on shelf-life, eating quality and water-holding capacity

3. Initiate development of capability in meat science at CSIRO

3 Slaughter factors that contribute to beef colour

This section summarises the findings from the research that was undertaken to investigate the slaughter factors that contribute to beef meat colour. The details of this research were published in Journal of Animal Production Science and the paper can be found in Appendix 8.1 (page 63).

An unfavourable beef meat colour is associated with negative impacts on eating quality, consumer acceptance and the value of the carcass. This is a major problem to the beef industry and is estimated to cost processors and producers up to $177 million per annum. For these reasons, this project aimed to identify the animal and slaughter factors that contribute to variations in beef colour.

From the 15 explanatory variables tested, the significant factors in the model were determined to be final pH (pH_F), temperature at pH 6, time from slaughter to grading, feeding regime and animal maturity. The occurrence of unacceptably dark meat dropped nearly 3 fold (from 8% to 3%) when the time of grading was increased from ‘14’ hours to ‘31’ hours post-slaughter ($P<0.01$). This highlighted the importance of the time from slaughter to grading on determining the value of the carcass. A proposed mechanism was outlined, describing the structural changes that occur during the early post-mortem period, which are believed to drive the lightening effect, observed with time post-slaughter. Consequently, recommendations were made to extend the time from slaughter to grading, especially when a dark colour is observed.

Dark cube roll muscles at carcass grading were observed where a high temperature at pH 6 (rigor temperature) or high final pH (pH_F) was measured (P<0.05). A high temperature at pH 6 was associated with lighter carcasses and an optimal temperature around 25°C was recommended to minimise meat colour issues such as dark-cutting and heat-induced toughening. Less than 30% of carcasses had a non-compliant pH_F and a dark non-compliant meat colour >3, indicative of an opportunity to determine the mechanism behind this pH-induced colour development and thus reduce the incidence of non-compliance.

In addition, carcasses were also darker when they were obtained from animals that were older or pasture fed (P<0.05). This indicates the animals which may be “at risk” of dark cutting and which could be targeted to improve beef meat colour.

4 Literature Review

4.1 Summary

Unfavourable meat colour is associated with a reduced value of the carcass, with non-compliant carcasses being a major issue for processors and producers. Carcasses can lose value up to $400 per carcass and the estimated impact to the industry could be up to $177m/ annum.

Consumers also view either very pale or extremely dark colours unfavourably and such meat is often associated with being unacceptable. Pale colours can experience excess in surface wetness or weep can also be excessively soft and associated with toughening, especially after aging. Dark cutting beef can also have variable tenderness and a flavour which is less meaty and has more “bitter”, “sour” or “off” descriptors. Therefore the industry has tried to minimise the occurrence of non-compliant meat through improved animal handling and minimising pre-slaughter stress. In addition, optimised feeding regimes have been recommended to improve the glycogen stores of the animal and ensure a full pH decline is achieved post slaughter.

Meat colour is determined by primarily by pigment myoglobin, with the majority of research focusing on protein oxidation mechanisms and interventions. Myoglobin reacts with oxygen in the air and is either oxygenated to a bright red colour or oxidated to a brown colour. The extent of this reaction is dependent upon the oxygen consumption rate and the structure of the muscle. Techniques to optimise the red oxymyoglobin have been employed, and include improved packaging and addition of antioxidants.

The structure of the muscle is also a determinant of meat colour as it can impact the light scattering properties of the muscle and is much less researched. The extent of light scattering is determined by the extent of myofibril shrinkage, anisotropy of the sarcomere and the refractive index of the sarcoplasm and extracellular space. During the early post-mortem period, there is extensive muscle fibre shrinkage and contraction of the sarcomere during rigor. Thus, in the time from slaughter to grading, there are major structural changes in the muscle, which are thought to impact on the lightness of the meat. The mechanisms behind colour development of low and high pH meat are believed to be different and are thought to arise from variations in myofibrillar and cytoskeletal protein integrity which control lattice spacing. In addition, sarcoplasmic proteins are thought to bind to myofibrillar proteins and could be involved in changes in refractive index and

the extent of light scattering. The mechanisms and techniques to optimise the structural characteristics are yet to be substantiated and provide an interesting area for future research into optimisation of meat colour.

4.2 Importance of meat colour to the industry

4.2.1 Cost to Industry

Within the beef industry, the colour of the meat is of critical importance to the value of the carcass as it reflects both the consumer preference for purchase and the acceptability in terms of eating quality and shelf-life. If the colour of the meat is poor, producers and processors can bear a large economic loss and consumers of meat can suffer the consequences of a poorer quality product. For this reason, an assessment scheme has been developed to improve the reliability of product.

Meat Standards Australia (MSA) have developed a grading system known as the chiller assessment language (AUS-MEAT 2005) where qualified graders assess the carcass for traits such as meat colour. Carcasses are quartered and allowed to bloom for at least 20 minutes (≤12 °C). The *longissimus thoracis* (cube roll) at the $10^{th}/11^{th}$ rib or at the $12^{th}/13^{th}$ rib is graded using colour chips on a colour scale (Figure 1, page 8). The scale ranges from 1A to 7 scored by the most predominant colour present. Muscles are considered non-compliant by MSA if the colour is not in the range of 1B to 3. Colour score 1A is considered to be pale, soft, exudative (PSE) or heat toughened and scores ≥4 are known as “dark cutters” (DC) or “dark, firm and dry (DFD)”. Both of these conditions are considered unfavourable for meat quality and consumer satisfaction and also economically.

Generating DC meat is a considerable difficultly for processors due to export limitations. The higher pH conditions associated with DC can cause issues with more bacterial growth and hydrogen sulphide formation, which can result in green spots and spoilage (Nicol, Shaw et al. 1970). Consequently, DC meat is normally not used for whole sale cuts of meat, but instead diverted to secondary markets for incorporation into meat products or small goods (Brownlie 1989). But, inclusion of DC meat into meat products can be problematic, due to the unfavourable quality properties. For these reasons, there are economic implications for processors and producers.

A DC carcass is estimated to cost approximately $50-400/ carcass (industry sources). During 2011-12, MSA non-compliance for meat colour was 3.6% (Meat and Livestock Australia Limited 2012). In the same year, 8.5 million cattle were slaughtered nationally, meaning approximately 300, 000 carcasses would be failing meat colour (ABARES 2011-12). Thus, the national impact of downgrading carcasses (from industry source values) is estimated to be in the region of $15 to 177 million annually. This has a detrimental impact on one of Australia’s largest domestic and export industries**.**

4.2.2 Consumer acceptability

The appearance of beef to consumers is of critical importance during the purchasing process. When beef is presented to a consumer, a number of factors contribute to the acceptability of the product. These factors can include the appearance of fat, degree of marbling, meat colour, a wet or moist appearance of the surface and also the presence of weep in the bag or tray. All of these factors influence the consumers preference and therefore the purchasing power of the product and hence the overall price.

In relation to meat colour, researchers have performed studies correlating dark colours with a low desirability score (Jeremiah, Carpenter et al. 1972). The highest level of desirability was

associated with beef loins that were a pink or pale red colour. This is consistent with the findings of (Viljoen, de Kock et al. 2002) who also found higher consumer acceptability scores associated with normal (pH <5.8) raw longissimus thoracis and lumborum steaks compared to high pH (≥ 5.8) DC steaks, which had an old or not fresh appearance. Consumers are seeking an attractive red colour, and show less preference for steaks that are either extremely dark nor extremely pale (Jeremiah, Carpenter et al. 1972). These findings highlight the importance of an optimal colour for consumer satisfaction.

4.2.3 Relationship between meat colour and eating quality

Water holding capacity

Meat colour is also closely associated with the surface tension and the water holding capacity (WHC) of the muscle. The appearance of excess weep associated with muscle is viewed negatively by consumers, as is often the case in pale, soft and exudative (PSE) conditions (Hunt and Hedrick 1977). Excessive amounts of weep are normally associated with a loose, open structure which is correlated to lighter colours of muscle. In contrast, a darker coloured muscle with a closed, tight structure is associated with a higher WHC capacity.

On the surface of the muscle, the size of pores or space between fibres can also influence the amount of water retained in the structure (Trout 1988). Larger pore sizes tend to be associated with lower water retention and an increased weep loss. These pores are of considerable interest and could be directly related to light scattering properties. This can result in an unfavourable product in terms of texture. The loss in moisture during the mastication is related to juiciness, texture and flavour release.

Juiciness and tenderness

As meat colour is directly influenced by the water holding capacity and the expressible moisture, so too is the texture. The density of the structure will influence the overall eating quality by influencing both the tenderness and mouth-feel. Some studies have been conducted on both the eating quality of DC and PSE beef.

The eating quality of DC beef has been viewed extensively, in relation to toughness, texture, juiciness and flavour (Dransfield 1981). Upon cooking, 4 to 5 day post-mortem DC steaks loose moisture and become more open in structure and have higher panel tenderness scores (Hunt and Hedrick 1977). When aged for 7 days and frozen, DC steaks from the *longissimus*, *gluteus medius* and *semimembranosus* have displayed higher shear force values, with a corresponding reduction in sensory panel tenderness (Wulf, Emnett et al. 2002).

In relation to pale, soft, exudative (PSE) beef, there has been a reported decrease in shear values of the cube roll compared to normal beef (Hunt and Hedrick 1977; Aalhus, Best et al. 1998). In terms of sensory attributes, an increase in loose water has been correlated with a decrease in taste and texture (Wismer-Pedersen 1959).

Both the PSE and DC conditions are examples of the importance of meat colour and the inter-relationship with other quality attributes of the muscle. The significant effects that meat colour have on WHC, juiciness and tenderness of the overall product have been highlighted. In addition, these attributes can also be related to the overall perception of flavour.

Flavour

One of the key components of eating quality is the flavour perception of the meat. From the literature, sensory panellists tend to prefer the stronger beef flavour of normal beef compared to DC beef (Dransfield 1981; Wulf, Emnett et al. 2002). Consumers have described normal steaks as “meatier” and DC steaks as having an “off- flavour”. This reduced meaty flavour is a result of

lower carbohydrate content in the meat and a subsequent reduction in the Maillard reaction. DC steaks from various muscles are described as “peanutty”, “sour” and “bitter” in comparison to normal steaks (Wulf, Emnett et al. 2002) and semitendinosus roasts having strong aftertastes associated with a “bittery”, “livery” and “off” flavour (Jeremiah, Aalhus et al. 1997). An increased rancidity flavour has also been associated with high pH muscles (Yancey, Dikeman et al. 2005). These rancid and bitter flavours have been associated with beef that has been aged and is no longer desirable (Spanier, Flores et al. 1997), thus highlighting the lower preference for a high pH meat.

It is interested to consider some of the compounds, which could determine the altered flavour properties of high pH meat. Lactic acid formation is positively correlated with sour flavours and also, bitter and astringent flavours are associated with red, slow-oxidative muscles, possibly due to the higher carbonyl, iron and free fatty acid (Chikuni, Oe et al. 2010). Considering these compounds is therefore important to the sensory characteristics of the muscle.

In summary, colour is only one aspect of consumer acceptability and is determined by numerous structural and functional properties of the muscle. It is for this reason, that differences in colour impact the eating quality aspects of the muscle. Thus, determining the mechanism of colour development and optimising colour could minimise negative eating quality attributes exerted upon the meat and improve both economic impact to the industry and consumer satisfaction.

4.3 Muscle composition

The muscle is a complex system and must be examined in relation to quality attributes, especially colour components. In particular, myoglobin has been extensively researched, but there is evidence to suggest structural proteins are also involved. This review will summarise current research methods and the possible mechanisms behind variations in colour.

4.3.1 Pigment

There are two main pigment compounds found within the animal, which largely participate in the observed colour of the muscle. Haemoglobin is found within the red blood cells (erythrocytes) in the circulatory system and myoglobin is a sarcoplasmic protein found in the muscle cell. Both pigments are involved in the maintenance of optimal oxygen concentrations in the tissues of the animal.

Haemoglobin

Haemoglobin is responsible for the transport of oxygen and carbon dioxide to and from the tissues and is thus located in the circulatory system (erythrocytes). It is a metalloprotein that contains four haem groups per molecule, thus giving it a quaternary structure. Each globular haem protein contains a central iron atom. The oxidation of this central iron atom upon exposure to oxygen results in bright red appearance of the blood. A delayed bleeding after slaughter can result in bacteraemia (Lawrie 1977). Such spoilage of otherwise a sterile medium, promotes a “gamey” flavour and discolouration. However, others have shown delayed bleeding (3 to 6 minutes) was not correlated with an elevated total pigment or haemoglobin content and no differences in lean colour were observed in heifer cube roll, striploins and *biceps femoris* muscles (Williams, Vimini et al. 1983). Also, a trained panel could not significantly discriminate the flavour, juiciness or tenderness, between carcasses bleed at 0, 3 and 30 minutes post stunning**.**

Myoglobin

Myoglobin is the primary colour pigment in both heart and skeletal muscle of vertebrates. Myoglobin content in three beef muscles was determined to be between 2-5mg/g wet weight

(Hunt and Hedrick 1977). The total pigment of the *longissimus* was determined to contain approximately 80% myoglobin (Livingston and Brown 1981). Therefore, myoglobin can be considered to be the primary pigment and is a key determinant in colour of beef muscle.

The colour displayed by the pigment is quite often due to the ligand present in the binding pocket (Figure 2). During muscle post-mortem degradation and storage, a series of autoxidation reactions occur which alters the colouration of the meat. When myoglobin is in the native deoxygenated form (no oxygen present) it appears as a purple colour and is known as deoxymyoglobin (DMb). DMb contains no ligand in the sixth position of the iron complex and utilises only five binding sites. With exposure to oxygen, the protein becomes oxygenated and forms a bright cherry red colour known as oxymyoglobin (OMb). In both DMb and OMb, the central iron atom is found in the ferrous form (Fe^{2+}). Furthermore, when the iron atom contains many unpaired electrons and is in the reduced ferric form (Fe^{3+}), it results in the formation of metmyoglobin (MMb), which is brown in colour.

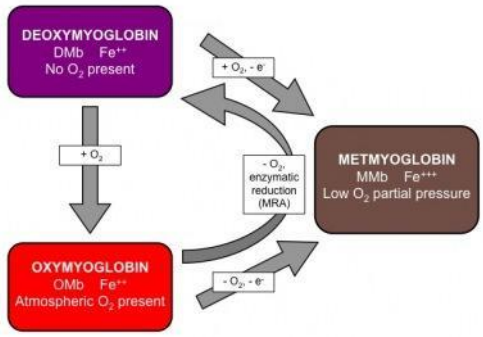


Figure 2: Oxidation states of myoglobin, modification of image from (Raines 2009), originally from (Mancini and Hunt 2005).

During post-mortem degradation of the muscle, the various oxidation states of myoglobin occur at different depths of the muscle. Myoglobin has a high affinity for oxygen, so when muscle is first exposed to the air, the purple DMb becomes exposed and undergoes oxidation to form an OMb layer on the surface Figure 3. The time for the bright red colour to form is known as blooming and is associated with the freshness and quality of the meat. Recommendations for blooming time in beef muscle prior to grading are between 20 minutes and 3 hours (AUS-MEAT 2014).

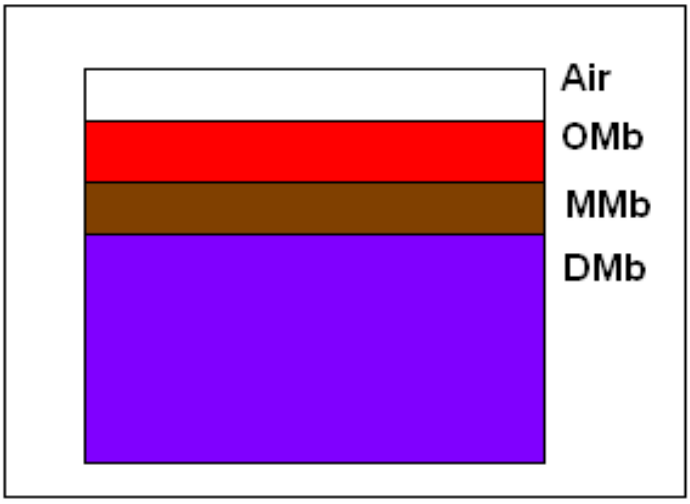


Figure 3: Surface layers of myoglobin on exposure to oxygen in the air (oxymyoglobin, OMb; metmyoglobin, MMb; deoxymyoglobin, DMb).

The depth of the OMb layer is dependent upon the oxygen penetration into the muscle. The depth at which the oxygen level is almost zero is normally around 2-4mm (Brooks 1929), and hence the OMb layer is normally this deep from the surface. Below this point oxygen concentration is not high enough to saturate the myoglobin (Young and West 2001). During storage of meat a MMb layer starts to develop between the DMb and OMb layers. This brown MMb layer increases both towards the centre and the surface of the muscle during storage. This process is dependent upon numerous factors and can be investigated in relation to the reducing capability of the muscle and also the oxygen consumption rate (OCR).

The speed at which oxygen penetrates into the muscle is known as the oxygen consumption rate (OCR). The depth of penetration was originally determined in an ox flexor muscle and was found to be 2mm (0°C) and after 100 hours increased linearly to 4mm (Brooks 1929). These authors noted as time progressed, and depth of penetration increased so did the oxygen consumption of the muscle. Thus, when OCR is lower the depth of penetration is higher and the MMb layer forms lower in the tissue and minimises colour deterioration with storage. This has led to a considerable amount of research in this area to investigate factors influencing OCR.

The OCR process is dependent upon several factors, such as the partial pressure of oxygen (pO_2) (Brooks 1935), the pH and temperature of the meat. As seen in Figure 4, the effect of pO_2 is correlated to the reaction speed of OMb to MMb (George and Stratmann 1952; Young and West 2001). The steep rise of the curve is indicative of the initial stages of blooming when meat is first exposed to air and has been investigated relative to storage conditions (Feldhusen, Warnatz et al. 1995). In cold pre-rigor conditions, the rate of ADP generation by the ATPase limits mitochondrial respiration and results in a decrease of ATP and OCR. In warmer conditions, (15 to 38°C), ATP turnover is the rate limiting factor which determines OCR. Therefore, increasing temperature results in an increased OCR (Bendall 1972a) and thus would result in more oxygenation and a redder appearance.

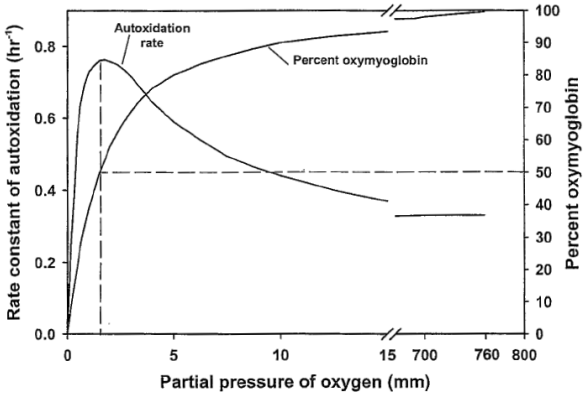


Figure 4: Rate of autoxidation of myoglobin as influenced by pO_2. Modified by (Young and West 2001), original from (George and Stratmann 1952).

In post rigor muscles, the lower pH alters the OCR in a different manner. Due to the higher demand in energy and lower content of available metabolites, the rate of ATP generation, as determined by the rate of mitochondria respiration, is the primary factor determining OCR. Also, increasing pH towards neutral results in an increase of 50-100% in OCR (Bendall and Taylor 1972b). Thus, as the pH of the muscle is higher, the OCR is greater, but this may be inhibited by the dense structure of the muscle fibres, which would limit oxygen penetration into the structure, hence the thick DMb layer often observed in high pH muscles.

Like all cellular pathways, the oxidation of myoglobin may be impacted by lipid oxidation pathways due to the interactions and common metabolites involved (Baron and Andersen 2002). Peroxides, such as hydrogen peroxide (H_2O_2), are thought to react with myoglobin to promote it as a pro-oxidant species. These reactants are thought to contribute to the oxidation of unsaturated fatty acids and breakdown of lipid hydroperoxides. Other molecules generated by lipid oxidation reactions, such as 4-hydroxy-2-nonenal (HNE), have been shown to induce redox destabilising of myoglobin in beef (Alderton, Faustman et al. 2003). The presence of HNE was shown to accelerate the oxidation of OMb to MMb in both acidic (pH 5.6) and at physiological pH (pH 7.4) with low temperatures (4 and 25 °C). At 37°C, oxidation was only enhanced at physiological pH. The results indicated that within bovine myoglobin, preferential adduction of HNE to the proximal His 93 was “suggested that lipid oxidation-induced OMb oxidation was potentially more critical in beef than pork”.

These reactions and their interactive relationships are reviewed by (Faustman, Sun et al. 2010). They examined both myoglobin as a facilitator of LOX and vice versa and the use of antioxidants such as α-tocopherol and lycopene, incorporated into muscle in order to minimise unfavourable oxidation reactions. For these reasons, the importance of feeding regime and antioxidant availability has received considerable attention (Mohan, Hunt et al. 2010; Bekhit, Hopkins et al. 2013; Ponnampalam, Norng et al. 2014).

4.4 Muscle structure and light scattering properties

In addition to the pigment composition of the muscle, the structural proteins are also involved in light scattering and translucency of the muscle (Swatland ; Macdougall 1970; Krzywicki 1979). Light scattering is the deflection of photons from a straight path (incident light) to multiple diffuse patterns as normally caused by some sort of matter, in this case muscle. The light is either reflected back to the observer or else it is dispersed throughout the muscle and can undergo

refraction, absorption or transmission. The light scattering properties will be determined by the degree of attenuation or loss of intensity of light that occurs in the muscle, with low pH muscles associated with more light scattering than high pH muscles (Swatland 2008).

However, few scientists have researched the influence of light scattering properties to the colour of muscle. Work has been conducted in the areas of photometry and the movement of light around the muscle, using x-ray diffraction, goniophotometers and various microscope devices (MacDougall and Jones 1981; Diesbourg, Swatland et al. 1988; Swatland 1988; Swatland 1990). Such methods provide insight into light scattering and the structural mechanisms involved. However, there are still many uncertainties around the major determinants and the mechanism, both molecularly and at the macromolecular level. The key mechanisms are proposed around the myofibrillar spacing, anisotropy of the sarcomere, integrity of cytoskeleton and cell adhesion proteins and refractive index of the extramyofibrillar or extracellular space. These are outlined below.

4.4.1 Myofibrillar spacing

The spacing between the myofilaments (e.g. actin and myosin) is about 40 nm apart and is insufficient to allow for penetration of large amounts of light. For this reason, this space is thought to have minimal involvement in the light scattering properties of the muscle. However, the intermyofibrillar space is larger (100 to 400nm) and provides a large enough space for light to be scattered. The diameter of the myofibrils and hence the space that is between them will primarily determine the light scattering properties (Offer, Knight et al. 1989). Myofibrils that are swollen (as in high pH or DC meat) will have a larger diameter and lie adjacent to each other and have a close packing arrangement (Figure 5). If the myofibrils are butting, there is less ability for the light to be diffracted between the myofibrils. This reduces scattering, maximises the depth of light transmittance and minimises reflectance, and hence decreases the lightness or L* value of the meat.

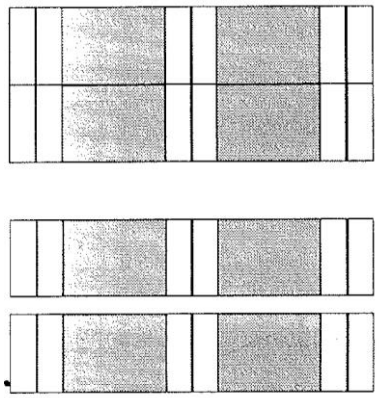


Figure 5: Variability in the parallel packing of myofibrils relative to adjacent myofibril. Top: A close packing arrangement reduces light scatter & Bottom: Gaps between myofibrils may contribute to different refractive indices and a higher degree of scattering.

In comparison, in a low pH or PSE condition, there is more space between the myofibrils and light is able to pass easily through the tissue there is a higher level of scattering. The myofibrils and the gaps between them display a large variation in refractive index and allow for the passage

of light around the structure of the myofibril. This can be caused by a shrinking process or some method of separation which allows for more reflectance and light scattering in the structure.

The rate and degree of pH fall is also influential to this process. A higher level of myofibrillar shrinkage occurs when the pH declines from 6 to 5.5, compared to that from pH 7 to 6 (Diesbourg, Swatland et al. 1988). Needless to say, higher pH muscles incur less shrinkage. Also, faster glycolysing muscles with lower WHC tend to show earlier membrane damage, a higher level of extracellular water and larger gaps between myofibrils which could be contributing to more light scattering (Tornberg, Wahlgren et al. 2000). The higher level of denatured proteins and lower ability of these proteins to bind water could be contributory to the lighter colour observed. It is interesting to note that little change in lattice spacing is observed when muscles are held around physiological pH (pH 7.2), and signifies the importance of the pH decline on the myofibrillar shrinkage.

4.4.2 Anisotropy and integrity of the sarcomere

In the longitudinal section, light scattering properties are also affected by the striations of the muscle and hence the integrity of the sarcomere. Low pH muscles (pork & beef) show more degradation around the M-line whilst the Z-lines remain intact (Cloke, Davis et al. 1981; Yu and Lee 1986). In comparison, high pH muscles show the opposite, with Z-lines being degraded and M-lines more preserved. These findings suggest differences in integrity of the structural proteins could be causative of various proteins of the myofibril being degraded at different rates (Table 1).

Desmin degradation is also more prevalent in high pH pork *longissimus* muscle, which could be responsible for the loss in integrity of the Z-line in these muscles (Bee, Anderson et al. 2007). This weakening of the sarcomere structure is associated with elevated levels of μ-calpain and increased myofibrillar fragmentation and illustrates the susceptibility of the structure which could play a role in altering light scattering properties.

In fast glycolysing or low pH muscles, there is more myosin denaturation and faster troponin T degradation (Ho, Stromer et al. 1994; O'Halloran, Troy et al. 1997). In comparison, there is more tropomyosin and α-actinin. This modification of structural proteins could alter the extent of sarcomere contraction, anisotropy and the extent of muscle fibre shrinkage during the early post-mortem period, thus impacting the extent of light scattering.

Table 1: Summary of intact and rate of degradation of various proteins (myofibrillar, cytoskeletal or sarcolemma) with different rates of pH decline.

Protein	Slow glycolysing or high pH or high WHC muscle	Fast glycolysing or low pH or low WHC muscle	Reference
Myofibrillar / contractile proteins			
Myosin	More myosin	Less myosin (higher levels of denaturation of	(Stabursvik, Fretheim et al.

		subfragment 1 & 2) in PSE pork	1984; Warner, Kauffman et al. 1997)
Troponin T (Tn T)	More Tn T	Less Tn T (faster breakdown)	(Ho, Stromer et al. 1994; O'Halloran, Troy et al. 1997)
Tropomyosin (TM)	Less TM	More TM	(Yu and Lee 1986)
α-actinin	Less α-actinin	More α-actinin	(Yu and Lee 1986)
Desmin	Less desmin	More desmin, increased shrinkage, increased drip	(Bee, Anderson et al. 2007)
Cytoskeletal or sarcolemma proteins			
Nebulin	More nebulin	Less nebulin/ more degradation (PSE pork and electrically stimulated beef)	(Ho, Stromer et al. 1996; O'Halloran, Troy et al. 1997; Warner, Kauffman et al. 1997)
Titin	More titin	Less titin/ more degradation	(Ho, Stromer et al. 1996; Watanabe and Devine 1996; O'Halloran, Troy et al. 1997)
Talin	Less talin	More talin, lower degradation, more drip loss.	(Bee, Anderson et al. 2007)
Integrin		Less integrin (more degradation of β1-chain)	(Lawson 2004)

4.4.3 Integrity of the cytoskeletal and cell adhesion proteins

The extent to which the shrinkage occurs is dependent upon the degree of restraint to which the myofibrils are exposed to. Restraining transverse structural proteins will maintain shrinkage until the weakest of these elements is compromised (Offer and Cousins 1992). Cell adhesion, cytoskeletal or extracellular proteins (such as those of the endomysial- perimysial junction) all play a role in myofibrillar spacing. The denaturation of some of these proteins (e.g. titin and nebulin) in the low pH condition is believed to be partially responsible for less restraint of the myofibril lattice and consequently the ability to shrink. In comparison, high pH muscles display intact proteins which restrain the lattice and limit myofibrillar shrinkage.

In addition, the cell adhesion proteins integrin and talin have been involved in structural properties of the muscle. Integrin has a role in drip channel formation. Calpain mediated degradation of the β1 chain of integrin contributes to the opening of drip channels in pork muscle (Lawson 2004). Talin also appears to be involved in drip channel formation, with less intact protein being positively correlated to higher drip losses in the low pH condition. So, degradation

of these two proteins could be involved in generating a more open structure and contribute to lightness.

4.4.4 Refractive index of the extramyofibrillar / extracellular space

The sarcoplasmic proteins could also be partially responsible for changes in the refractive index of the extramyofibrillar and extracellular space and hence the light scattering properties. If they become modified, this could alter their solubility and binding properties (Hamm 1961; Clarke, Shaw et al. 1980; Warner, Kauffman et al. 1997) and change the viscosity and osmolarity of the sarcoplasm and distort the refractive index in the medium. A fast pH fall is associated with cell membrane damage and leakage of sarcoplasmic proteins (Tornberg, Wahlgren et al. 2000). In low pH conditions, sarcoplasmic protein insolubility increases and is associated with an increase in lightness and drip loss, indicative of their relationship to these attributes of the muscle (Joo, Kauffman et al. 1999).

Specifically, the sarcoplasmic proteins which have been observed in pork PSE condition were creatine kinase (CK), phosphorylase (PH), myokinase (MK) triosephosphate isomerise myokinase (TPI) and an unknown protein (26kDa protein) (Warner, Kauffman et al. 1997; Joo, Kauffman et al. 1999). In addition, aldolase and glyceraldehyde phosphate dehydrogenase (GAPDH) were also involved in the lightening effect of electrically stimulated meat (Clarke, Shaw et al. 1980). This highlights these proteins as possible culprits involved in increasing the light scattering properties of the muscle.

Also, the extent of transmittance could be impacted by organelles within the structure. Small diameter red oxidative fibres, which have a higher density of mitochondria within the extramyofibrillar space have a lower level of transmittance compared to those with a larger diameter (Swatland 2004). In addition, lysosomes or proteosomes may be influential on the light scattering properties of the muscle. In summary, any material which would alter the refractive index of the sarcoplasm or extracellular space could impact on the light scattering properties of the muscle.

4.5 Muscle glycogen concentrations

The quantity of glycogen present in the muscle tissue is important for overall post-mortem degradation. Excessive exercise or stressful circumstances can utilise glycogen stores present in both the muscle and the liver (Bate-Smith 1948; Walker, Warner et al. 1999). Low concentrations of muscle glycogen (approximately 0.6%) prior to slaughter minimises the amount of lactic acid formed in the muscle during post-mortem degradation. This results in a high ultimate pH as observed in the dark cutting (DC) condition in cattle (Ferguson, Bruce et al. 2001).

To prevent this DC condition, maintaining normal concentrations (1-1.5%) of muscle glycogen is extremely important (Ferguson, Bruce et al. 2001). Recommendations of feeding regimes and achieving optimal glycogen in the muscle for a suitable pH decline have been reported (Tarrant 1989; Knee, Cummins et al. 2007; Gardner, McGilchrist et al. 2014). From this information, recommendations have been made to industry to maintain glycogen levels at an optimum level in order to prevent the dark cutting condition (Meat and Livestock Australia Limited 2011).

4.6 Conclusion

In summary, both the pigment and the structure of the muscle is important in determining the colour of the muscle and hence the value of the carcass. Technologies currently being applied to improve meat colour have mainly focused on promoting optimal pigment and glycogen contents in the muscle, through optimal handling of the animal, adequate feeding regimes, antioxidant

additions to meat and favourable packaging regimes. Currently, there are few technologies focussing on improving the structure of the muscle and hence the light scattering properties of the meat. This is an opportunity for improving meat colour at grading and should be investigated further.

5 The influence of beef meat colour at grading on shelf-life, eating quality and water-holding capacity

5.1 Shelf-life and eating quality

This section summarises the findings from the research that was undertaken to investigate the influence of beef colour on shelf life and eating quality. The details of this eating quality research are accepted for publication in Australian Production Science and that paper can be found in Appendix (page 87) Currently, the majority of Australian beef is directed towards export markets, and predictions indicate there will be an increased demand from Asian markets like China and Indonesia to import Australian beef. Thus, the demand for beef processors to generate a product that has a long shelf-life which is suitable for consumption after ageing is increasingly important for the competitiveness of the Australian beef industry.

Previous research conducted by the CSIRO and MLA has indicated beef striploins could be successfully aged for up to 26 weeks, if optimal conditions were met early post-slaughter and during aging (Small, Jenson et al. 2012). Striploins were reported as having low microbiological counts and informal sensory analysis indicated suitability for consumption. Thus, this project aimed to assess the impact on eating quality of long aged striploins, using formal consumer sensory analysis.

In addition, high pH, dark beef has a variable eating quality with consumers often using sensory descriptors such as “off”, “sour” or “bitter” for flavour (Viljoen, de Kock et al. 2002; Wulf, Emnett et al. 2002). In addition, very pale carcasses have been associated with heat-induced toughening, which negatively impacts consumer eating quality (Warner, Dunshea et al. 2014). Unfavourable pH conditions of the muscles favour lactic acid bacteria and could contribute to early spoilage. Consequently, we aimed to investigate the impact of meat colour at grading on the ageing potential of the striploins.

Vacuum packed muscles were stored in the dark at -1.0±0.5°C for up to 20 weeks and sampled periodically for biochemical and sensory assessments. The effects of colour group, storage week and carcass traits were analysed. Although, dark muscles had a higher pH and lower lactate levels compared to the lighter colour group ($P<0.05$), there were no differences observed in eating quality between colour groups. The carcass traits dentition, feed type and fat depth did not influence the eating quality ($P>0.05$).

With increasing storage time, all eating quality attributes improved ($P<0.001$ for all). Lipid oxidation increased with storage time and although values at 20 weeks were slightly above

accepted levels for rancidity detection, MQ4 scores indicated the product would still be categorised as a 3 star product, indicative of the opportunity to store the striploin for this length of time, whilst maintaining an acceptable eating quality, regardless of meat colour at carcass grading.

5.2 Shelf-life and water holding capacity

5.2.1 Summary

In parallel, the effect of beef meat colour and ageing was investigated to determine the biochemical properties of the meat. The accumulation of purge in long aged striploins has been perceived negatively by consumers. Also, it is well known, the amount of purge can vary with meat colour, with dark meat colours retaining fluid and paler meat colours often being associated with high levels of purge (Warner, Dunshea et al. 2014). Therefore, we aimed to determine the effect of shelf-life on the biochemical properties of the meat, including pH, purge and retail colour stability.

As storage time progressed, the purge values increased up to around 3 to 3.5% at 12 weeks, and remained fairly stable thereafter. The increase in purge is due to the disintegration of the structure of the meat. Surprisingly, there was no difference between the three meat colour groups (P>0.05) in the % purge. Also, there was no interaction between treatments (P>0.05). This indicates the maximum purge loss was reached around the 12 week mark and stabilised.

The pH values for each colour group also increased during storage and stabilised after 12 weeks storage. As would be expected, the 'dark' meat group displayed higher pH values for all storage weeks (P<0.05). The 'light' and 'medium' groups had a lower pH at all the storage weeks. These observations are consistent with the expectation that high pH meat is generally associated with a dark appearance of the meat surface.

Retail colour stability showed a similar trend for all meat colour groups; however 'dark' meat colours showed a higher incidence of purple deoxymyoglobin. As aging increased throughout the 20 week shelf-life period, all muscles showed a reduction in retail colour stability. Consumer acceptability studies are required to validate a retail display life of 6, 4 and 3 days after 2, 12 and 20 weeks long aged storage.

Biochemistry analysis indicated the mechanisms behind variations in meat colour were due to a variety of factors, these being variations in myoglobin concentrations, oxidative/ antioxidant capacity of the muscle, denaturation and structural modifications to proteins. Further research is required to elucidate the specific structural proteins involved, to enable the development of strategies for improving meat colour at grading.

Therefore, in parallel with microbiological and eating quality shelf-life experiments, this report illustrates the potential for industry to age striploins for 20 weeks. This provides further opportunity for the industry to maximise shelf-life especially for export markets. As the demand for beef increases in Asian and overseas markets, long-aged shelf-life will improve Australia's competitiveness, whilst ensuring consumer satisfaction.

5.2.2 Background

The water-holding capacity (WHC) of the meat has been shown to impact on other meat quality properties, such as tenderness, juiciness and the appearance of meat (Offer, Knight et al. 1989). A reduction in WHC has been associated with more purge or press juice and has been positively correlated with pH (Boakye and Mittal 1993). So, as pH decreases there is an increase in purge, the mechanisms of which are well described elsewhere (Hamm 1961; Kristensen and Purslow 2001). Therefore, variations in pH can lead to alterations in WHC, colour and eating quality.

During ageing, muscles can lose water in the form of purge, which results in higher pressed juice and cook losses (Boakye and Mittal 1993). In parallel, there is also increased lightness, brightness and yellowness (Boakye and Mittal 1996) indicative of a possible relationship. However, this relationship is certainly questionable (van Laack, Kauffman et al. 1994), so we

aimed to further investigate the impact of meat colour at grading on the WHC during shelf-life. We also wanted to further investigate the proteins which may give rise to variations in meat colour and thus investigated protein and lipid oxidation status and colour stability within this experiment.

5.2.3 Methodology

Striploin and Sample collection

During autumn 2012, over 3 consecutive days, three Queensland beef processing plants were visited and a total of 162 beef striploins (*longissimus lumborum or LL*) were collected from 81 beef carcasses (Achilles hung) at ~24 hours post-mortem (6 carcasses from plant 1 were collected at ~72 hours, due to lack of availability of 'dark' colour scores on the first visit). The animals were between 0 and 7 tooth (dentition), were either pasture or grain fed and were a mix of *Bos Taurus* and *Bos indicus* x *Bos Taurus*. Unfortunately, MSA data was only obtained from two plants, where the Tropical Breed content (TBC) was reported to be 0-38%. Hormone Growth Promotants (HGP) were used in 61 animals. Fat depth (mm) of the carcass at the P8 site on the rump (AUS-MEAT 2005) was recorded as well as hot carcass weight (kg). Selection of carcasses was on the basis of meat colour at carcass grading (AUS-MEAT 2005). At each plant, colour assessment using AUS-MEAT colour chips was conducted by qualified plant personnel using the left side of the carcass approximately 8 hours prior to our arrival onsite. Due to the 8 hour gap in time, the right side of the carcass was quartered once we arrived on-site and we conducted colour measurements after approximately 0.5-1 hour bloom. Briefly, the left side of the carcass was quartered either between the 10^{th} and 11^{th} or between the 12^{th} and 13^{th} rib (depending on market destination), and the exposed *longissimus thoracis* (LT) or cube roll muscle was allowed to bloom at ~0-4°C for approximately 0.5-1 hour prior to grading. Muscles were graded by a Meat Standards Australia (MSA) qualified assessor and allocated to 3 different meat colour groups (Light, Medium or Dark), defined by AUS-MEAT colour (AMC) scores: Light, 1B or 1C; Medium, 2 or 3; Dark, >3 respectively (Figure 6); 1B being the palest and 5 being the darkest) (AUS-MEAT 2005). Generally, colour scores of >3 are considered unacceptable in appearance and are discounted by beef processing plants.

Figure 6: Showing the AUSMEAT colour chips on the left hand side and allocation to colour groups Light: meat colour 1B or 1C, Medium: meat colour 2 and 3 and Dark: meat colour ≥ 4. The photograph on the right hand side shows a representation of a striploin in each colour group.

Colour measurements (L* a* b* values and spectral scan from 400-700 nm) were also measured at approximately 10°C. This was conducted using a Hunterlab Miniscan EZ (light source A, observer angle 10°, aperture size 5 cm). Other colour parameters were calculated and are described below. The right side of the carcass was also quartered and left to bloom for between 0.5-1 hour prior to measurement. This freshly bloomed side was used to compare the assessments made by the MSA qualified assessor on the left side of the carcass earlier.

For each colour group, at 1-3 days post-slaughter, the *LL's* from both sides were boned out and were cut in half to generate one cranial and one caudal sample (from both left and right sides) and randomly allocated to one of 6 ageing time points; 0, 2, 8, 12, 16 or 20 weeks in an incomplete block of 2 units, the block comprising the cranial and caudal ends of the *LL*. This was to ensure an optimal comparison of storage weeks across sides and carcasses, giving a total of six replicates per plant, storage time and colour group combination (Figure 7). Thus at each plant, a total of 54 *LL*'s were collected, from 27 carcasses generating 108 half *LL*'s. Samples were vacuum packed, placed in cartons and subjected to standard plant storage and chilling regimes (Figure 8). Apart from week 0 samples, cartons of vacuum packed half *LL*'s were delivered to the laboratory using a chilled storage vehicle and stacked on a pallet in the dark at -1.0°C ± 0.5°C for up to 20 weeks.

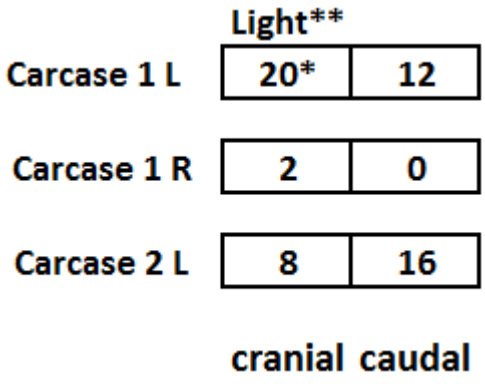


Figure 7: A diagram showing a representation of the allocation of treatments to storage week* (0, 2, 8, 12, 16 and 20) and colour group** (light, medium or dark) to 6 sections of striploin. Each striploin was cut into 2 pieces resulting in a cranial or caudal end, and were from 1 ½ carcasses; 2 from one carcass (L;left and R;right sides) and the other from a second carcass. Six half

striploins were collected for each colour group at each plant within storage time, yielding a total of 54 replicates from 81 carcasses.

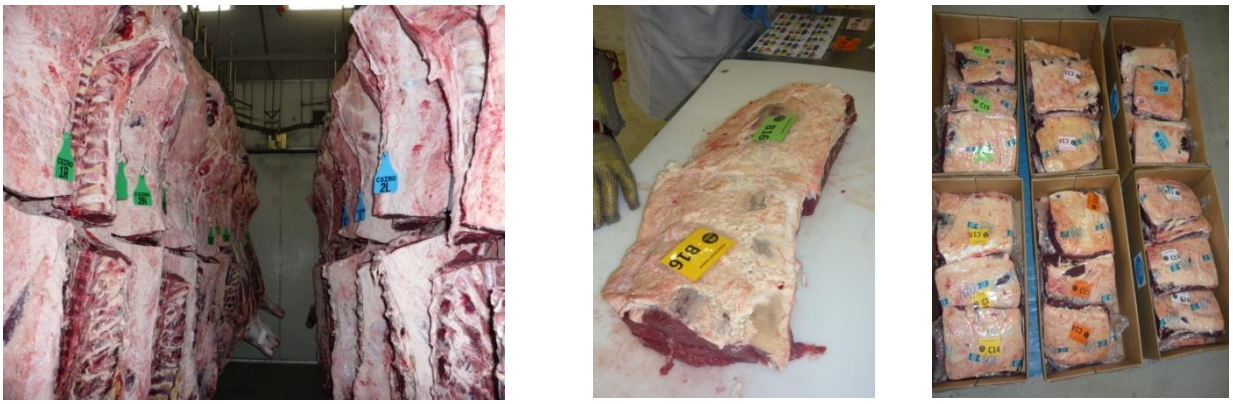


Figure 8: Far left: carcass identification and tagging. Centre: striploin halves were labelled and allocated into cartons for transport (far right).

Week 0 samples (54 striploin halves) were transported back to the laboratory in insulated containers and processed on the same day. Core samples were taken for microbiological analysis from 27 striploin halves (as part of another project, A.MIS.1004). Sub-samples were taken from all week 0 samples for protein solubility and extraction, tocopherol content, myoglobin concentration, and were frozen using liquid nitrogen and subsequently stored at -80°C until analyses could be completed. Only muscle samples, from plants 2 and 3, for storage week 0 were used for protein extraction and solubility. At all storage weeks, sub-samples for lipid oxidation (TBARS), glycogen and lactate contents were also collected. For TBARS collection, half of the striploins (n=9) were randomly selected for analysis. The pH, purge and colour of the samples were also measured. At weeks 2, 12 and 20 only, steaks for retail display were also cut and displayed for 12 days.

Retail display

For half striploins aged for 2, 12 and 20 weeks, after striploins were removed from vacuum packs, a 25mm thick steak from the central section of the muscle was bloomed at 5^{o}C for 60 minutes and the colour measured. At each week where the retail display occurred, six steaks were randomly selected per colour group (2 from each plant) for retail display. At week 12 and 20, a second steak was cut for TBARS analysis after 6 days, as described below. They were transferred into black foam trays (205 x 130 x 12mm) were over-wrapped with cling wrap and photographed using an Olympus digital SLR camera, model number E330, under 4 light struts angled at +/- 45° to the sample surface. Samples from each colour score group (light, medium, dark) were then placed on the top, middle and bottom shelves perpendicular to the light source (Figure 9). The light source in the cabinet was illuminated 24 hours a day and provided by 3 light tubes (Osram Natura L 36W/76) which were on the top and sides of the interior of the cabinet. These provided the highest intensity of light on the top shelf (1081 lux), medium level of light on the middle shelf (between 350-721 lux) and lowest intensity on the bottom shelf (290 -474 lux). Steaks from each colour group were rotated between top, middle and bottom shelves on a daily basis.

Steaks were displayed for 12 days and photographs were taken on a daily basis, using the camera described above. Colour measurements were also made daily, with the over-wrap intact on the meat, using colour equipment described above, standardised through similar over-wrap

film. At the end of the storage time, steaks were cut in half transversely and photographed through the depth of the steak to view the depth of browning.

Samples were taken for the determination of TBARS on day 0, 6 and 12 of the retail display samples from steaks aged for 12 and 20 weeks only. Separate steaks from the same striploin were used for this purpose. Week 2 retail display striploins only had samples taken for TBARS on day 12 of display.

Figure 9: Far left: retail display cabinet covered to minimise interference from external light. Centre: cabinet containing steaks across all 3 shelves and top shelf (far right).

Colour measurement

Triplicate colour measurements (L* a* b* values) and % reflectance at each wavelength from from 400-700 nm were measured at ~10°C. This was conducted using a Hunterlab Miniscan EZ (light source A, observer angle 10°, aperture size 5 cm). This was conducted on either a freshly cut 25mm steak after 60 minutes blooming at 5°C or on retail display over-wrapped steaks at ~20°C. The instrument was calibrated at the same temperature of measurement, using white and black calibration tiles, as supplied with the instrument (Novasys group Pty Ltd, Ferntree Gully, VIC, Australia). For retail display over-wrapped steaks, calibration was conducted through similar plastic.

Colour parameters were measured as follows:

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

In addition, the relative proportions of each myoglobin form (MMb – metmyoglobin; DMb – deoxymyoglobin; OMb- oxymyoglobin) were calculated as follows (Krzywicki 1979):

Reflectance (R) was converted to reflex attenuance (*A*) using equation 1:

Equation 1: $A = \log 1/R$

Where R was the reflectance at a specific wavelength expressed as a decimal fraction.

Equation 2: % MMb = (1.395 – ((*A*572 –*A*700)/(*A*525 – *A*700))) * 100

Equation 3: % DMb = (2.375 * (1-((*A*473 – *A*700)/(*A*525 – *A*700)))) *100

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

Purge

The purge for each striploin was calculated based on a weight loss basis only. Samples and bags were weighed prior to opening and post microbiological samplings. The weight of empty bag, drip keeper and microbiology samples were subtracted from initial weight. The weight difference or purge (g) was expressed as a percentage of the initial weight, as follows:

Purge (%) = [(wt striploin with drip /wt striploin no drip)/ wt striploin with drip] * 100.

pH

pH measurements were conducted using a TPS Model WP80 pH meter fitted with an Ionode IJ44 combination pH probe and temperature probe and temperature compensation. Calibrations were made at ~10°C, using pH 4 and 7 buffers (TPS Pty Ltd, product no. 121382 and 121388 respectively).

Thiobarbituric reactive species (TBARS)

The method of (Witte, Krause et al. 1970) was used to measure TBARS. Briefly, 2±0.1g samples were capped and cooked in 75°C water bath for 20 minutes and subsequently cooled for 30 minutes at 5°C prior to extraction. The concentration of malondialdehyde equivalents (mg/kg muscle) was calculated from absorbance readings at 530nm, using 1,1,3,3- tetraethoxypropane as a standard.

Glycogen content

The protein fraction of frozen muscle samples (1±0.05g) was removed by homogenisation (1:10 w/v) in 30mM HCl using an Ultra-turrax 22,000rpm for 2 x 15 second bursts. Samples were centrifuged (3,000 rpm, 4°C, 10 minutes) and supernatants containing free glucose and glycogen were frozen -20°C until assay could be performed. Thawed samples were analysed for total glucosyl units by incubating 50µl (37°C, 90 minutes) with the addition of 500µl of hydrolysing enzyme amyloglucosidase (1:200 in 40mM acetate buffer pH 4.8). The concentration (g/100 g muscle) was determined in duplicate using a glucose assay kit (sigma GAGO-20) and glucose as a standard. The total glucosyl units, as derived from glucose and glycogen, is referred to glycogen content, as described by (Cottrell, Dunshea et al. 2008). The absorbance of both samples and standards was measured at 540nm.

Lactate content

The L+ lactate determination within 1±0.05g muscle was conducted in accordance with the method of Noll and H.U.Bergmeyer (1985) and using enzyme concentrations as outlined by Bond and Warner (2007). The lactate content (µmol/g) of the muscle was determined stiochiometrically by measuring the absorbance of NADH at 340nm (extinction coefficient = 6.22 mM/cm).

Myoglobin content

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

α- tocopherol content

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

Protein extraction, solubility and SDS-PAGE

Samples were removed from -80°C storage and tempered for approximately 30 minutes. Duplicate 2g samples were used for total and sarcoplasmic protein solubility (Warner, Kauffman et al. 1997). The protein concentration (mg/g) of each fraction was determined spectroscopically using the Biuret method with bovine serum albumin as a standard (Gornall, Bardawill et al. 1949). Myofibrillar protein concentration was determined as the difference between total and sarcoplasmic fractions. For SDS-PAGE, a separate extraction procedure for myofibrillar protein purification was conducted, with the final suspension made in 1.1M NaCl, 0.1M $NaHPO_4$ at pH 7.2 buffer (Warner, Kauffman et al. 1997). Proteins were prepared to 2mg/ml and then were either diluted 1:1 with a final sample reagent (Laemmli 1970) with or without dithiothreitol to generate reducing or non-reducing conditions respectively. The protein (10µg) was loaded onto a Bio-Rad criterion Tris-HCl gel (4-20% linear gradient & 5% resolving gel, catalogue # 345-0027 and 345-0001 respectively) using a broad range molecular weight standard (6.5 to 200kDa, Bio-Rad, catalogue # 161-0317) for SDS-PAGE. Images were captured and viewed using a Bio-Rad GS-800 densitometer using Quantity One software for analysis.

Carbonyl content of myofibrillar proteins

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

Sulphydryl content of myofibrillar proteins

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

Volatile analysis

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

Statistical Analysis

Data analysis was completed using Genstat 15th edition (GenStat 2008). For colorimetric data (L*, a*, b*, hue, chroma), restricted maximum likelihood (REML) was used for comparison between colour groups and bloom times, using a random structure of plant/colour identity/carcass. To ensure valid comparison in the colour analysis, data from day one post-slaughter was used for all colour groups. One way analysis of variance (ANOVA) was used for the comparison of colour groups with the biochemical variates for week 0 data, using plant as the blocking structure. To check the attributes of the colour groups, for storage week 0, a principle component analysis (PCA) plot was generated. Variates used were pH, purge, L*, a* and b* values, as measured after vacuum packing and transport back to the laboratory. REML was also used for comparison between colour group and storage week treatments. Variates for lightness, chroma, hue, R630/580, myoglobin forms, pH, purge, TBARS, glycogen and lactate were compared in the model, using a fixed treatment structure (colour group* storage week) and a

random experimental structure of plant/colour /carcass/side/orientation from the cranial or caudal end. For retail display analysis, the model was reconfigured to take into account the day of retail display and tray (fixed treatment: colour group*day and random structure: plant/colour/tray/day). Least standard intervals (LSI) were generated to graphically display differences between colour groups.

When variates appeared to be heteroscedastic, a natural log transformation was used in the REML model, and subsequently means were back transformed and reported. Where data has been transformed, this is described in the results.

5.2.4 Results and Discussion

Carcass data for the colour groups

The dentition scores of the carcasses for the 'light', 'medium' and 'dark' colour groups were 0-4, 0-2 and 0-7 tooth respectively. The 'light' colour group had one carcass with a dentition score of 4 and the remainder were 0-2 tooth. Also, only one carcass from the 'dark' colour group had 7 teeth and the remainder of the 'dark' colour group were 0-4 tooth. Hot carcass weight for carcasses of the 'light', 'medium' and 'dark' colour groups were 324 ± 47, 314 ± 46 and 306 ± 43kg respectively. P8 fat depths for carcasses of the 'light', 'medium' and 'dark' colour groups were 13.3 ± 3.8, 13.1 ± 3.8 and 13.6 ± 4.8mm respectively.

Carcass data for plants

Cattle were either pasture or grain fed depending on the availability at the plant on the day of collection. Grain fed animals had all been fed for 100 days. Some carcasses from plant 3 were from animals with Brahman content (Brahman, Santa Gertrudis, Brahman euro cross), and were recorded as having a hump height of between 45 to 90 mm. Other carcasses from plant 3 were from animals described as cross bred Angus, Shorthorn or Angus, Hereford. Plant 1 had carcasses from animals described as Santa Gertrudis cross or charolais cross. Breed data was not available at plant 2. MSA data and vendor information were collected from plant 3 only, as other plants were unable to obtain this data. Thus data on plant-assessed MSA grading scores for AUSMEAT colour were only obtained from plant 3. For plants 1 and 2, the research teams allocation of AUSMEAT colour groups were used.

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

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

Plant	**1**	**2**	**3**

Pre-slaughter feed		Grain	Pasture	Grain & pasture
	Mean	348.9	294.5	300
Carcass weight (kg)	Range	294 to 434	221 to 440	255 to 331
	Mean	14.6	10.7	14.9
Fat depth (mm)	Range	10 to 22	6 to 20	9 to 21
	Mean	1.7	2.5	1
Dentition	Range	0-7	0-4	0-4

Some processing parameters for each plant are summarised in Table 3. All plants used the halal slaughter method, a hide puller rigidity probe (back stiffening) and spray chilled the carcasses. Plants 1 and 3 used immobilisation post-stunning and hot water decontamination unit and plants 1 and 2 used bleed rail electrical stimulation. The brand of vacuum bag specification also varied between plants. All the carton chilling regimes were below -1°C for at least 15 hours or more. All of these parameters should be contemplated when considering the potential variation in meat and eating quality between plants.

Table 3: Processing parameters from 3 plants

Parameter	**Plant 1**	**Plant 2**	**Plant 3**
Slaughter method	Halal	Halal	Halal

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

*Electrical stimulation

Effect of bloom time on colour

A summary of the carcass colour measurements can be seen in Table 4. Blooming time had a significant effect on all colorimetric values (P<0.05), highlighting the importance of a consistent bloom time. Overall, the L*, a*, b*, hue and chroma increased with a longer bloom time. Furthermore, there was an interaction between colour group and bloom time for L* (P<0.05) which was most interesting. The 'light' and 'medium' colour groups showed no change in L* with blooming for 8 hrs (P>0.05), but the 'dark' colour group showed a significant lightening with 8 hrs of blooming (P<0.05). In fact, the 'dark' colour group had an equivalent surface lightness to the 'medium' and pale groups, after 8 hrs of blooming.

After 0.5-1.0h blooming, the 'dark' group (AMC≥4) had significantly lower L*, a* and b* values, indicative of the dark, less red, more blue colour of the meat. This was also reflected in a lower chroma score, however hue scores were not significantly different. These colour measurements are consistent with expectations of the lower AMC scores ≤1C having a lighter, more red appearance and the higher AMC scores ≥4 having a dark, less red appearance.

The AUS-MEAT chiller assessment language recommends at least 20 minutes bloom time after refacing (AUS-MEAT 2014). These results highlight the importance of a standard bloom time for colour development, as variations in bloom time may alter the colour, which is consistent with other findings (Lee, Apple et al. 2008).

Table 4: Effect of bloom time (BT) and meat colour groups (CG; AUS-MEAT colour (AMC) scores Light, ≤ 1C; Medium, 2 or 3; Dark, ≥4) on Lightness (L*), redness (a*) and yellowness (b*), hue and chroma. The left side of the carcass was graded by the qualified assessor and was bloomed for 7.5-8.0h at ~5°C, whereas the right side was bloomed for 0.5-1h in the same conditions. Values are least squares means and the LSD (least significant difference) and P-values are shown.

	Bloom time 0.5-1.0 h			**Bloom time 7.5-8.0 h**			**LSD**			***P*-value**		
	Light	Medium	Dark	Light	Medium	Dark	CG	BT	CG*BT	CG	BT	CG*BT

L*	36.2	34.9	31.4	37.2	35.5	36.5	2.06	2.18	2.18	0.043	<0.001	0.004
a*	29.1	29.0	27.2	30.4	29.0	28.5	1.49	1.50	1.50	0.064	0.042	N.S
b*	21.4	20.9	19.4	23.6	22.2	21.8	1.53	1.53	1.53	0.003	<0.001	N.S.
Hue	36.3	35.7	35.2	37.7	37.3	37.3	0.72	0.72	0.72	0.01	<0.001	N.S
Chroma	36.1	35.8	33.4	38.5	36.5	35.9	2.09	2.09	2.09	0.002	0.002	N.S

Quality traits associated with the colour groups

Five components were used for the PCA (Figure 10) and these were the variates deemed most important for colour formation, namely ultimate pH, purge, L*, a*, b* values. Overall, separation of colour groups based on these quality variates was most apparent in the ‘dark’ colour group, where high pH and lower lightness values were observed. The separation of ‘light’ and ‘medium’ groups was not as pronounced, with both groups having a lower pH and higher lightness compared to the ‘dark’ group. From these results, we can distinguish that the majority of muscles in the ‘dark’ colour group had higher ultimate pH and lower L*, however there were some outliers. These findings should be taken into consideration for future analysis.

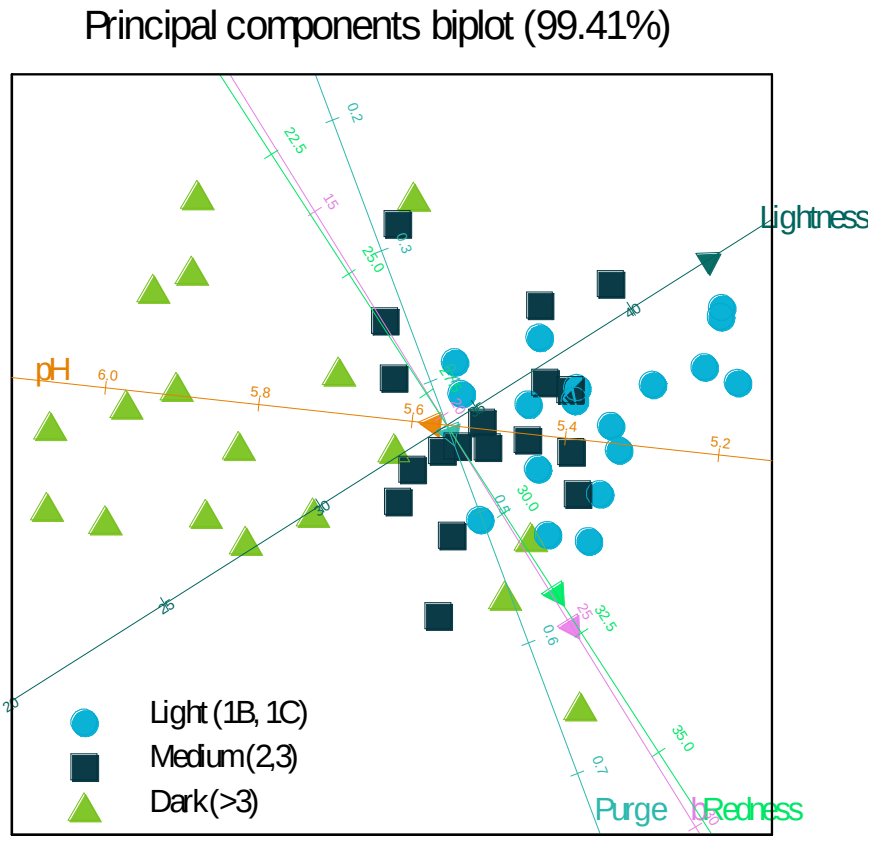


Figure 10: Principle component biplot of pH, purge and colorimetric values from striploin muscles from different meat colour groups, as defined by AUS-MEAT colour (AMC) scores: Light: 1B,1C; Medium: 2 or 3; Dark: >3, where n=18 per colour group. Colorimetric values were for lightness, redness and yellowness (b*), the latter two attributes being closely associated. Other quality variates were purge and pH (ultimate pH).

Chemical characterisation of colour groups

The myoglobin content (mg/g) for each of the muscles from each colour group and dentition scores are displayed in Figure 11. The myoglobin content was found to be significantly different between treatments ($P< 0.05$). The 'light' colour group had the lowest mean myoglobin content (4.8mg/g), followed by the intermediate colour group (5.2mg/g) and the 'dark' colour group having the highest content (6.6mg/g). This group also had one carcass with dentition 7, which would have increased the mean of the group. Unfortunately, this design feature of the experiment could not be avoided, due to availability of the carcasses on the collection day, and may contribute to the dark colorimetric values observed in the group.

(a) (b)

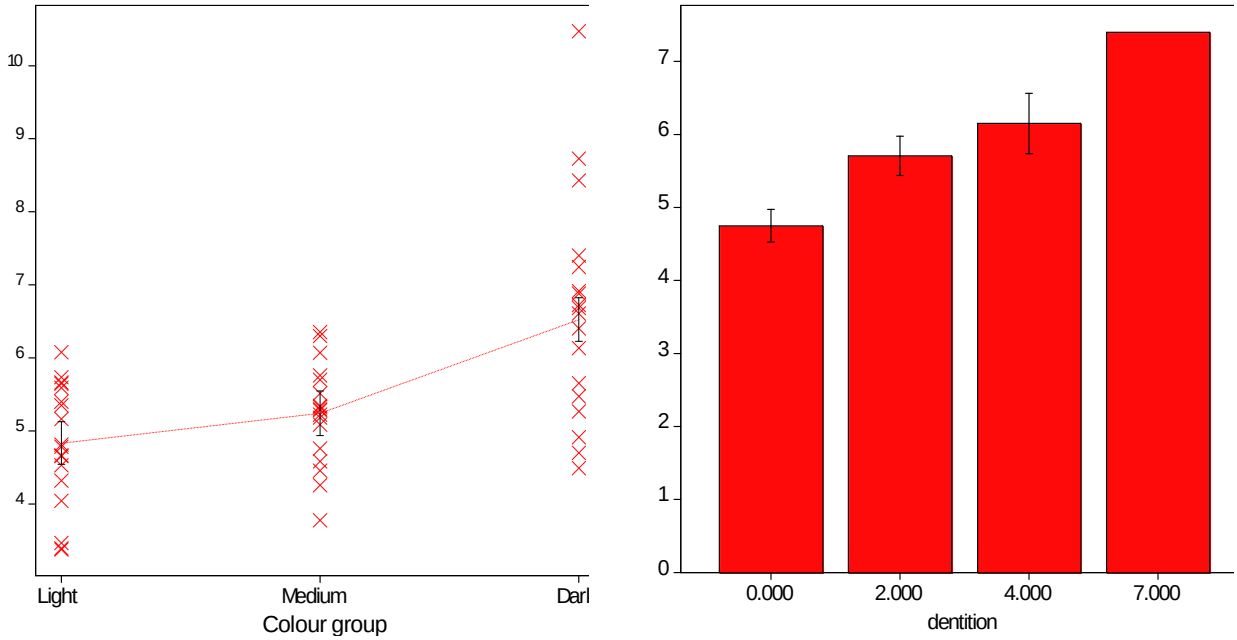


Figure 11: Variation in myoglobin content (mg/g) of the striploin amongst (a) colour groups or (b) dentition scores. Colour groups are light, medium and dark (AUS-MEAT colour (AMC) scores 1B & 1C, 2 & 3 or >3 respectively, n=18 per colour group). The mean is shown with the vertical bar representing ±s.e. Each data point is shown on graph (a) with colour group having a F probability <0.05. For graph (b), each dentition score (0, 2, 4 or 7 tooth) had n=13, 19, 8 and 1 respectively for each dentition group. Variation in vitamin E (α- tocopherol) content is shown in Figure 12. Regardless of colour score, pasture fed animals had a significantly higher (P<0.001) tocopherol content (3.6µg/g) within the muscle compared to grain fed animals (2.2µg/g). These results are comparable with other data published (Descalzo and Sancho 2008). There was no difference between colour groups and there was no interaction between colour group and feeding regime (P≥0.05). At levels of muscle tocopherol below 3.0 mg/g the shelf-life of the meat during display can be substantially reduced, due to oxidation onset (Ponnampalam, Norng et al. 2014). Thus the striploins from the grain-fed cattle were likely to have a reduced shelf-life, but unfortunately this was not tested in the retail display model due to the small sub-set used.

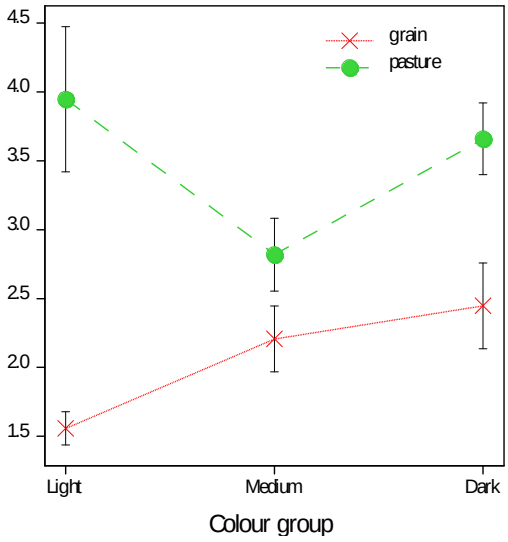


Figure 12: The variation in Vitamin E (α-tocopherol) in the striploin with feeding system (pasture vs 100 days grain) and colour group. Colour groups are light, medium and dark (AUS-MEAT colour (AMC) scores 1B & 1C, 2 & 3 or >3 respectively, n=18 per colour group). The mean is shown with the vertical bar representing ±standard error. Each data point is shown on the graph. Feeding regime, F probability $P<0.001$, colour group and interaction, F probability $P\geq0.05$.

Protein extraction & solubility

To evaluate the effect of colour score on protein properties, both the extractability and solubility were assessed (Table 5 and Figure 13). There was no difference observed in the sarcoplasmic protein solubility between the 3 colour groups, however 'dark' muscles had a lower total and myofibrillar protein solubility ($P<0.05$). Others have found lower protein extractability with light coloured muscles (Warner, Kauffman et al. 1997), however this was not observed in this study. The reason for this is unknown, although it would be interesting to observe if this is the case using a range of different pH buffers for the extraction process, as this extraction procedure was only completed at a relatively high pH of 7.2.

Protein oxidation, as assessed using the carbonyl and sulphydryl content of the myofibrillar prep, was also found to be similar between treatments ($P>0.05$). Similar levels of carbonyl content have been observed in pork striploin myofibrillar preps when different levels of drip were observed (Traore, Aubry et al. 2012). However, cooking can result in more oxidation (Sante-Lhoutellier, Astruc et al. 2008), with high drip muscles being particularly susceptible (Traore, Aubry et al. 2012), and could be a consideration for future studies investigating meat colour and oxidation in the cooked product and implications this may have on flavour characteristics of the meat.

Table 5: Comparison of protein properties from colour groups (light, medium or dark), as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively at week 0. Protein analysis was for mean total, sarcoplasmic and myofibrillar protein solubilities and the sulphydryl and carbonyl contents of myofibrillar extracts from striploin muscles.

	Light	Medium	Dark	LSD	FPr
Total protein solubility (mg/g)	222	223	213	7.7	0.022

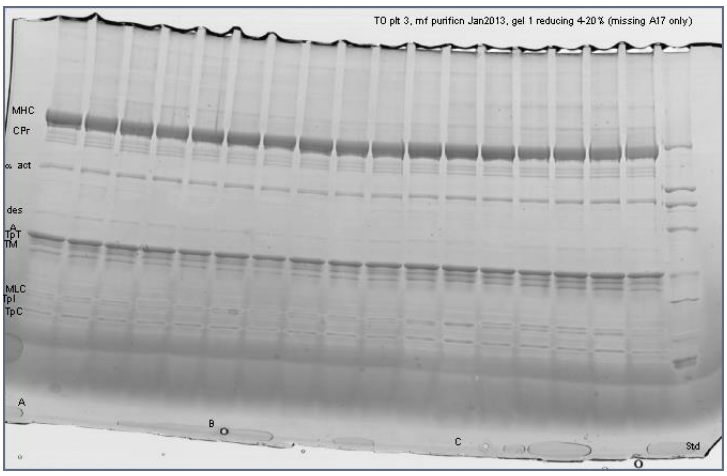
Sarcoplasmic protein solubility (mg/g)	75	73	74	4.3	N.S
Myofibril protein solubility (mg/g)	147	150	139	8.4	0.035
Sulfhydryl content (moles/10^5 g protein)	26	26	24	4.0	N.S
Carbonyl content (nmoles/mg protein)	0.79	0.79	0.80	0.09	N.S

Using the purified myofibril preparations from plant 3 only, the protein degradation products from both reducing and non-reducing conditions were assessed (Figure 13). Under reducing conditions, the 'dark' meat colour group appeared to show slightly more lower molecular weight proteins, such as myosin light chain and troponins I and C. Under non- reducing conditions, as expected, there were more high molecular weight proteins at the top of the gel, but a distinction between colour groups was not apparent. Overall, under both reducing and non-reducing conditions the degradation products appeared to be relatively similar, with no obvious differences.

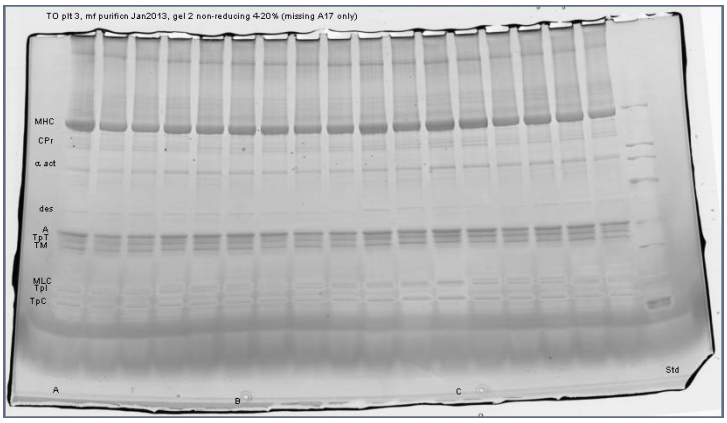
Proteins run with the 5% gel (Figure 13c) displayed the higher molecular weight proteins more clearly. The 'dark' meat colour group appeared to have a higher quantity of titin band T2, and possibly more nebulin present, which was not present in the other colour score samples. This most likely indicates less degradation (and less proteolysis) in these samples (Warner et al., 1997) and is consistent with others (O'Halloran, Troy et al. 1997). The integrity of these proteins could be associated with structural differences at the myofibrillar level and influence the rate of myofibrillar shrinkage which occurs during the early post-mortem period.

In addition, there is a band in the region of phosphorylase ~95kDa in the 'light' and 'medium' colour score samples, which is not present in the other 'dark' colour score samples. This most likely indicates denaturation of sarcoplasmic proteins and irreversible binding onto the myofibril, as described by Warner et al (1997) and Joo et al (1999). This can also be visualised in the region slightly below C-Protein ~135kDa, whereas the 'dark' colour group shows no extra bands.

(a)



(b)



(c)

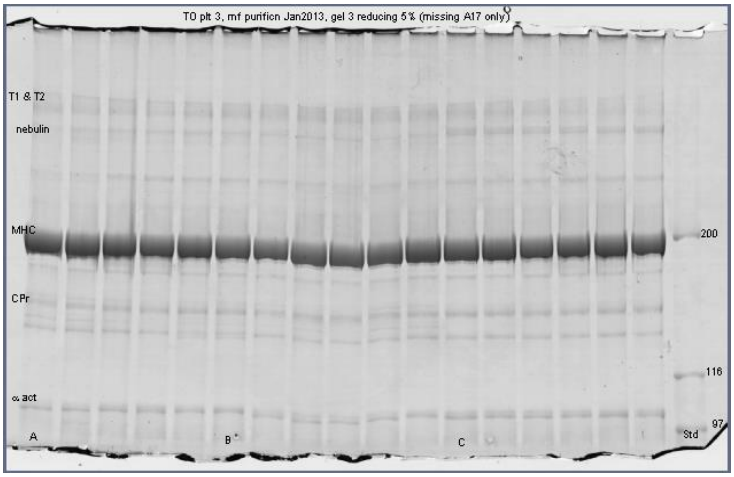


Figure 13: SDS-PAGE by colour group 'light' (lanes 1-5 from left to right), 'medium' (lanes 6-11) or 'dark' (lanes 12-17) for week 0 myofibrillar extracts. Broad range standard (6.5-200kDa) displayed in far right (lane 18). Gels were either 4-20% (a)-reducing and (b)-non-reducing or (c) 5% polyacrylamide. Myofibril proteins in order of ascending molecular weight are: Troponin C (TpC), troponin I (TpI), myosin light chains (MLC), tropomyosin (TM), troponin T (TpT), actin (A), desmin (des), α-actinin (α-act), C- Protein (C-Pr), myosin heavy chain (MHC), nebulin and titin 1 & 2 (T1 & T2).

Correlations between colorimetric data, protein solubility and oxidation.

Table 6 shows the correlations between colour, pH, protein solubility measures, oxidation and tocopherol values. Correlations were observed between the colour attributes (L*, a* and b* values) and pH (P<0.001). Lightness was positively correlated to myofibrillar protein concentration, suggesting proteins from of 'light' coloured meat had a higher solubility in the high salt buffer. Correlation did not exist between colorimetric (L*, a* and b*) values and protein or lipid oxidation measures. An increase in tocopherol content was associated with a reduced sulphydryl content and reduced myofibrillar and total protein concentration. The presence of sulphydryl groups have been previously associated with reduced protein aggregation, so in this study increasing the antioxidant content of the meat appears to be changing the properties of the myofibrils, which is impacting the solubility. Determining the mechanism involved in this process is outside the scope of this study, but could be interesting to consider in future work. Sulphydryl content and carbonyl contents were positively associated (P<0.001). In addition, total protein concentration was negatively correlated to metmyoglobin concentration (R=-0.048, data not

shown). Together these findings illustrate a relationship between protein solubility, protein oxidation and lipid oxidation which needs to be further investigated.

Table 6: Correlation Table depicting correlation coefficients (r) for protein solubility, protein oxidation and tocopherol measurements. If the |r| is >0.48, the probability value is *P*<0.05 and if |r| is >0.72, the probability value is *P*<0.001. n=18

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

Volatile analysis

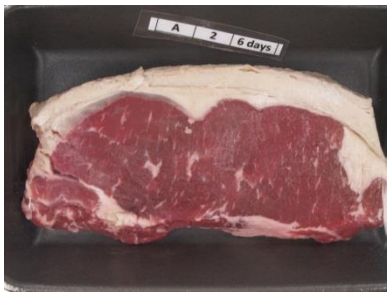
The volatile profile of each sample from 0 and 20 weeks was measured by GC-MS. No significant volatile compounds were detected in any sample. This was surprising since it was anticipated that there would some present in the profile yet this was not the case. One reason for this could have been the extraction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

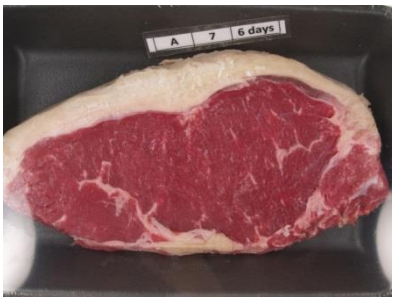
Retail display

Visual appearance of striploins during retail display showed that the striploins aged for 2, 12 and 20 weeks became unacceptable in colour after 6, 4 and 3 days respectively, but these observations would need to be verified using a formal consumer visual assessment. The reason steaks became visually unacceptable was mainly as a result of discolouration to surrounding fat and browning at the edges of the steaks, rather than large meat discolouration at the surface of the muscle. Typical images for steaks on day 3 of display, after 20 weeks ageing are shown in

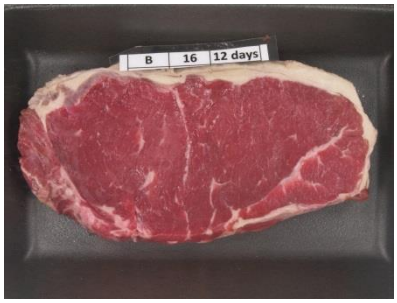
Figure 14. Colour group did not appear to have a large impact on the retail display life. The photographs highlight the potential for extending the shelf-life of striploin regardless of meat colour at grading. Further research into consumer acceptability could be conducted in order to confirm such findings with larger sensory assessment panels.

‘Light’ meat colour (AMC ≤1C)





‘Medium’ meat colour (AMC 2 or 3)



‘Dark’ meat colour (AMC ≥4)

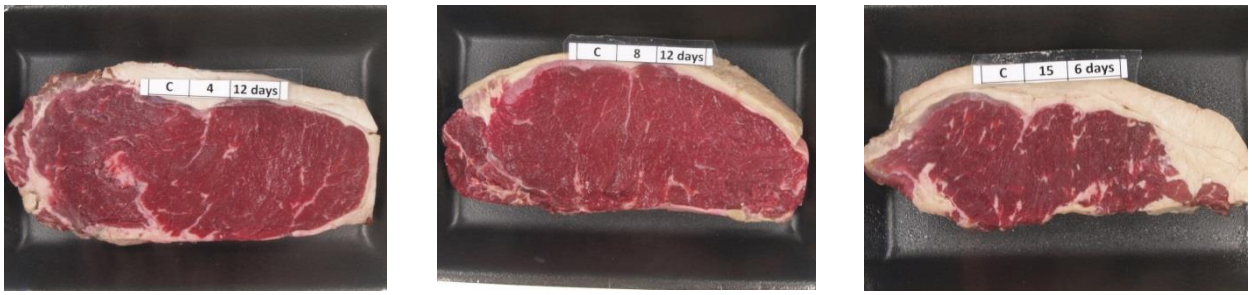


Figure 14: Comparison of representative retail display steaks from beef striploin muscles, segregated into colour groups 'light', 'medium' or 'dark', as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively. Muscles were stored vacuum packed in the dark at -1.0±0.5°C for 20 weeks. The steaks were cut and bloomed for 60 minutes at 5°C, then placed in black trays and over-wrapped and stored in the retail display cabinet for a total of 12 days. These steaks are after 3 days storage in the cabinet.

For all weeks, lightness (L*) values were significantly higher (P<0.001) in the 'light' meat colour group and lowest in the 'dark' meat colour group, with the 'medium' colour group being intermediate (data not shown). The reflectance ratio (R630/580) was considered to be of primary interest, due to the reported relationship with consumer acceptability score in other studies (Morrissey, Jacob et al. 2008) and is illustrated in Figure 15, 16 and 17 for striploins aged for 2, 12 and 20 weeks respectively. Lamb *longissimus* colour is reported as being unacceptable when R630/580 values (oxy/met ratio) were below a cut point of 3.5 (Warner, Jacob et al. 2010) and a red indicator line is shown at 3.5 in all Figures.

Overall, the 'light' colour samples generally had the lowest R630/580 ratio and the 'dark' colour score samples had the highest R630/580 ratio. This indicates that striploins assessed as light red (or pale, AUSMEAT colour score 1B, 1C) went brown more quickly (and had a shorter shelf-life), which agrees with literature that PSE, low pH meat generally has a shorter shelf-life (Kim et al, 2014). The results also indicate that striploins assessed as dark (AUSMEAT colour score >3) went brown more slowly (and thus had a longer shelf-life), which agrees with data for sheep *longissimus* (Warner et al, 2007).

The striploin steaks showed evidence of dropping below the R630/580 acceptability line after 10, 6 and 5 days in display for the steaks aged for 2, 12 and 20 weeks. These display times are different to the duration of acceptability in colour using visual appearance of the steaks, which appear to discolour around the edges of the meat after 6, 4 and 3 days in display. It would appear that the cut-off of R630/580 =3.5 for colour acceptability may not be appropriate for beef. It is evident, from the Figures that on days 6, 4 and 2 of display for the 2, 12 and 20 week aged product, the 'light', 'medium' and 'dark' colour groups are similar in R630/580 and the LSI has substantially reduced. We suggest that this is the day when the colour actually becomes unacceptable, which agrees closely with visual appearance.

In summary, ageing for longer than 2 weeks substantially reduced the shelf-life of the steaks. In addition, the 'dark' steaks had longer shelf-life and the pale steaks had shorter shelf-life.

Oxidation during retail display (TBARS)

The data for TBARS across all the storage weeks and 3 storage days (0, 6 and 12) was heteroscedastic, and so was transformed using a natural log, for the purpose of the REML analysis and back-transformed for the trellis plot (Figure 18). For all storage weeks, there was no effect of colour group on TBARS values (P>0.05). Compared to weeks 12 and 20, storage week

2 displayed a different trend, as TBARS values for all colour groups decreased at day 12. This could be either an error in the measurement, or could be due to the measured reactive species becoming utilised in other chemical reactions. At storage weeks 12 and 20, lipid oxidation increased with retail display, from 1.6 to 2.8mg/kg. Although values at 20 weeks were slightly above accepted level for rancidity detection (2.28mg/kg), MQ4 scores from the parallel eating quality study indicated the product would still be categorised as a 3 star product or having a "good everyday" eating quality. In summary, oxidation in the meat increased with days on display. The TBARS levels on days 6 and 12 of display for 12 and 20 week aged steaks are above the level of 0.5 mg/kg where consumers are able to detect off-odours and flavours.

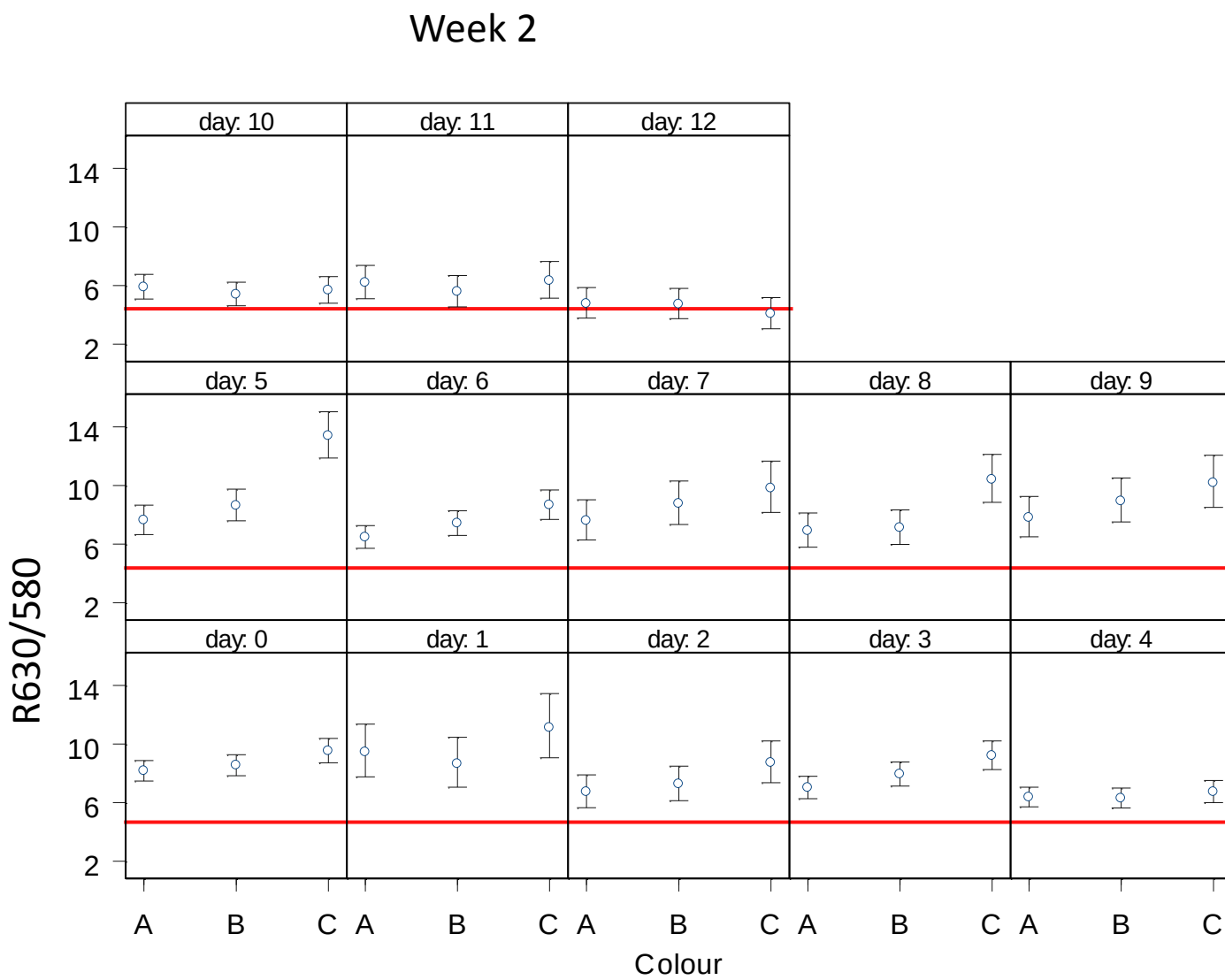


Figure 15: Effect of colour group (A: light; B: medium or C: dark, as defined by AUS-MEAT colour scores: ≤1C; 2 or 3; ≥4 respectively) and day of display (day 0 to day 12) on the R630/580 ratio

for striploin after 2 weeks storage in the dark at -1.0±0.5°C. The means presented are backtransformed means and the vertical lines are +/- LSI (Least Significant Interval). The steaks were cut and bloomed for 60 minutes at 5°C, then placed in black trays and over-wrapped and stored in the retail display cabinet for a total of 12 days. The red horizontal line indicates the cut-point (R630/580=3.5) for consumer acceptability

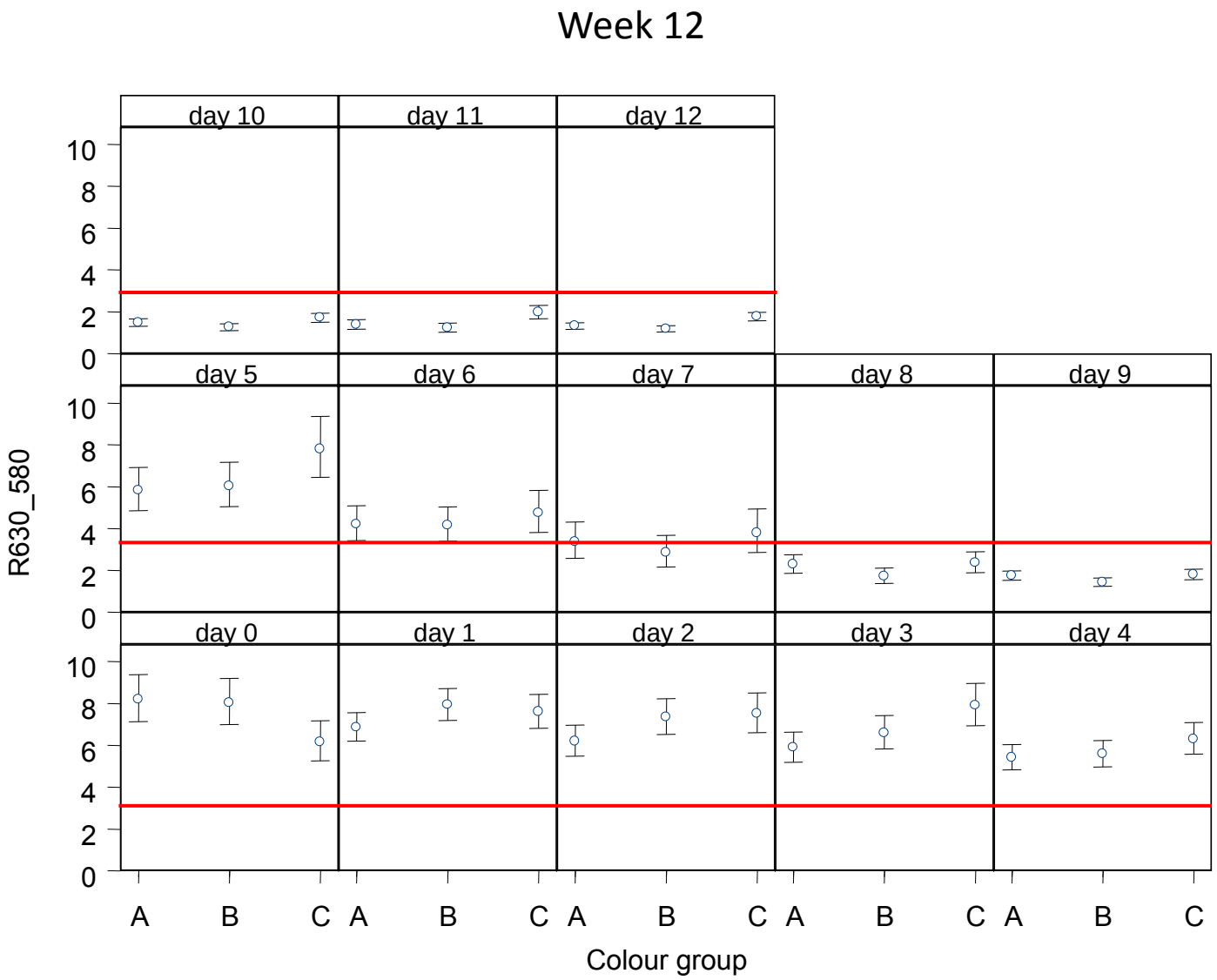


Figure 16: Effect of colour group (A: light; B: medium or C: dark, as defined by AUS-MEAT colour scores: ≤1C; 2 or 3; ≥4 respectively) and day of display (day 0 to day 12) on the R630/580 ratio for striploin after 12 weeks storage in the dark at -1.0±0.5°C. The means presented are backtransformed means and the vertical lines are +/- LSI (Least Significant Interval). The steaks were cut and bloomed for 60 minutes at 5°C, then placed in black trays and over-wrapped and stored in the retail display cabinet for a total of 12 days. The red horizontal line indicates the cut-point (R630/580=3.5) for consumer acceptability.

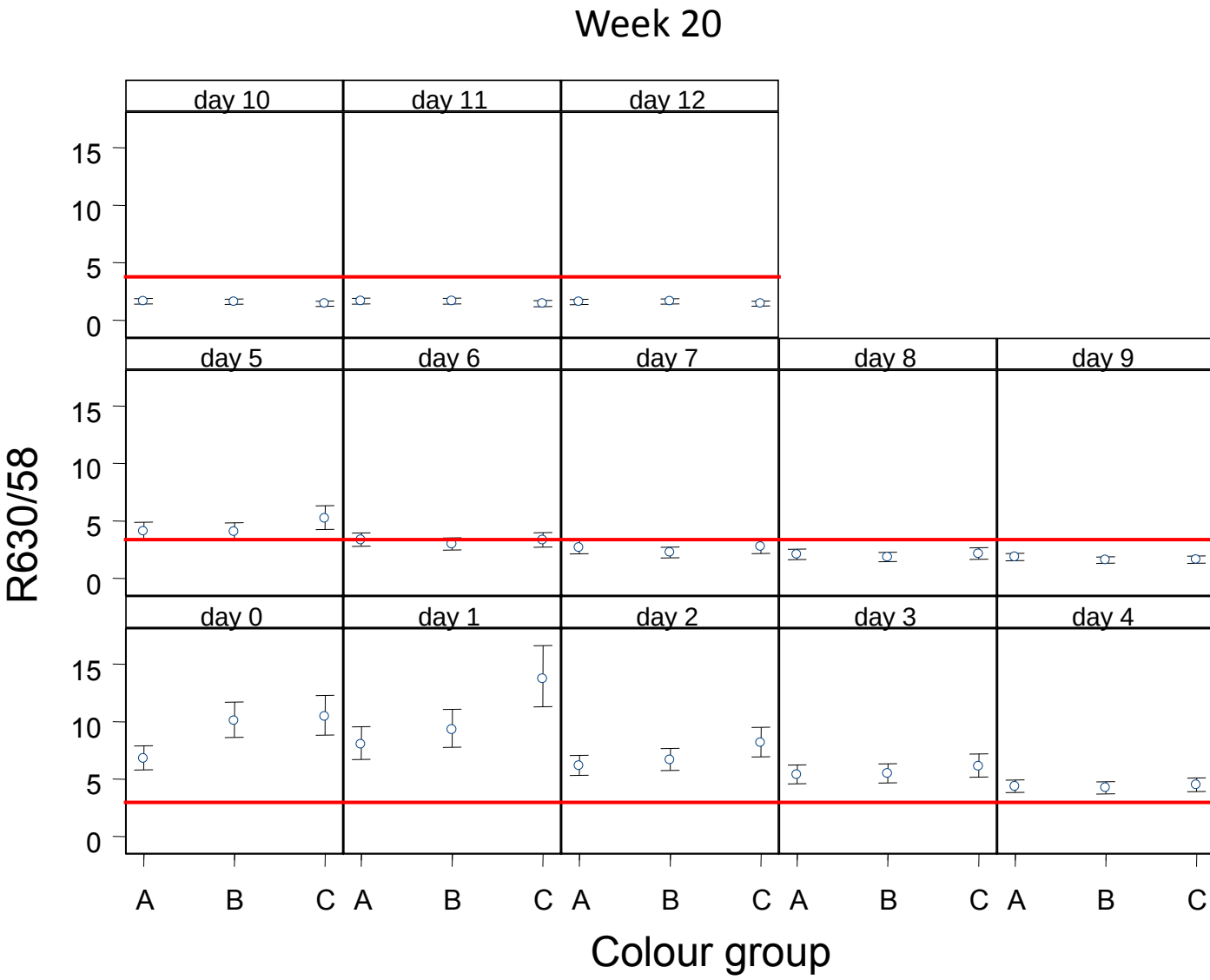


Figure 17: Effect of colour group (A: light; B: medium or C: dark, as defined by AUS-MEAT colour scores: ≤1C; 2 or 3; ≥4 respectively) and day of display (day 0 to day 12) on the R630/580 ratio for striploin after 20 weeks storage in the dark at -1.0±0.5°C. The means presented are backtransformed means and the vertical lines are +/- LSI (Least Significant Interval). The steaks were cut and bloomed for 60 minutes at 5°C, then placed in black trays and over-wrapped and stored in the retail display cabinet for a total of 12 days. The red horizontal line indicates the cut-point (R630/580=3.5) for consumer acceptability.

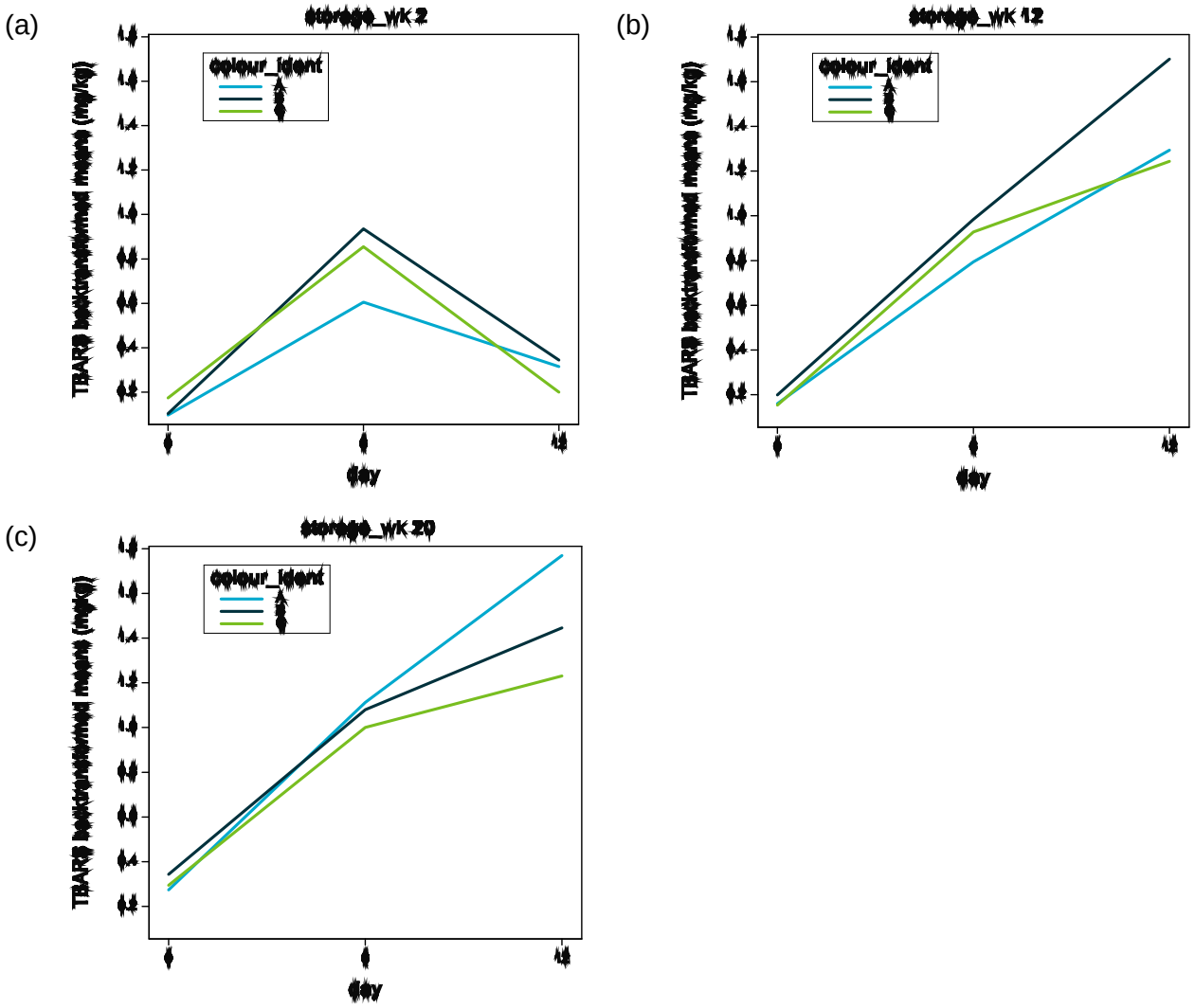


Figure 18: Effect of colour group (A:light; B:medium or C:dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively), storage week and day of display on TBARS values for beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C for (a) 2, (b) 12 or (c) 20 weeks and steaks were subsequently overwrapped for retail display over a total of 12 days, with TBARS being conducted on samples at 0, 6 and 12 days. Storage week, F probability <0.001, Day of display, F probability<0.001, Colour group, F probability = 0.547.

Colorimetric attributes

Colorimetric attributes (lightness, chroma, hue and R630/580) from each colour group and storage week are summarised in Table 7. Colour group influenced all colour attributes ($P<0.05$), with steaks from the 'light' colour group having higher lightness, hue and chroma and lower R630/580 values, relative to the 'dark' colour group.

In terms of storage week, all four variates showed highly significant ($P<0.001$) differences over the storage period. With storage time, all colour groups were lighter and less red (higher hue, lower R630/580) and also displayed an increase in chroma, indicative of more saturation in the primary hue of the sample, which is consistent with other findings (Boakye and Mittal 1996). This difference with storage time was most evident in the change between 0 and 2 weeks of storage. This change in colour with storage has been reported to be due to "the increased retention of oxygen in the outer layers", but could be a result of a reduction in muscle fibre diameter, increased extracellular space and associated purge generation during the ageing process. It is interesting to note, all colour groups displayed a R630/580 well above the consumer acceptability minimum score of 3.5 (Morrissey, Jacob et al. 2008), indicative of a visually acceptable product.

When the interaction between colour group and storage week was considered, there appeared to be a significant effect on chroma ($P<0.001$), hue and R630/580 ($P<0.05$) but not on lightness ($P>0.05$). For the R630/580 trait, it was evident that the decrease in this trait with storage time is only evident in the 'light' colour group samples. Thus it appeared that for 'medium' and 'dark' colour groups, the colour of aged steaks when the vacuum pack is first opened was similar after 20 weeks of ageing, to that of 0 week aged steaks. For 'light' colour group steaks, after opening, the colour of aged steaks was more brown (more metmyoglobin) than the steaks were at 0 weeks, indicative of reduced colour stability and more oxidation during ageing.

Table 7: The effect of colour group (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week (0, 2, 8, 12, 16, 20) on colorimetric measurements on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C for 0, 2, 8, 12, 16 or 20 weeks. At each time point, steaks were cut and bloomed for 60 minutes at 5°C prior to colour measurement.

week	Light	Medium	Dark	storage week	Colour group	Storage week. Colour group

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

Spectral colour data

The reflectance spectral data from 400 to 700nm for each storage week is displayed in Figure 19. As would be expected, the lowest reflectance values were observed in the wavelength range (530-590nm) associated with green colours, indicative of the maximum absorption at these wavelengths. In comparison, the maximum reflectance occurred at the higher end of the spectrum (>610nm), indicative of minimum absorption and higher reflectance of red wavelengths.

Between colour groups, reflectance values at the higher end of the spectrum were different, with the ‘dark’ meat colour group displaying a lower reflectance. This could be due to higher transmittance or absorption of red light. This effect is related to less light scattering that occurs

with high pH muscles, which can allow for a longer light path into the structure and more absorption by myoglobin (Swatland 2004; Hughes, Warner et al. 2014). In comparison, the light colour group displays more light scattering and less absorption, and hence presents higher reflectance values.

When comparing spectrum across the storage weeks, there was little differences observed. Although, week 0 does show slightly lower values compared to the other time points. This could be due to the earlier time post-mortem and could be associated with the reducing activity and structural differences of the muscle.

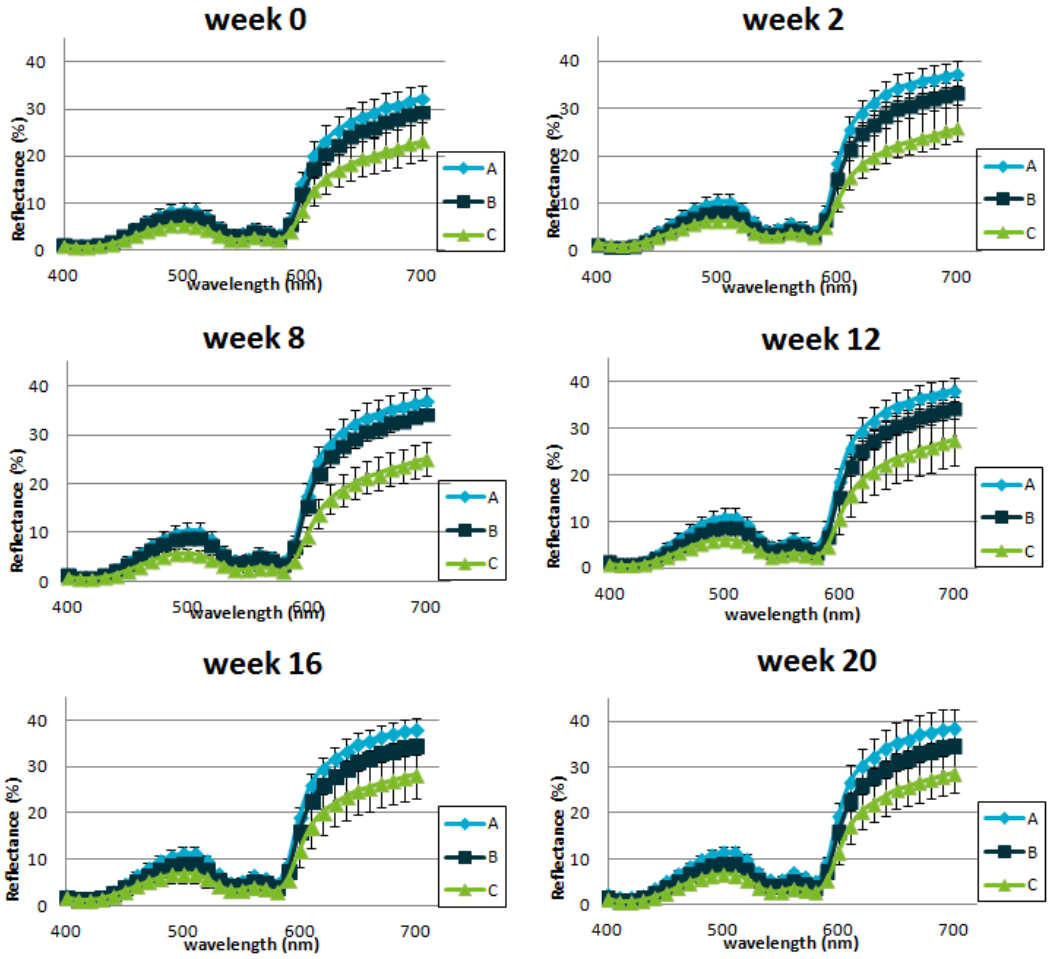


Figure 19: The effect of colour group (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week (0, 2, 8, 12, 16, 20) on reflectance (%) means ± s.d. on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C. At each time point, steaks were cut and bloomed for 60 minutes at 5°C prior to colour measurement.

Myoglobin colour attributes

The concentration of the three forms of myoglobin (being deoxymyoglobin, DMb ; oxymyoglobin, OMb ; metmyoglobin, MMb) in the surface of the steaks were determined for each colour group at each storage week and this is shown in Figure 20. As would be expected, the red OMb was the predominant form of the pigment and was >60% for all colour groups at all storage weeks. The purple DMb was the least prominent form. The brown MMb was similar in its levels in the surface of the steaks to the DMb levels.

Storage week had a significant effect of all three pigments levels ($P<0.001$) with increases in the %OMb and MMb and decreases in % DMb over storage time. Colour group also influenced the three pigment levels in the steak surface with the 'dark' colour group having the highest %DMb and MMb and the 'pale' and 'medium' colour groups having the highest %OMb. The higher % MMb in the 'dark' colour group was not expected, as this was indicative of more oxidation. In the literature, a dark pork meat colour has been associated with a reduced MMb content (Zhu and Brewer 1998), due to a higher oxygen consumption rate (OCR) and metmyoglobin reducing activity (MRA), so the reason for the difference here was not known. The 'dark' colour group's higher %DMb was due to the thin OMb layer and "close structure" of the meat as observed in meat with a high pH (Lawrie 1958).

There was an interaction between colour group and storage time for all three pigments ($P<0.05$). The interaction was most evident in the changes in the % pigments between 0 and 2 weeks of storage. In the 'light' and 'medium' colour groups, the %DMb dropped to a low level at 2 weeks of storage and remained low until 20 weeks of storage. In the 'dark' colour group, the %DMb remained higher throughout the storage period. This suggests that for 'light' and 'medium' colour groups a 0.5 to 1 hr bloom time was not sufficient time for them to develop a 'true' colour. This was supported by the increase in %OMb for the 'light' and 'medium' colour groups, between 0 and 2 weeks of storage. This increase was not evident in the 'dark' colour group.

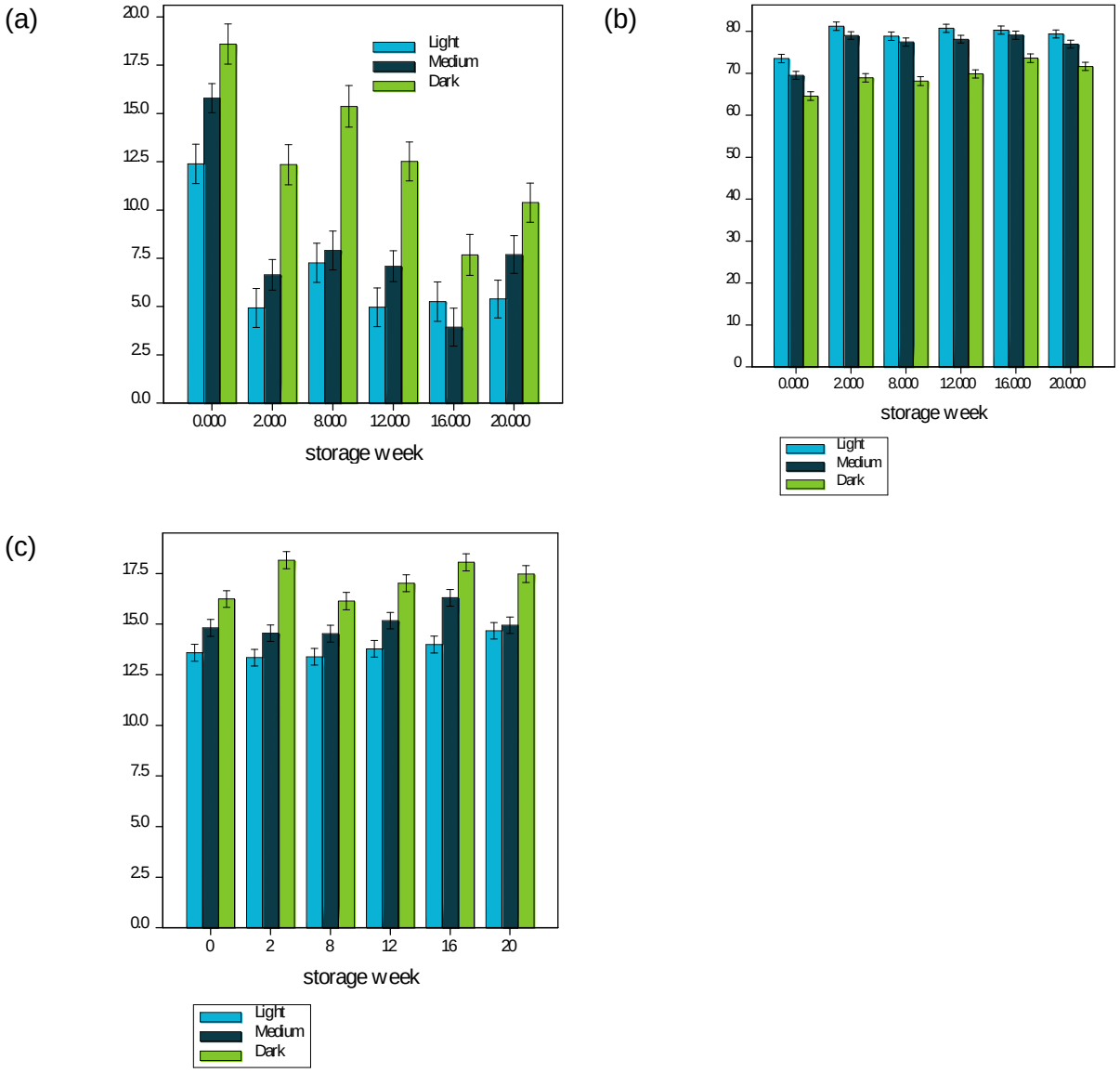


Figure 20: The effect of colour group (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week (0, 2, 8, 12, 16, 20) on colorimetric measurements on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C. At each time point, steaks were cut and bloomed for 60 minutes at 5°C prior to colour measurement. Predicted means ± s.e.d for percentage of 3 myoglobin forms, being (a) DMb- deoxymyoglobin; (b) OMb-oxymyoglobin or (c) MMb-metmyoglobin. Storage week for all forms, F probability <0.001; Colour group, F probability <0.001 (DMb and OMb) and 0.002 for MMb; interaction F probability <0.05 (DMb and OMb) and <0.001 for MMb.

Purge

The percentage purge for each meat colour group at each storage time is shown in Table 8. The data was observed to be heteroscedastic and so was transformed using a natural log, so the back transformed means are shown. At week 0, purge scores were only 0.5% for all colour

groups and were the lowest of any storage time. As time progressed, the purge values increased up to around 3 to 3.5% at 12 weeks, and remained fairly stable thereafter. The increase in purge was believed to be due to the disintegration of the structure of the meat, but this is a complex process and the mechanisms are outside the scope of this study and are well described elsewhere (Hamm 1961; Kristensen and Purslow 2001; Huff-Lonergan and Lonergan 2005). There was no difference between the three meat colour groups (P>0.05) in the % purge. Also, there was no interaction between treatments (P>0.05). This indicates the maximum purge loss was reached around the 12 week mark and stabilised. It was interesting that the 'light' colour group did not have higher purge %, as we expected that steaks of this colour group would exhibit low water-holding capacity and high purge.

Table 8: The effect of colour group, CG (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week, SW (0, 2, 8, 12, 16, 20) on purge (%) on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C. At each time point, predicted means ± s.e.d for purge (g) was expressed as a percentage of the initial weight, as follows: Purge (%) = [(wt striploin with drip /wt striploin no drip)/ wt striploin with drip] * 100.

	Back transformed means			Transformed state (natural log)					
CG	Light	Medium	Dark	S.e.d			F Pr		
SW				SW	CG	SW*CG	SW	CG	SW*CG
0	0.4	0.5	0.5	0.13	0.11	0.24	<0.001	N.S.	N.S.
2	1.6	1.3	1.3						
8	2.3	2.5	2.3						
12	3.5	2.8	3.0						
16	3.2	3.3	3.4						
20	3.2	2.9	3.1						

pH

The pH values for each colour group at each storage week are shown in Figure 21. As would be expected, the 'dark' meat group displayed higher pH values for all storage weeks (P<0.05). The 'light' and 'medium' groups had a lower pH at all the storage weeks. These observations are consistent with the expectation that high pH meat is generally associated with a dark appearance of the meat surface.

Between week 0 and 2, the pH of all the muscles increased. This increase in pH was most predominant in the 'light' and 'medium' coloured groups. The increase in pH is unexpected; normally a pH decline is observed with vacuum packed storage due to formation of organic acids and other compounds formed by the specific bacterial populations (Jones 2004). However, vacuum packing of lamb *longissimus* muscle also resulted in an increase in pH for up to 12 weeks (Moore and Gill 1987). In this study, the pH increase was relatively small, increasing by 0.07, 0.05 and 0.01 for 'light', 'medium' and 'dark' coloured groups respectively, between 2 to 20 weeks. It was interesting that lactate levels also declined during this period, and a decline in lactate has previously been associated with reduced colour stability (Moore and Gill 1987). In addition, during the first 10 days of storage, an increase in pH post-mortem has been reported to progress more slowly in normal compared to dark-firm-dry (high pH) beef (Livisay, Xiong et al. 1996) indicative of different metabolic processes occurring during storage. This result was

difficult to explain, but was believed to be due to the different biochemical properties of the muscle which may influence bacterial species and catabolites formed during the aging process (Moore and Gill 1987; Livisay, Xiong et al. 1996; Jones 2004).

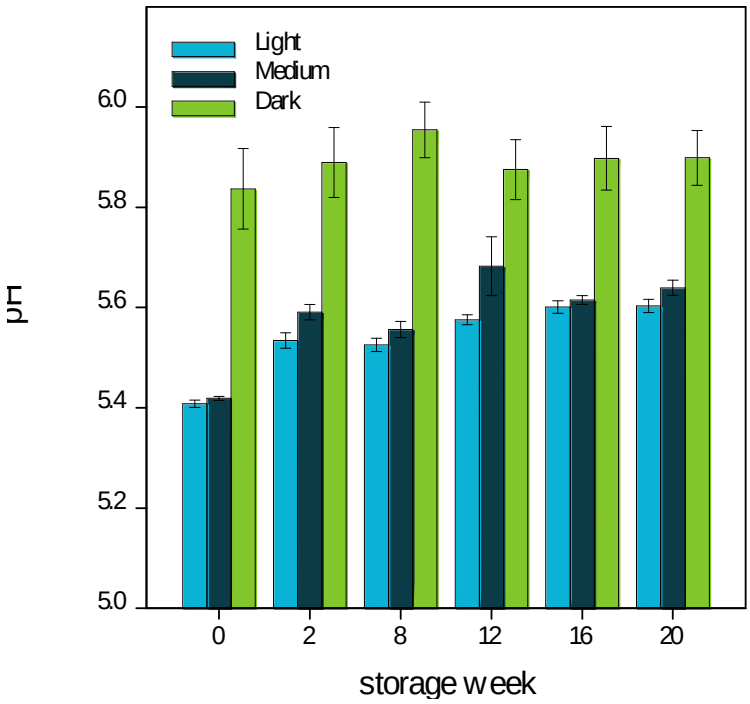


Figure 21: The effect of colour group (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week (0, 2, 8, 12, 16, 20) on pH means ± s.e.d. on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C. At each time point, steaks were cut and bloomed for 60 minutes at 5°C prior to measurement. pH was measured upon opening using a spear tip pH probe ~10°C. Storage week, F probability <0.001, Colour group, F probability <0.001 and interaction F probability <0.002.

Thiobarbituric acid species (TBARS)

The lipid oxidation values (as measured by TBARS) for each colour group over the storage period are displayed in Figure 22. Results illustrated lipid oxidation appeared to increase with storage time, reaching a maximum at week 20.

Between colour groups, oxidation measurements indicated similar oxidation levels in all of the groups, with no distinct difference or trend. High pH meat has previously been associated with a

reduction in meaty flavour and increase in bitterness or rancid flavours (Yancey, Dikeman et al. 2005). Lipid oxidation, as measured by TBARS, is reported to be a good predictor of sensory perception of rancidity (Campo, Nute et al. 2006). Early sensory studies on beef patties indicate a TBA value between 0.6-2.0mg/kg has been perceived with an oxidised or rancid flavour (Greene and Cumuze 1982). However, more recently in beef loin a TBARS value greater than 2.28mg/kg was associated with a decrease in beef flavour and increase in rancid flavour (Campo, Nute et al. 2006). The results obtained at week 2, 8 and 12 were below this level, however, panellists consuming meat at 20 weeks did not express any problems with the flavour (see appendix).

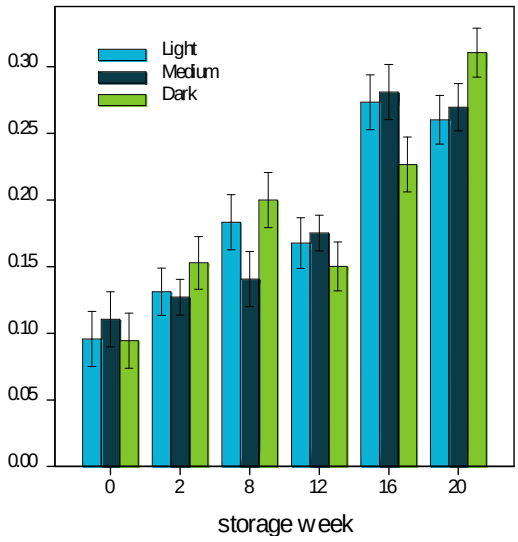


Figure 22: The effect of colour group (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week (0, 2, 8, 12, 16, 20) on TBARS means ± s.e.d. on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C. At each time point, steaks were cut and frozen at -80°C prior to lipid oxidation analysis. Thiobarbituric acid reactive species (TBARS) as measured after cooking (75°C for 20 minutes) was expressed as malondialdehyde equivalents (mg/kg muscle). Storage week, F probability <0.001, Colour group, F probability <0.001 and interaction F probability <0.002.

Glycogen and lactate content

A summary of the glycogen and lactate content within the muscle for each colour group and storage week are shown in Figure 23 and 24. The ‘dark’ colour group had a significantly lower lactate (P<0.001) content. In order to prevent dark cutting, the recommended muscle glycogen content is 1-1.5% and at values below 0.6% the likelihood of dark cutting is largely increased (Ferguson, Bruce et al. 2001). The mean scores for glycogen content are all less than 0.6%, whereas the ‘light’ and ‘medium’ groups show means above this value (data not shown). Correlations reveal both the glycogen and lactate contents were negatively correlated with pH value (P<0.001). Thus, from the data, the ‘dark’ meat colour group displayed a glycogen and lactate content which was characteristic of dark cutting meat.

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

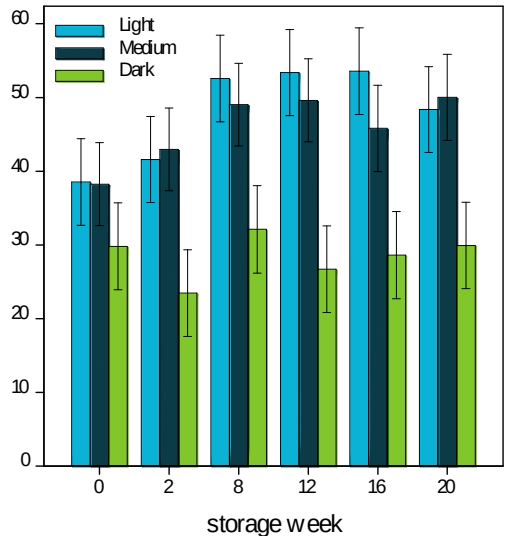


Figure 23: The effect of colour group (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week (0, 2, 8, 12, 16, 20) on glycogen means ± s.e.d. on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C. At each time point, steaks were cut and frozen at -80°C prior to analysis. Predicted means ± s.e.d for glycogen as measured after hydrolysis, with amyloglucosidase, expressed as µmol glycogen/g muscle. Storage week, F probability <0.001, Colour group, F probability 0.067 and interaction F probability <0.009.

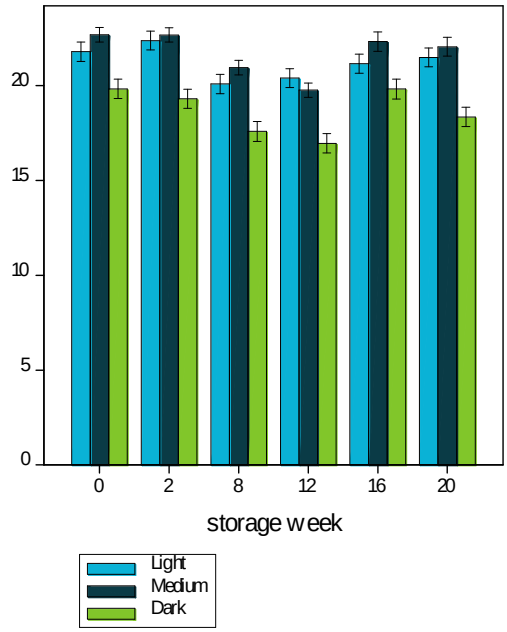


Figure 24: The effect of colour group (light; medium or dark, as defined by AUS-MEAT colour (AMC) scores: ≤1C; 2 or 3; ≥4 respectively) and storage week (0, 2, 8, 12, 16, 20) on lactate means ± s.e.d. on the beef striploin. Muscles were stored vacuum packed in the dark at -1.0±0.5°C. At each time point, steaks were cut and frozen at -80°C prior to analysis. Lactate was measured stiochiometrically by measuring the absorbance of NADH at 340nm. Storage week, F probability <0.001, Colour group, F probability <0.001 and interaction F probability 0.355.

5.2.5 Conclusions

Regardless of meat colour at grading, ageing increased the lightness, chroma, hue and R630/580. The purge also increased with storage, however no differences were observed between colour groups. The pH of the muscle also increased with storage time, which could be a result of bacterial populations on the meat.

Biochemical properties of the meat indicated the 'dark' colour group had more purple deoxymyoglobin levels and reflected more light in the red wavelengths. This group also had a reduced protein solubility. In comparision, the 'light' colour group had more red oxymyoglobin present and myofibril protein separation, indicated the presence of sarcoplasmic binding to some structural proteins.

Retail display, results indicated ageing for longer than 2 weeks substantially reduced the shelf-life of the steaks. In addition, the 'dark' steaks had longer shelf-life and the pale steaks had shorter shelf-life.

Week 0 analysis:

- Ensure adequate blooming time prior to grading, and where possible keep a consistent blooming time prior to grading for all carcasses.
- Older animals could have higher myoglobin levels in the muscle, which could contribute to a dark, less red appearance.
- Meat colour at grading appeared to have no difference in sarcoplasmic protein solubility, but 'dark' muscles did have a lower total and myofibrillar protein concentration.
- Meat colour at grading did not influence levels of sulphydryl or carbonyl groups, indicative of similar levels of protein oxidation.

Retail display:

- Vacuum packing at -1.0°C for 2, 12 and 20 weeks, yielded meat which was visually appealing. Preliminary evidence suggests steaks may be suitable after retail display for 6, 4 and 3 days respectively.
- Subtle differences in colour stability during retail display where the 'light' colour group had appeared to discolour faster compared to the other colour groups.
- For all colour groups, lipid oxidation (TBARS) increased with day of retail display.

All weeks biochemical analysis:

- With storage, all colour groups were lighter and redder. Purge increased and reached a plateau around week 12, but was not over 3.5% for all meat colour groups.
- The mechanisms behind the formation of 'light' and 'dark' colour groups appear to be different. 'Light' colour groups appear to show sarcoplasmic binding to myofibrils, whereas 'dark' colour groups had intact cytoskeletal proteins present, which could result in less restraint during myofibril shrinkage and less light scattering. Unfortunately, 'dark' muscles also had a higher myoglobin concentration, so this is confounded in the findings.
- Between colour groups, there was no obvious difference in lipid oxidation, but all muscles showed increased oxidation over the storage period. These levels were not detrimental to the eating quality.
- Between colour groups, spectral scan data exposed differences in myoglobin forms, with 'dark' muscles having lower reflectance in the red wavelengths. The 'light' colour group

had more red oxymyoglobin, whereas the 'dark' colour group showed higher levels of the purple deoxymyoglobin.

5.2.6 Recommendations

In terms of industry recommendations, there are still many unknowns in relation to colour at grading, both from a pre- and post-grading perspective.

In short, recommendations are as follows:

- This report illustrated the importance of bloom time on the colour generated at grading. A standard or more consistent bloom time should be considered to minimise variability in measurement.
- This study illustrates that striploins can be stored for up to 20 weeks, whilst still having a retail display of approximately 3 days. However, this value is provided as a guide on a small selection (n= 6) of steaks and further consideration should be given in order to confirm the findings of this study on a larger scale, perhaps with a more subjective consumer panel.
- Aging striploins for up to 20 weeks was not detrimental to the quantity of purge in any colour group and there was little evidence of discolouration of meat. Hence, processors can age striploins for this period without any negative impacts on excessive purge.
- With aging, all muscles increased in lightness, so this provides a potential opportunity for improving appearance of dark coloured striploins with increased storage.

Therefore, in parallel with microbiological and eating quality shelf-life experiments, this report illustrates the potential for industry to age striploins for 20 weeks. This provides further opportunity for the industry to maximise shelf-life especially for export markets. As the demand for beef increases in Asian and overseas markets, long-aged shelf-life will improve Australia's competitiveness, whilst ensuring consumer satisfaction.

6 Development of capability in meat science at CSIRO

As is evident from the research described above, undertaking research on meat colour has increased the capability of meat science team at CSIRO-Coopers Plains. New methods for meat colour analysis and biochemistry techniques to identify proteins and extend knowledge in the meat space have been developed. In addition, Joanne Hughes has published 2 papers as an outcome of this project and has developed excellent skills in research and development for the meat industry. She has communicated these results to the meat industry in several forums and has started a PhD, under the supervision of Professors Robyn Warner (now at Melbourne University) and Frank Clarke (Griffith University).

These papers are as follows:

Slaughter factors that contribute to variations in beef colour: Hughes, J. M., Kearney, G., & Warner, R. D. (2014). Improving beef meat colour scores at carcass grading. *Animal Production Science, 54*(4), 422-429.

Influence of beef meat colour at grading on shelf-life, eating quality and water-holding capacity: Hughes, J. M., McPhail, N. G., Kearney, G., & Warner, R. D. (2014). Beef longissimus eating quality increases up to 20 weeks storage and is unrelated to meat colour at carcass grading. *Animal Production Science, In press*.

Presented at ISNH/ISRP conference 8-12 Sep 2014.

7 Recommendations

The report highlights animal, carcass and processing factors contributing to meat colour scores at grading and provides recommendations to reduce the incidence of dark meat colour at grading for beef carcasses.

It is recommended that:

- The time of slaughter to grading could be lengthened from 14 to 31 hours to minimise economic penalties to carcasses with meat colour score >3 when graded ~14 hours post-mortem.
- Animals, especially older and pasture fed animals are handled carefully to minimise stress, as these animals are already prone to darker colour scores.
- Heavier grain fed animals that are more prone to high rigor temperatures, should be moved to cold chillers as fast as possible to reduce the temperature of the carcass. High rigor temperatures which can result in reduced tenderness and a failure to age. We recommend plants to target a pH 6.0 at 25°C to maximise the incidence of meat colours 2 and 3.

The report also indicates meat colour at grading is not related to eating quality during long-aged shelf-life. Thus, from this data it is recommended that:

- The beef industry can store striploins for up to 20 weeks (in dark vacuum packed conditions at -1.0±0.5°C), whilst still achieving acceptable eating quality for the consumer.

- ‘Light’ (1B or 1C) meat colours at grading are most likely to improve in eating quality, especially for flavour and overall liking with storage, so plants should consider these muscles for long aged shelf-life. Other darker (>1C) meat coloured striploins can also improve in eating quality with storage, but not as pronounced as the lighter colours. All muscles become lighter with aging, illustrating the potential to improve the appearance of darker meat colours.
- Suppliers and retailers of beef striploins should consider aging beef to improve eating quality.

8 Appendices

8.1 Slaughter factors that contribute to beef colour

Improving beef meat colour scores at carcass grading.

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Short summary

Unacceptably dark meat colour scores are a major cost to the beef industry. Beef carcasses (n=1,512) were assessed for meat colour and 10% were non-compliant for colour. The main causative factors were a short time from slaughter to grading, high final pH (pH_F), low rigor

temperature, older animals and pasture feeding. Attention to these factors can improve meat colour scores at grading.

Abstract

Unacceptable meat colour scores at the time of carcass grading are associated with reduced meat quality and consumer rejection. We hypothesised that the meat colour at carcass grading would be influenced by the pH and temperature decline post slaughter, as these would be determined by animal and processing factors. Beef carcasses (n=1,512) at 7 Australian processing plants were assessed, at grading, for the meat colour of the *longissimus thoracis*. Statistical modelling determined the animal, carcass and processing factors contributing to the meat colour score at carcass grading. The occurrence of unacceptably dark meat dropped from 8% to 3% when the time of grading was increased from‘14’ hours to ‘31’ hours post-slaughter ($P<0.01$). A high temperature at pH6 (rigor temperature), high final pH (pH_F), pasture feeding and older animals were associated with dark *longissimus thoracis* muscles at carcass grading ($P<0.05$ for all). Less than 30% of carcasses with non-compliant pH_F displayed a dark non-compliant meat colour >3, indicative of an opportunity to determine the mechanism behind this pH-induced colour development and thus reduce the incidence of non-compliance. It is recommended that when there is a high occurrence of carcasses with a dark meat colour >3 that the time from slaughter to grading is checked to ensure carcasses are in full rigor at the grading point. This will assist in minimising economic penalties due to dark coloured carcasses. Finally, animal factors, such as maturity and feeding regime also had a considerable impact on the meat colour at carcass grading.

Keywords: pH, temperature, dark cutting, myoglobin, maturity, feeding regime

Introduction

Beef meat colour is an important attribute for the visual appearance and consumer acceptability. In Australia during 2012-13, Meat Standards Australia (MSA) reported the main reason for carcasses not meeting specification was due to unfavourably dark meat colour (MC > 3) and/or a

high pH (pH > 5.70), with 5.3% carcasses failing for one of these reasons or a combination of both (Meat and Livestock Australia Limited 2013). The cost to the industry of dark meat colour, based on MSA graded carcasses, is estimated to be approximately $400 per affected carcass (industry sources) and to be in the region of $35 million/annum for MSA carcasses alone. As MSA carcasses are approximately only 24% of the beef carcasses in Australia, the cost to the national beef industry is much higher. Consequently, there is a need to minimise the incidence of beef carcasses failing colour specification. Dark and light meat colours are associated with the unfavourable conditions known as dark cutting and heat-induced toughening, respectively. Both of these quality conditions negatively impact water-holding capacity, visual appearance, shelf-life and eating quality aspects such as tenderness (Purchas and Aungsupakorn 1993; Ferguson, Thompson et al. 1999; Kim, Warner et al. 2014; Warner, Thompson et al. 2014b). It is well known that pre-slaughter stress in cattle depletes glycogen reserves and is associated with a high pH of the meat, particularly in animals on poor nutritional feeding regimes prior to slaughter (Lawrie 1958; Knee, Cummins et al. 2007). In comparison, heavier grain fed animals can be exposed to post-slaughter processing, which can generate unfavourable high temperature, low pH conditions in the muscle (Warner, Gutzke et al. 2014a). .

The surface colour of meat is largely determined by the concentration and chemical status of myoglobin, and depth of the myoglobin layers (see Faustman and Cassens (1990) for a review) and also by the changes in the structure of the muscle post-mortem, which is much less known and understood. During early post slaughter pH decline of the muscle, there is evidence of structural alterations and drip channel formation (Heffron and Hegarty 1974; Bertram, Schafer et al. 2004), which could result in differences in absorbance, reflectance and oxygen penetration into the muscle. As the muscle enters rigor, there can be a reduction of 14-16% in muscle fibre diameter and consequently an increase in extracellular space (Heffron and Hegarty 1974). In addition, myofibrils can undergo shrinkage, creating the opportunity for drip formation and loss (Diesbourg, Swatland et al. 1988; Bertram, Schafer et al. 2004). The magnitude of these

changes is a pH-dependent process and is a primary driver in the change in colour of muscle post-mortem. A limited pH decline results in a high pH (pH > 5.7) and a dark colour at grading, as opposed to a full pH decline to 5.4-5.5 which results in a bright cherry-red colour at grading (Murray 1989). Also, the rate of this pH decline is influential in the process, as the temperature of the muscle is important (Hamm 1961; Renerre 1990). A rapid post-mortem metabolism (fast pH fall) can result in denaturing conditions, resulting in changes in light scattering and also loss of sarcoplasmic proteins such as myoglobin from muscle (Swatland 2008) and, hence, a paler colour. For these reasons, we hypothesised that the meat colour at grading would be influenced by the pH and temperature decline post slaughter, as these would be determined by animal and processing factors. Although the influence of animal and processing factors on beef meat colour have been reported, the factors relevant to Australia under modern processing conditions need to be known. Therefore, the aim of this study was to test the factors which could contribute to beef meat colour at grading, in Australian beef processing plants. From this information we can look to optimise beef meat colour and, hence, supply information to industry to improve the meat colour at grading.

Methods

Animals and treatments

Data from 1512 beef carcasses were obtained from seven beef processing plants across five states in Australia. This study was a component of a larger study described by Warner, Gutzke et al. (2014a). In brief, data were collected from 103 mobs of cattle over a total of 28 days from March to June 2006 (autumn and winter months). Data on pre-slaughter feeding were collected and comprised a finishing feed type of either milk (n=58), pasture (n=364) or grain (n=1090). Animals were allocated into one of four categories; vealer, young/prime, cow/ox or grain. Each category was determined by the plant, depending upon dentition, feeding regime and carcass weight. ‘Vealers’ had no erupted permanent incisor teeth and their carcasses weighed no more

than 170 kg (still suckling the cow but have access to pasture). Cattle categorised as 'young/prime' were animals that were grazing pasture pre-slaughter and had 0-4 permanent incisors. Cattle categorised as 'cow/ox' were grazing pasture pre-slaughter and had 6-8 permanent incisors. The animals in the 'grain' category were grain-fed (in a feedlot) and had 0-4 permanent incisors. Within the 'grain' category the range of 'days on feed' were between 60 and 350 days. No bulls, exhibiting secondary sexual characteristics, were included in the study.

Animals were stunned using a captive bolt pistol, and were either immobilised (n=904) or directly shackled by the hind limb immediately prior to exsanguination without immobilisation (n=568). The time of exsanguination was recorded and defined as the time of slaughter. Some animals (n=309) were also exposed to electrical stimulation and almost all animals had a rigidity probe (n=1409 animals) attached to the carcass during hide pulling. The durations of electrical inputs at the immobiliser, electrical stimulation and hide puller were recorded (sec). Carcasses were then halved, weighed (hot carcass weight), the gender (female or castrate) was recorded and the fat depth (mm) was measured at the P8 site on the rump of the carcass (Meat and Livestock Australia Limited 2011). The time of entry into the chiller was also recorded.

Temperature and pH decline

After placement in the chiller, two pH, temperature and time measurements were recorded in the *m. longissimus lumborum* in proximity to the 2nd to 5th lumbar vertebrae on individual carcasses. Where possible, the second measurement was timed to ensure the muscle pH was below 6.0. For each measurement, care was taken to place the pH meter into a fresh incision rather than just using the site where the last measurement was recorded. A Jenco TPS pH meter (WP-80), with a polypropylene spear-type gel electrode (Ionode IJ 44) and temperature probe (part no 121247), all manufactured and purchased from TPS Pty Ltd, Springwood, Qld, Australia, were used to measure pH and temperature. The pH meter and electrode were calibrated at ambient temperature using buffers of 4.00 and 7.00 and checked regularly for re-calibration. After overnight chilling, a pH was measured at the time of grading which occurred 14 to 31 hours post

slaughter defined as the pH_F. The temperature at which a carcass passed through pH 6 was defined as the temperature at pH 6 (rigor temperature) and the calculation is described in (Warner, Gutzke et al. 2014a).

Grading assessments

Carcass sides were quartered at the $4^{th}/5^{th}$ ribs (vealer only) or between the $10^{th}/11^{th}$, $11^{th}/12^{th}$ or $12^{th}/13^{th}$ ribs. The exposed *longissimus thoracis* was allowed to bloom for a minimum of 120 minutes prior to colour assessment. Assessment was carried out with the aid of light from a torch (1400-3000 lux, 12 volt, 20 watt diachronic glass faced low voltage, tungsten halogen lamp, part number: 01-E710A2, Emroth Technologies Pty Ltd, Kenmore, Australia) held at an angle of approximately 45° to the muscle surface and at a distance of 30-45cm from the meat surface. Meat colour was subjectively scored on a scale from 1 (split into 1A, 1B, 1C) to 7 using AUS-MEAT colour chips by a qualified assessor according to the AUS-MEAT chiller assessment language (AUS-MEAT 2005). Meat colour was considered to be paler at the lower end of the scale (meat colour scores 1A, 1B and 1C) and unacceptably dark at the higher end of the scale (meat colour scores > 3). Meat Standards Australia (MSA) classifies carcasses as dark cutting carcass when the meat colour score > 3 and/or having an ultimate pH > 5.70. The time of assessment was recorded and was between 14 to 31 hours post slaughter with *longissimus thoracis* temperatures 7.4 ± 3.3°C. Graders also measured the size (cm^2) of the exposed *longissimus thoracis* and defined this as eye muscle area (EMA).

Statistical analysis

Due to the minimal numbers of muscle colour grades 5 (n=40) and 6 (n=18), these grades were pooled. For simplicity of illustration, meat colour scores > 3 were pooled for tables and graphs. The method of generalised linear model with a multinomial distribution and logit link function was used to analyse the data for the dependent variable meat colour score. A total of 15 explanatory variates were tested in the initial model; 10 quantitative and 5 qualitative. The initial analysis

included the terms; plant, mob, gender, category, days on feed (nested within grain category), time from slaughter to chiller, time from slaughter to colour grading, temperature at pH 6, pH_F, hot carcass weight, EMA, P8 fat depth, duration of immobiliser, duration of stimulator and duration of rigidity probe and relevant interactions. Grader was tested and found to be confounded with plant and thus could not be included in the model. Extensive examination of mob was undertaken but it was removed for several reasons, but mainly for parsimonious reasons. Terms were only included in the final model if they were statistically significant ($P < 0.05$). All statistical analyses were performed using Genstat (GENSTAT Committee 2008). Plant was included in the model in order to adjust for its effect, but the results are not presented as the focus of the study was on finding influencing factors, not on finding differences between plants.

Using the significant terms within the meat colour model, the predicted percent of each meat colour score was calculated. The rate of change between meat colour scores was calculated by dividing the predicted percentage difference of two meat colour score groups by the actual time (hours) interval.

Results

A summary of all the qualitative and quantitative variates tested are given in Tables 1 and 2, respectively. From all the explanatory variables tested, six variables were found to be significant ($P < 0.05$; Tables 1 and 2). These were; plant, time from slaughter to carcass grading, temperature at pH 6, pH_F, category and 'days on feed' within the grain-fed category. The remaining variables (the time from slaughter to chiller, hot carcass weight, EMA, P8 fat depth, gender, duration of immobiliser, electrical stimulation or rigidity probe) and relevant interactions were found to have no effect on the meat colour score ($P > 0.05$). From all the carcasses

measured, 10% had a meat colour score >3 and would be considered non-compliant, using the Meat Standards Australia grading system (Meat and Livestock Australia Limited 2011).

Time from slaughter to grading

The time from slaughter to grading influenced the meat colour score (P<0.01; Table 1) and was highly variable (14 to 31 hours). The predicted percent of each meat colour score over the time of grading is presented in Table 3. Carcasses graded early (14 hours post slaughter) showed a higher incidence of meat colour scores > 3 (8%), compared to carcasses graded at 31 hours post slaughter which showed fewer meat colour scores >3 (3%).

Carcasses graded at 14 hours post slaughter had 22% light meat colour scores (1B/1C) whereas those carcasses graded at 31 hours post slaughter had 43% light meat colour scores (1B/1C). The majority of the carcasses (75%) graded after 31 hours post slaughter were of meat colour 1C or 2, whereas if carcasses were graded after only 14 hours post slaughter, the majority (71%) were meat colour scores 2 or 3, indicative of a darker appearance.

The rate change in the occurrence of meat colour scores also appeared to vary between 14 to 31 hours and for the purpose of presentation has been segregated into 4 groups (Table 4). Between 14 to 19 hours post slaughter the rate of decrease in occurrence of meat colours >3 was largest (0.4%/hour), whereas between 24 to 31 hours post slaughter only 0.2%/hour changed from having meat colour >3 to <3. In addition, more carcasses per hour moved into the 1B or 1C meat colour in the 26 to 31 hour post slaughter window, compared to the 14 to 19 hour post slaughter window. Thus, more dark colours (>3) become lighter from 14 to 19 hours and more intermediate meat colours (>1C) become lighter (1B or 1C) from 26 to 31 hours post slaughter.

Temperature at pH 6

The temperature of the muscle during the development of rigor, as measured by temperature at pH 6, had an effect on the meat colour score (P<0.001; Table 1). The range in temperature at pH 6 was from 15 to 40°C and the effect on the predicted meat colour score is shown in Fig. 1.

High temperature at pH 6 carcasses (40°C) displayed the lightest colours, with 38% of carcasses being graded as either 1B or 1C and the fewest dark colours, with only 4% having a meat colour score >3. This temperature also generated the lowest percentage (59%) of carcasses with a meat colour of 2 or 3. In comparison, a low temperature at pH 6 (15°C) displayed the highest percentage (18%) of dark meat colour scores > 3 and smallest percent (10%) of lighter meat colour scores 1B and 1C. The largest percent (73%) of meat colour scores 2 or 3, were observed at 15 and 25°C, but at 25°C there were only 10% with meat colour score >3, meaning there were 8% fewer dark coloured carcasses.

Final pH (pH_F)

The pH_F in the *longissimus thoracis* at grading influenced the meat colour score (P<0.001; Table 1). Carcasses with a higher pH_F had a higher incidence of darker meat colour scores (Fig. 2). In those carcasses having pH_F above the dark cutting reference point, the percent of meat colour scores > 3 increased, with pH_F values of 5.8, 6.0 and 6.2 having 28%, 74% and 96% respectively. In comparison, those carcasses with a lower pH_F of 5.4 and 5.6 had only 1 and 5% of meat colour scores >3. It is interesting to note, that almost half (47%) of carcasses with a pH_F of 5.80 had a meat colour score of 3. But, generally, a darker meat colour score was typically observed when the pH_F of the carcass was high.

Carcasses with a low pH_F (5.4) had the highest percent of lighter meat colours; with colour score 1B and 1C occurring in 20 and 56% carcasses at this pH_F, respectively. As the pH_F increased, the lighter meat colours declined, with no meat colour 1B and 6% of meat colours 1C being observed in carcasses with a pH_F > 5.7. However, over 70% of carcasses with a pH_F of 5.8 had a meat colour score <3 and this group had the highest incidence of meat colour score 3 which composed 47% of all carcasses in the group. Thus, a pH_F~5.8 or greater did not correlate well with dark meat colours.

Category and feeding regime of animal

The category of the animal, and the 'days on feed' within the grain-fed category, influenced the meat colour at grading (*P*<0.001, Table 2 and P<0.05, Table 1, respectively) and are summarised in Fig. 3 and Table 5. The younger animals (vealer category) displayed the largest percent (54%) of pale meat colour scores 1B (9%) and 1C (45%) and the lowest percent of meat colour scores >3 (2%). In comparison, the older animals, cows and ox, displayed a very high percent of darker meat colour scores >3 (33%).

Darker meat colour scores >3 were twice as prevalent in the young/prime (pasture fed) category (6%) compared to the grain categories (2 to 3%). The grain categories displayed a large percentage (43 to 53%) of light meat colour scores 1B and 1C. Thus, approximately half of all grain fed cattle had a light meat colour and these values were comparable to the vealer category. In comparison, the young/prime category had only 26% of carcasses with a meat colour 1B or 1C, but had the most number of carcasses graded as meat colour 2 or 3 (68%).

Discussion

Influence of time from slaughter to grading on the occurrence of 'dark coloured' meat

In our study, delaying the grading time from 14 to 31 hours post slaughter reduced the occurrence of 'unacceptably' dark coloured meat from 8% to 3%, almost a three-fold reduction. Thus we can accept the hypothesis that the time post slaughter when grading occurs influences the meat colour score at grading. This is not a novel finding as (Murray 1989) reported that the incidence of dark coloured beef was three times higher at 15-18 hr than at 23-26 hr post stunning in the Canadian beef industry. While this phenomenon is well-known world-wide, the AUS-MEAT chiller assessment requirements state that a non-stimulated carcass cannot be assessed until 18 hours post-slaughter (Food Science Australia 2004). Furthermore, AUS-MEAT states that a carcass that has received full stimulation can be assessed at 8 hours post-slaughter (Food Science Australia 2004). Our results show that even 14 hours is too early to grade beef carcasses for meat colour. Thus it is recommended when grading a carcass after approximately

14 hours post slaughter and observing a dark meat colour score >3, the time from slaughter to grading could be lengthened to achieve the ultimate pH and therefore minimise economic penalties to that particular carcass.

Influence of time from slaughter to grading on lightness of meat and rate of colour change

The rate of meat colour change from dark colour scores (>3) to lighter meat colour scores (1B or 1C) was apparent between 14 to 31 hours. The % dark colour scores (>3) decreases and the % pale colour score increases from 14 to 19 hours and between 26 to 31 hours post slaughter, intermediate meat colour scores (>1C) decrease and pale colour scores (1Bor 1C) increase. Thus, the muscle appears to become lighter during this early post slaughter period and is consistent with other findings (Young, Priolo et al. 1999). The mechanisms which are primarily responsible for the lightening effect observed are not currently known. However, it is believed that it could be due to the progression of the pH decline and the effect this would have on both the structure and the myoglobin status of the muscle.

A proposed mechanism for lighter meat colour scores

During the early post slaughter period, the pH of the muscle usually falls from 7 to ~5.4, which coincides with a reduction in muscle fibre diameter, increase in extracellular space, myofibrillar shrinkage and drip formation (Heffron and Hegarty 1974; Offer and Cousins 1992; Bertram, Schafer et al. 2004). Consequently, there is an increase in light penetrating or being transmitted into the structure, and light can be either absorbed by pigments such as myoglobin or scattered by the structural elements. The deeper the light is transmitted into the structure, the higher the absorption by myoglobin and the less light is scattered, hence the darker the appearance (Swatland 2004). This is a pH-dependent process, with high pH muscles having swollen fibres and proportionately less light scattering, whereas muscles with a lower pH undergo more myofibrillar and fibre shrinkage and consequently scatter more light (Swatland 2008).

Furthermore, if denaturing conditions occur, denaturation of the myosin head results in further shrinkage in the myofibrillar lattice (Offer 1991).The magnitude of these structural changes would depend on the progress of the pH decline and thus would be time dependent (Diesbourg, Swatland et al. 1988; Offer and Cousins 1992). In our study, a longer time from slaughter to meat colour grading may provide the muscle with more time to undergo shrinkage of these structural elements compared to those graded earlier. Thus, a greater time between slaughter and grading allows for further progression of the pH decline (and more time allowed for shrinkage of the structural elements) which should correspond to the development of lightness observed at the surface of the meat. Additionally, as this shrinkage occurs, the looser structure could allow for an increase in oxygen diffusion and subsequent pigment oxygenation, promoting red oxymyoglobin formation (Lawrie 1958; MacDougall 1982; Young and West 2001). In comparison, muscles graded earlier would have undergone less shrinkage and consequently appear darker due to the higher density of the lattice. These pH-dependent changes in the structure and pigment occurring during the early post slaughter period are believed to be contributory to the lightening of the meat colour score over the time period investigated. Further research is required to validate these theories.

Temperature at pH 6 effects

A higher carcass temperature at pH 6 was associated with a higher incidence of lighter meat colours. High temperatures at rigor can result in reduced tenderness and a failure to age (Kim, Warner et al. 2014; Warner, Thompson et al. 2014b). This can generate a condition known as heat-induced toughening and can create denaturing conditions for the muscle proteins and lead to aggregation of sarcoplasmic proteins and precipitation onto the surface of myofibrillar proteins (Bendall and Wismer-Pedersen 1962). This lowers the ability of the proteins to bind water molecules, generating drip and increased light scattering (Bendall and Wismer-Pedersen 1962; Kim, Warner et al. 2014). In comparison, muscles going into rigor at lower temperatures had a higher incidence of darker meat colours and have been associated with a thinner red

oxymyoglobin layer (Renerre 1990). In addition, the rate of these structural changes within the muscle and variations in metabolic activity between carcasses could also be influential to the incidence of meat colour at grading. Hence, the time to reach pH 6 could be confounded with the temperature at pH 6, and would need to be further investigated. For this study, we focused purely on the effect of temperature at pH 6 and the relationship to meat colour at grading. In short, a high temperature (40°C) at pH 6.0 generated the largest percent of light meat colours 1B and 1C (38%) and a low temperature (15°C) generated the largest percent of darker meat colours >3 (18%), therefore conditions should be created to minimise such extremities. From the data, the temperature for generating the largest percent of meat colours 2 and 3 and minimal meat colours >3 was 25°C and therefore plants should target a pH 6.0 around this carcass temperature. This is also a suitable temperature for meat tenderness (Geesink, Bekhit et al. 2000).

pH_F

As discussed above, obtaining an optimal pH decline in the muscle is desirable and so is achieving a pH_F which is neither too high nor low. The relationship of pH and meat colour interaction is widely researched, with detrimental effects of pre-slaughter stress on utilisation of energy stores and consequently formation of meat with a high pH_F being documented (Lawrie 1958; Wismer-Pedersen 1959). A high pH_F is associated with tightly packed muscle fibres, reduced light scattering and a only a thin layer of red oxymyoglobin on the surface of the meat (Renerre 1990). In comparison, those carcasses with a low pH_F (5.4) have a larger percent of lighter colour scores 1B and 1C (76%) in our data, most likely due to the acidic environment created in the muscle causing denaturation of structural proteins and visualised as having a lighter meat colour that is more exudative (Kim, Warner et al. 2014). As discussed previously, there appears to be an effect of the rate of pH decline and the time post slaughter in which changes to the structure of the muscle occur which determine the colour observed at grading. The results of this study highlight the inconsistencies that occur between high pH meat and dark meat colours of the carcass. Consequently, an opportunity exists to determine the mechanism of

this pH-induced light scattering effect in order to understand ways to reduce the incidence of non-compliant carcasses.

Animal factors

Finally, animal factors such as maturity and feeding regime also affected the meat colour score at grading. Vealers and grain-fed animals had approximately 50% of the lighter 1B and 1C meat colour scores and older animals had the highest incidence of dark cutting (33%). Muscles from older animals are well known to exhibit darker coloured meat (Page, Wulf et al. 2001; Mlynek and Gulinski 2007; McGilchrist, Alston et al. 2012). This reduction in lightness is most likely to be caused by an increase in myoglobin concentration and lower numbers of large “white” fast glycolytic fibres and more “red” slow oxidative fibres (Moon, Yang et al. 2006; Mlynek and Gulinski 2007). Together, this age induced transition to a slower, more oxidative condition of the muscle could be contributory to the darker meat colour observed.

Furthermore, pasture fed animals had a lower percent (26%) of lighter meat colours compared to grain (43-53%) and usually have higher muscle concentrations of antioxidants, such as tocopherol and ascorbic acid, and thus have higher myoglobin colour stability and lower levels of lipid and protein oxidation (Descalzo and Sancho 2008). This could minimise protein denaturation, thus preserving the native structure and function of the proteins thereby improving colour stability and in this study is favourable to intermediate colour scores 2 and 3. Additionally, depending upon seasonal variation of pasture and pre-slaughter animal treatment, these animals could have depleted glycogen stores, which is associated with an increase in dark cutting (Knee, Cummins et al. 2007). In comparison, grain feeding can increase the glycogen and fat content of the animals, resulting in a thicker subcutaneous fat layer (increasing P8 fat depth), a higher core body temperature (Jacob, Surridge et al. 2014) and thus more insulation during the carcass chilling. Although hot carcass weight and P8 fat depth were not significant within the meat colour model, as the temperature at pH 6 increased, so too did the occurrence of lighter meat colours. Also, grain fed animals maybe less prone to stressful circumstances due to exposure and

habituation to new environments, transportation and human interaction. Therefore, animal specific pre-slaughter factors, such as animal handling, maturity and feeding regime can also impact the meat colour at carcass grading.

Conclusion

A longer time from slaughter to grading was found to result in a lighter meat colour. Muscles graded at 14 hours post slaughter had a dark-cutting incidence (MSA colour score >3) of 8%, whereas those graded at 31 hours post slaughter had an incidence of 3%. We postulate this was due to structural shrinkage of the muscle fibres and myofibrils which increases the light scattering properties of the muscle. Also, a high temperature at pH 6 was associated with lighter carcasses and an optimal temperature around 25°C was recommended to minimise meat colour issues such as dark-cutting and heat-induced toughening. Less than 30% of carcasses with non-compliant pH_F displayed a dark non-compliant meat colour score >3, indicative of an opportunity to determine the mechanism behind this pH-induced colour development and thus to reduce the incidence of non-compliance. From these results, when grading a carcass after approximately 14 hours post slaughter and observing a dark meat colour score >3, it is recommended that the time from slaughter to grading could be lengthened to minimise economic penalties to that particular carcass. Finally, animal specific pre-slaughter factors, such as animal handling, maturity and feeding regime can impact meat colour observed at carcass grading.

Acknowledgements

The assistance of Janine Lau, Rob Strachan and Linden Cowper and funding from Meat Livestock Australia and Australian Meat Processors Corporation is gratefully acknowledged.

List of tables and figures

Table 1: The effect of quantitative variables tested in the meat colour model of the *longissimus thoracis* (n = 1512) .

Variates showing mean, s.d. and significance from the initial statistical model.

Quantitative variable	Count	Mean	s.d.	Min	Max	Significance
Processing factors						
Time from slaughter to chiller (hours: minutes)	1008	0:50	0:17	0:33	2:37	NS
Time from slaughter to grading (hours)	1478	22	4	13	32	<0.01
Duration of immobiliser (seconds)	944	12	7	1	65	NS
Duration of electrical stimulation (seconds)	309	15	5	13	30	NS
Duration of rigidity probe (seconds)	1440	8	2	2	21	NS
Animal factors						
Days on feed (grain fed only)	1090	159	100	60	350	<0.05
Hot carcass weight (kg)	1512	298	95	96	560	NS
Eye muscle area (cm^3)	1507	69	13	20	108	NS
P8 fat depth (mm)	1512	19	9	1	53	NS
Factors influenced by both animal and processing conditions						
Temperature at pH 6 (°C)	1492	36	5	6	40	<0.001
pH_F	1508	5.55	0.32	5.34	6.44	<0.001

Table 2: The effect of qualitative variables tested in the meat colour model of the *longissimus thoracis* (n = 1512).

Table displays the categories and counts within each variable and the associated significance from the initial statistical model.

Qualitative variable	Categories within variable	Count	Significance
Plant	1	280	<0.001
	2	262	
	3	175	
	4	265	
	5	202	
	6	134	
	7	194	
Gender	Female	383	NS
	Male	1073	
	Missing values	56	
Category	Vealer	58	<0.001
	Young/ Prime	233	
	Grain	1090	
	Cow/ Ox	131	

Table 3: Predicted percent of meat colour scores (1B being palest to >3 being unacceptably dark) of the *longissimus thoracis* for carcasses (n=1512) graded between 14 to 31 hours post slaughter from the meat colour model. These predictions were calculated using mean values in the model for plant, pH_F 5.6, temperature at pH 6 35°C, category and for animals which were grain-fed for 159 days.

Time from slaughter to grading (hours)	Meat colour score				
	1B	1C	2	3	> 3
14	2	20	40	31	8
19	3	24	41	26	6
24	4	29	41	22	5
26	4	31	40	20	4
31	6	37	38	16	3

Table 4: Rate of change in the occurrence of meat colour scores (1B and 1C being palest to >3 being unacceptably dark) of the *longissimus thoracis* for carcasses (n=1512) graded between 14 to 31 hours post slaughter, using predicted percentages from the meat colour model.

Four time intervals were allocated based on prediction values in the model (calculated using mean for plant, pH_F 5.6, temperature at pH 6 35°C, category and for animals which were grain-

fed for 159 days). The rate of change in meat colour score was calculated as the difference in predicted percentages at each time point divided by the actual time interval (as a decimal).

Time interval (hours post-slaughter)	Actual time interval (hours: minutes)	Rate of increase in occurrence of meat colours 1B &1C (% /hr)	Rate of decrease in occurrence of meat colours > 3 (% /hr)
14-19	4:48	1.1	0.4
19-24	4:48	1.2	0.3
24-26	2:24	1.3	0.2
26-31	4:48	1.4	0.2

Table 5: Predicted percent of meat colour scores (1B being palest to >3 being unacceptably dark) of the *longissimus thoracis*for carcasses (n=1512) from animals fed on grain between 70 and 340 days prior to slaughter from the meat colour model. These predictions were calculated using mean values in the model for plant, pH_F 5.6, temperature at pH 6 35°C and time from slaughter to grading 22 hours.

Days on Feed	Meat colour score				
	1B	1C	2	3	> 3
70	6	37	38	16	3

150	6	40	37	14	3
210	7	41	36	13	2
270	8	43	35	12	2
340	8	45	33	11	2

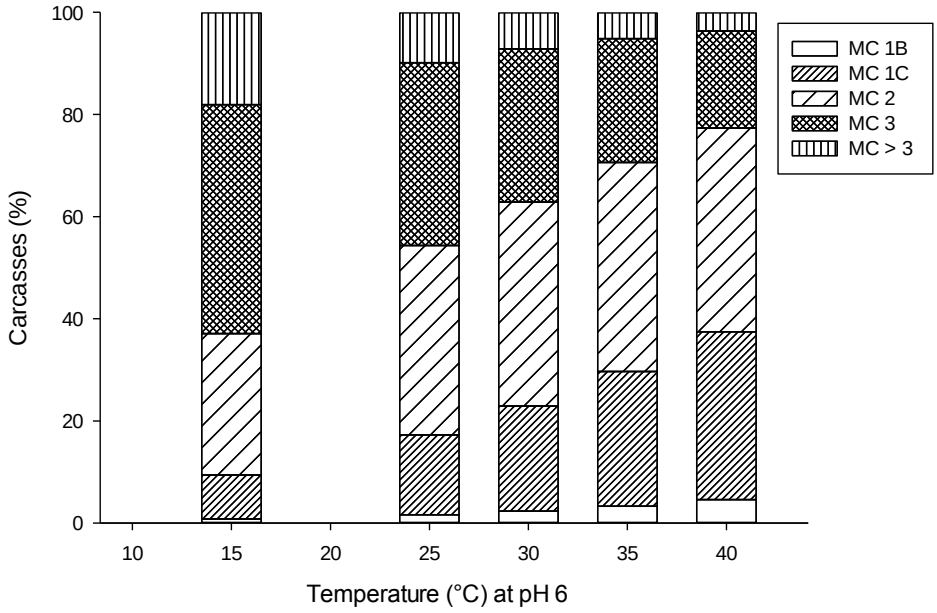

Figure 1: Predicted percent of carcasses (n=1512) with each meat colour score (1B being palest to >3 being unacceptably dark) in the *longissimus thoracis* for a range in temperature at pH 6 from 15 to 40°C from the meat colour model.

These predictions were calculated using mean values in the model for plant, pH_F 5.6 and time to from slaughter to grading 22hours, category and for animals which were grain-fed for 159 days.

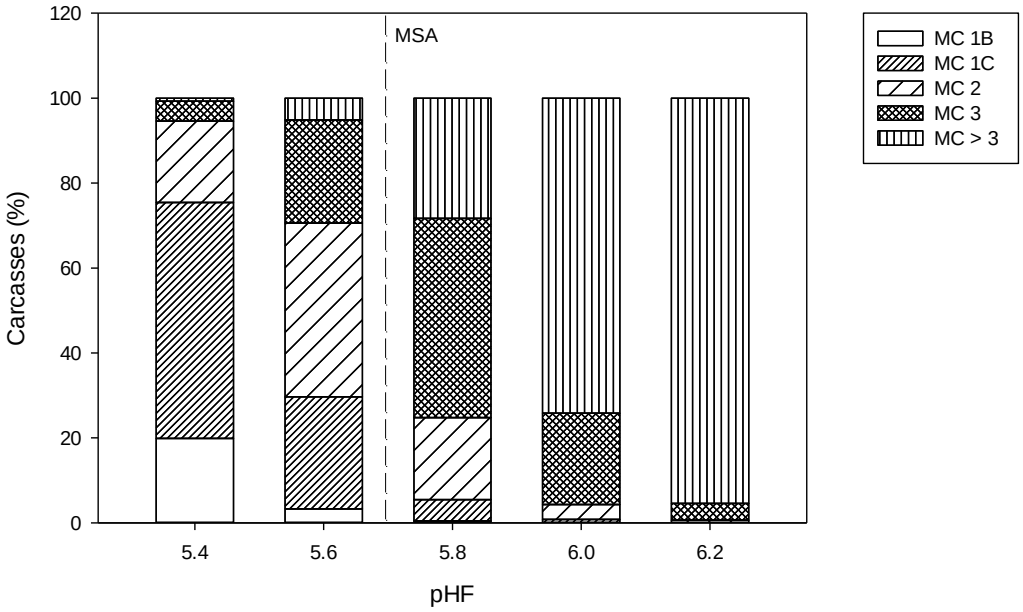

Figure 2: Predicted percent of carcasses (n=1512) with each meat colour score (1B being palest to >3 being unacceptably dark) in the *longissimus thoracis* for a range in pH_F from 5.4 to 6.2 from the meat colour model.

The vertical dotted line represents the Meat Standards Australia (MSA) cut off where carcasses pHu > 5.70 are classified as unacceptably high in pHu and are not eligible for grading. These predictions were calculated using mean values in the model for plant, temperature at pH 6 35°C, time from slaughter to grading 22 hours, category and for animals which were grain-fed for 159 days.

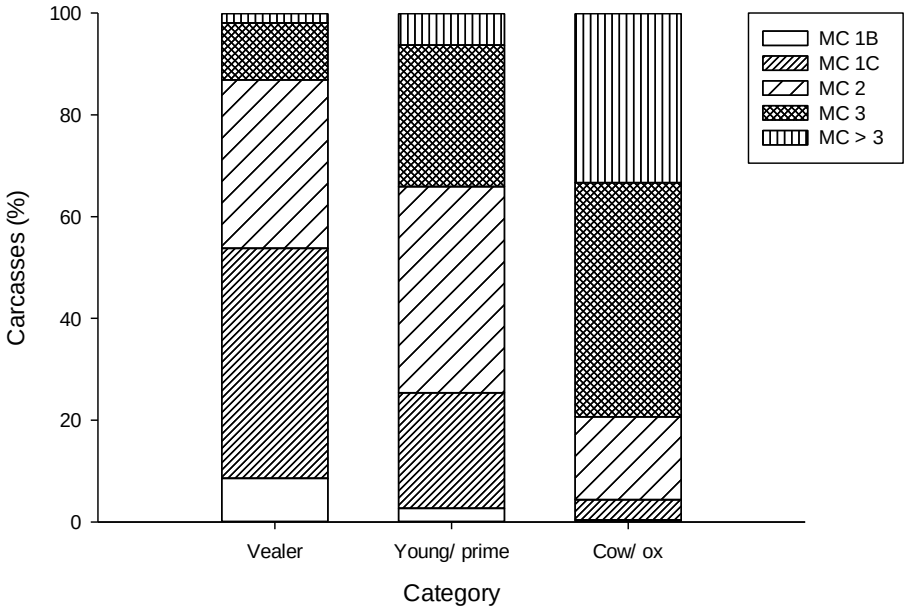

Figure 3: Predicted percent of each meat colour score (1B being palest to >3 being unacceptably dark) of the *longissimus thoracis* for carcasses (n=1512) from each category of animal within non-grain feed types from the meat colour model.

The categories were vealer (no evidence of eruption of permanent incisor teeth, hot carcass weight < 170 kg), young/ prime (grazing pasture pre-slaughter and 0-4 permanent incisors) or cow/ox (grazing pasture pre-slaughter and had 6-8 permanent incisors). These predictions were calculated using mean values in the model for plant, pH_F 5.6, temperature at pH 6 35°C and time from slaughter to grading 22 hours.

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8.2 The influence of beef meat colour at grading on eating quality

Beef *longissimus* eating quality increases up to 20 weeks storage and is unrelated to meat colour at carcass grading.

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Short Summary

Beef meat colour is important for consumer acceptance, with unfavourable colours associated with rejection. Meat of a dark colour can have a reduced shelf-life and eating quality. When stored for up to 20 weeks, loins scored as light, medium and dark at grading all had a “good everyday” eating quality. We have demonstrated that beef aged for 20 weeks is still acceptable to consumers, regardless of meat colour at carcass grading.

Abstract

Optimal beef meat colour is associated with increased consumer acceptance, whereas dark or pale meat has a reduced desirability. Dark beef also has a variable eating quality and reduced shelf-life. We hypothesised that a poor meat colour at carcass grading would generate an unacceptable eating quality after vacuum packed chilled storage for up to 20 weeks, due to the unfavourable pH conditions commonly associated with light and dark muscles. At three beef processing plants, beef *longissimus* muscles from 81 pasture and grain fed cattle (mix of *Bos taurus* and *Bos indicus* x *Bos taurus*) were graded at ~24hours post-slaughter for meat colour. The carcasses were allocated to light, medium and dark colour groups with n=27 carcasses per colour group. From the 81 carcasses, a total of 162 *longissimus lumborum* (*LL*) muscles were collected and half *LL*'s were randomly allocated to three ageing times (2, 12, 20 weeks) within colour group and six half *LL*'s were used per colour group within storage period and plant. Vacuum packed muscles were stored at -1.0±0.5°C for the designated period and sampled for biochemical and sensory assessments. The effects of colour group, storage week and carcass traits were analysed. Dark muscles had higher pH compared to the lighter colour group ($P<0.05$). The carcass traits dentition, feed type and fat depth did not influence the eating quality ($P>0.05$). After 2, 12 and 20 weeks of vacuum packed chilled storage; eating quality was similar for all 3 meat colour groups ($P>0.05$). With increasing storage time, all eating quality attributes improved ($P<0.001$ for all). Lipid oxidation increased with storage time and although values at 20 weeks were slightly above accepted levels for rancidity detection, MQ4 scores indicated the product would still be categorised as a 3 star product, indicative of the opportunity to store the *longissimus lumborum* (*LL)* for this length of time, whilst maintaining an acceptable eating quality, regardless of meat colour at carcass grading.

Keywords: Shelf-life, aging, lipid oxidation, MSA consumer assessment, sensory assessment.

Introduction

Beef meat colour is a fundamental criterion of consumer acceptability, with darker or paler meat colours being associated with a lower consumer acceptance compared to meat colours which are bright red in colour (Jeremiah, Carpenter et al. 1972; Viljoen, de Kock et al. 2002). Some studies have shown no difference in eating quality between colour groups (Jeremiah, Carpenter et al. 1972); however other studies have described dark beef as being more tender (Hunt and Hedrick 1977), the flavour as "bitter", "sour" or "off" (Viljoen, de Kock et al. 2002; Wulf, Emnett et al. 2002) or lacking in flavour (Young and Braggins 1993), and the associated high pH of the meat being detrimental to storage (Rey, Kraft et al. 1976). In addition, pale beef muscles (rump and loin), can be less acceptable to the consumer in terms of flavour and tenderness compared to normal muscles, especially after ageing for 21 days (Warner, Thompson et al. 2014).

In order to obtain maximum shelf-life, it is recommended that packaged beef primals be stored at temperatures close to -0.5°C, with shelf-life reported up to 26 weeks at this temperature (Small, Jenson et al. 2012). Chilled vacuum packed meat favours diverse subpopulations of lactic acid bacteria (LAB) which can metabolise glucose and peptides within the meat to form compounds such as lactic acid, acetic acid and ethanol (Labadie 1999). These metabolites have been associated with the formation of spoilage odours and flavours. To date, there is no information describing the meat colour at carcass grading to muscle biochemical properties in relation to the eating quality after vacuum packed chilled storage for over 12 weeks. In addition, although processors state that vacuum packed beef has a shelf-life up to 20 weeks, recommendations state that maximum shelf-life for vacuum packed beef is 12 weeks (Food Science Australia 2002; Meat and Livestock Australia Limited 2011). We hypothesised that a poor meat colour at carcass grading would generate an unacceptable eating quality after vacuum packed chilled storage for up to 20 weeks, due to the unfavourable pH conditions commonly associated with light and dark muscles. Thus, the aim was to determine the effect of meat colour at grading on eating quality after vacuum packed storage for 2, 12 and 20 weeks.

1. Materials and Methods

Sample collection & colour measurement

During autumn 2012, over 3 consecutive days, three Queensland beef processing plants were visited and a total of 162 beef striploins (*longissimus lumborum or LL*) were collected from 81 beef carcasses (Achilles hung) at ~24 hours post-mortem (6 carcasses from one plant were collected at ~72 hours, due to lack of availability of 'dark' colour scores on the first visit). The animals were between 0 and 7 tooth (dentition), were either pasture or grain fed and were a mix of *Bos Taurus* and *Bos indicus* x *Bos Taurus*. Unfortunately, MSA data was only obtained from two plants, where the Tropical Breed content (TBC) was reported to be 0-38%. Hormone Growth Promotants (HGP) were used in 61 animals. Fat depth (mm) of the carcass at the P8 site on the rump (AUS-MEAT 2005) was recorded as well as hot carcass weight (kg). Selection of carcasses was on the basis of meat colour at carcass grading (AUS-MEAT 2005). Briefly, the left side of the carcass was quartered either between the 10^{th} and 11^{th} or between the 12^{th} and 13^{th} rib (depending on market destination), and the exposed *longissimus thoracis (LT)* muscle was allowed to bloom at ~0-4°C for approximately 0.5-1 hr. Muscles were graded by a Meat Standards Australia (MSA) qualified assessor and allocated to 3 different meat colour groups (Light, Medium or Dark), defined by AUS-MEAT colour (AMC) scores: 1B or 1C; 2 or 3; >3 respectively (1B being the palest and 5 being the darkest) (AUS-MEAT 2005). Generally, colour scores of >3 are considered unacceptable in appearance and are discounted by beef processing plants.

Objective colour measurements were made on the exposed *LT* after blooming at 0-4°C, for 0.5-1.0 hours. A Hunterlab Miniscan EZ 45/0 LAV (light source A, observer angle 10°, aperture size 5 cm) was used to measure lightness, redness and yellowness attributes in triplicate (L*, a*, b* values respectively). The instrument was calibrated at ~4°C, using white and black calibration

tiles, as supplied with the instrument (Novasys group Pty Ltd, Ferntree Gully, VIC, Australia). Colour parameters were calculated as follows: hue = [arctangent (b*/a*)] and chroma = $(a^{*2} + b^{*2})^{1/2}$. For each colour group, at 1-3 days post-slaughter, the *LL's* from both sides were boned out and were cut in half to generate one cranial and one caudal sample (from both left and right sides) and randomly allocated to one of 6 time points; 0, 2, 8, 12, 16 or 20 weeks in an incomplete block of 2 units, the block comprising the cranial and caudal ends of the *LL*. This was to ensure an optimal comparison of storage weeks across sides and carcasses, giving a total of six replicates per plant, storage time and colour group combination (Fig. 1). Thus at each plant, a total of 54 *LL*'s were collected, from 27 carcasses generating 108 half *LL*'s. These samples were collected as part of a larger study, but for this paper only storage weeks 2, 12 and 20 were of interest. Samples were vacuum packed, placed in cartons and subjected to standard plant packing and chilling regimes. Cartons of vacuum packed half *LL*'s were delivered to the laboratory using a chilled storage vehicle and stacked on a pallet in the dark at -1.0°C +/- 0.5°C for up to 20 weeks.

At the allocated time points, half *LL's* were removed from cold storage and processed. The pH of the interior of the muscle was measured using a TPS WP-80 pH meter with a polypropylene spear-type gel electrode (Ionode IJ 44) and temperature probe (all from TPS Pty Ltd, Springwood, QLD, Australia). The pH meter was calibrated at ~10°C, using pH 4 and 7 buffers (TPS Pty Ltd, product no. 121382 and 121388 respectively). Sub-samples of ~50g were taken from all samples for biochemical analysis and were frozen using liquid nitrogen and subsequently stored at -80°C.

Lactate content

The L+ lactate determination on 1±0.05g of muscle was conducted in accordance with the method of Noll and H.U.Bergmeyer (1985), based on measurement of enzyme activities and

concentrations as outlined by Bond and Warner (2007). The lactate content (µmol/g) of the muscle was determined stoichiometrically, using the absorbance of NADH at 340nm (extinction coefficient = 6.22 mM/cm).

Thiobarbituric reactive substances (TBARS)

As a measure of lipid oxidation, the concentration of thiobarbituric reactive substances (TBARS) was determined on the frozen subsamples (Witte, Krause et al. 1970). Briefly, duplicate 2±0.01g samples were capped and cooked in 75°C water bath for 20 minutes and subsequently cooled for 30 minutes at 5°C prior to extraction. The concentration of malondialdehyde equivalents (mg/kg muscle) was calculated from absorbance readings at 530nm, using 1,1,3,3-tetraethoxypropane as a standard.

Meat Standards Australia (MSA) consumer sensory assessment

After 2, 12 and 20 weeks storage at CSIRO facilities and sub-sampling for biochemistry, all remaining *LL* samples were vacuum packed and transported (4.0 ± 1.1°C) to Cosign Pty Ltd (Coffs Harbour, NSW, Australia) and fabricated into standardised steaks for MSA consumer assessment and frozen (Watson, Gee et al. 2008). After thawing, samples were cooked using the standard grill procedure (Gee, Porter et al. 2005) and tested by 60 consumers over 3 sessions. One sample was tasted by 10 consumers and each sample was served 5 times in a different presentational order with each consumer tasting 6 sub-samples. All samples were scored for tenderness, flavour, overall liking, juiciness and a combination of these 4 attributes was calculated (MQ4) using the respective weightings (0.3, 0.3, 0.3 & 0.1). Clipped scores were used in the analysis (Watson, Gee et al. 2008).

Statistical analysis

Data analysis was completed using Genstat 15th edition. For colorimetric data (variates L*, a*, b*, hue, chroma), analysis of variance (ANOVA) was used for comparison between colour groups, using plant as a block. To ensure valid comparison in the colour analysis, data from day one was used for all colour groups. Restricted maximum likelihood (REML) was used for comparison between colour group and storage week treatments. For the analysis of the variates pH, lactate content and TBARS, a fixed treatment structure colour group*storage week was used and feeding regime, dentition and fat depth were also tested in the models. A random structure taking into account the 'orientation' of the *LL* half from either cranial or caudal end (plant/colour identity/carcass/side/orientation) was also included. For consumer score analysis, the model had a fixed treatment structure of colour group*storage week and also included fixed effects of feeding regime, dentition and fat depth. The random structure was the same as above but also took into account the session involved in the sensory panel. The sensory variates tested were MQ4, tenderness, overall liking, flavour, juiciness and satisfaction scores. The final model only included the significant effects (P<0.05). Due to the lack of data for one plant, it was not possible to include TBC in the statistical analysis.

2. Results

Population

The dentition scores of the carcasses for the light, medium and dark colour groups were 0-4, 0-2 and 0-7 tooth respectively. The light colour group had one carcass with a dentition score of 4 and the remainder were 0-2 tooth. Also, only one carcass from the dark colour group had 7 teeth and the remainder of the dark colour group were 0-4 tooth. Hot carcass weight for carcasses of the light, medium and dark colour groups were 324 ± 47, 314 ± 46 and 306 ± 43kg respectively. P8

fat depths for carcasses of the light, medium and dark colour groups were 13.3 ± 3.8, 13.1 ± 3.8 and 13.6 ± 4.8mm respectively.

Colour measurements

The colour attributes for the *LT* muscle bloomed for 0.5-1 hr are summarised in Table 1. Between colour groups, there was separation of the data for all colour attributes, but due to the limited experimental units in this stratum of the output, these were not significant. From the design used, any information on the main effect of colour group alone would have far less precision than the treatment structure colour group by storage week. Hence, the separation observed in the data was expected. Also, although the darker colour group contained more animals of dentition score ≥4, the term dentition was not significant (P>0.05) for any colour attribute.

Biochemical measurements

The variates feeding regime, dentition and fat depth were found to have minimal influence (P>0.05) on the biochemical attributes measured. Treatment effects on biochemical analyses are summarised in Table 2. The dark coloured group had a significantly higher pH (P<0.01), and lower lactate content of the muscle (P<0.05). For both pH and lactate, storage week had a significant effect (P<0.01), with week 2 samples having a lower pH and higher lactate content compared to other weeks. However, there was no significant storage week and colour group interaction for either pH or lactate content (P>0.05).

In all meat colour groups, loins from longer storage times had higher TBARS concentrations (P<0.001), but no significant difference (P>0.05) in values were found between colour groups.

Meat Standards Australia (MSA) eating quality attributes

Inclusion of the variates feeding regime, dentition and fat depth were found to have no influence (P>0.05) on any of the eating quality attributes. The effects of colour group and time of storage on the MSA eating quality attributes of satisfaction, tenderness, overall liking, flavour, juiciness and the combined MQ4 scores from the four latter attributes are displayed in Fig. 2.

For all eating quality attributes, there was no significant difference (P>0.05) observed between colour groups, however there was a change with storage time (P<0.001).

Between 2 and 12 weeks, there was an improvement in all eating quality attributes (P<0.001), with no further improvement observed at 20 weeks. There were no significant interactions (P>0.05) between colour group and storage week for any of the eating quality scores.

For all storage weeks, the tenderness and MQ4 scores were positively correlated with TBARS values, with 9 and 13% respectively of the variation being determined by this attribute (data not shown).

3. Discussion

Regardless of meat colour at grading, after 20 weeks storage, all samples had an acceptable MQ4 score categorised as having a MSA 3 star rating (Watson, Gee et al. 2008).

The MQ4 values observed at week 2 appeared to be low compared to those observed in other studies (Legrand, Hocquette et al. 2012). The reason for this is unknown, although it could be due to the combination of breed, and use of HGP's which are known to reduce eating quality (Thompson, Polkinghorne et al. 1999; Polkinghorne, Thompson et al. 2008; Thompson, McIntyre et al. 2008) but unfortunately these variables were not able to be included in our statistical

modelling. Although, differences between colour groups were not significant, after 2 weeks aging, only the dark group had a MQ4 score of 46, which is the border between 'no grade' and '3 star' in the Australian market (Watson, Gee et al. 2008; Legrand, Hocquette et al. 2012). Both the light and medium colour groups had MQ4 scores below this value and would be considered by Australian consumers to be unsatisfactory. These results are interesting and possibly a result of the sampling and wide variance observed in the consumer data.

After aging for 12 or 20 weeks, all muscles would be considered to have a "good everyday" eating quality indicative of eating quality assured (Watson, Gee et al. 2008; Meat and Livestock Australia 2014). In a previous study, a small informal sensory panel found *LL*'s stored for 26 weeks were organoleptically acceptable (Small, Jenson et al. 2012). The results of this study are the first MSA consumer assessment for eating quality conducted using 20 week aged *LL* from different meat colour groups. Compared to week 2, all eating quality attributes for all colour groups, improved and were significantly higher at 12 and 20 weeks. Thus, by storing the meat for longer than 2 weeks, scores were higher for tenderness, overall liking, flavour, juiciness and satisfaction. All of these attributes contributed to the MSA 3 star rating of the meat assessed after 20 weeks, and were not impacted by the meat colour at grading.

Initial carcass muscle colour did not have a significant effect on any of the eating quality scores and similar findings have been reported in beef *longissimus* muscles for either 0 or 7 days aging for overall acceptability, tenderness and juiciness (Jeremiah, Carpenter et al. 1972; Viljoen, de Kock et al. 2002; Wulf, Emnett et al. 2002). This leads us to reject the hypothesis that meat colour observed at grading influences the eating quality after 2, 12 or 20 weeks storage. However, the considerable variation between muscles, for attributes flavour, satisfaction and overall liking, especially at week 20, leads us to consider a future study with more replicates may be required. In this study, any properties of the muscle which gave rise to the colour at carcass grading were not detected by the sensory panel after the various aging periods.

Over the storage time from 2 to 20 weeks, the increase in pH was most predominant in the light and medium coloured groups. The increase in pH is unexpected; normally a pH decline is observed with vacuum packed storage due to formation of organic acids and other compounds formed by the specific bacterial populations (Jones 2004). However, vacuum packing of lamb *longissimus* muscle also resulted in this increase in pH for up to 12 weeks (Moore and Gill 1987). In this study, the pH increase was relatively small, increasing by 0.07, 0.05 and 0.01 for light, medium and dark coloured groups respectively, between 2 to 20 weeks. It is interesting that lactate levels also declined during this period, and a decline in lactate has previously been associated with reduced colour stability (Moore and Gill 1987). In addition, during the first 10 days of storage, an increase in pH post-mortem has been reported to progress more slowly in normal compared to dark-firm-dry (high pH) beef (Livisay, Xiong et al. 1996) indicative of different metabolic processes occurring during storage. This result is difficult to explain, but is believed to be due to the different biochemical properties of the muscle which may influence bacterial species and catabolites formed during the aging process (Moore and Gill 1987; Livisay, Xiong et al. 1996; Jones 2004).

Between colour groups, oxidation measurements indicated similar oxidation levels in all of the groups, with no distinct difference or trend. High pH meat has previously been associated with a reduction in meaty flavour and increase in bitterness or rancid flavours (Yancey, Dikeman et al. 2005). Lipid oxidation, as measured by TBARS, is reported to be a good predictor of sensory perception of rancidity (Campo, Nute et al. 2006). Early sensory studies on beef patties indicate a TBA value between 0.6-2.0mg/kg has been perceived with an oxidised or rancid flavour (Greene and Cumuze 1982). However, more recently in beef loin a TBARS value greater than 2.28mg/kg was associated with a decrease in beef flavour and increase in rancid flavour (Campo, Nute et al. 2006). The results obtained at week 2 and 12 were below this level, however panellists consuming meat at 20 weeks did not express any problems with the flavour.

In conclusion, these are the first results illustrating that irrespective of meat colour at grading, *LL* muscle can be chilled stored under vacuum for up to 20 weeks whilst maintaining a suitable eating quality.

4. Conclusion

Contrary to our hypothesis, meat colour at grading did not influence the eating quality of the *LL* after aging for 2, 12 or 20 weeks. Between 2 and 12 weeks, all colour groups showed an improvement in eating quality attributes as measured using MSA consumer assessment. Lipid oxidation, as indicated by TBARS measurement, increased with storage time and although values at 20 weeks were slightly above accepted level for rancidity detection (2.28mg/kg), MQ4 scores indicated the product would still be categorised as a 3 star product or having a “good everyday” eating quality, indicative of the opportunity to store the *LL* for this length of time, whilst maintaining a suitable eating quality.

Acknowledgments

CSIRO acknowledge funding provided by The Australian Meat Processor Corporation and matching funds provided from the Australian Government, via Meat and Livestock Australia, to support the research and development detailed in this publication. The authors would also like to acknowledge the technical assistance of Vicki Eggleston, Anita Sikes and Janet Stark.

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List of tables and figures

Table 1: Effect of colour group (light, medium, dark) on colorimetric measurements (L*, a*, b*) of beef *longissimus thoracis* muscles after 0.5-1 h bloom at 0-4^{o}C. 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 predicted means and the least significant differences (LSD) and P-values are shown (where non-significant or N.S. is *P*>0.05).

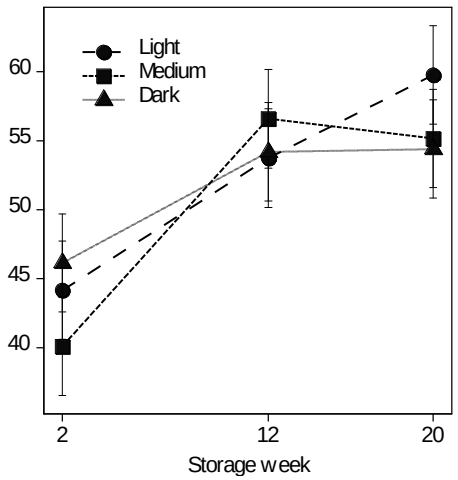
	Light	Medium	Dark	LSD	P-value
L*	36.3	34.9	31.1	5.55	N.S.
a*	29.2	29.0	27.3	4.05	N.S.
b*	21.4	20.9	19.4	3.88	N.S.
Hue	36.3	35.7	35.2	1.60	N.S.
Chroma	36.2	35.7	33.5	5.52	N.S.

Table 2: Effect of storage week (SW; 2, 12, 20) and colour group (CG; light, medium, dark) on biochemical measurements of beef *longissimus lumborum* muscles. 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 predicted means and the least significant differences (LSD) and P-values are shown (where non-significant or N.S. is *P*>0.05).

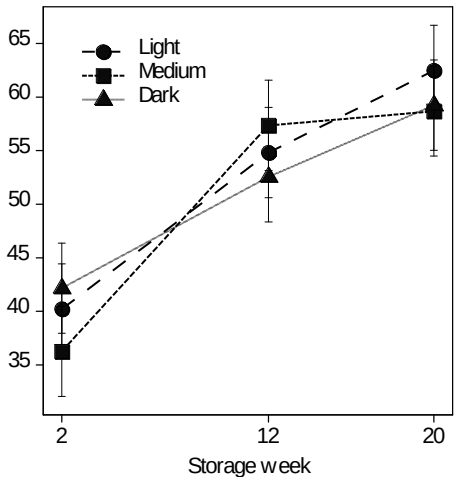
	SW	Light	Medium	Dark	LSD			P-value		
					SW	CG	SW*CG	SW	CG	SW*CG
pH	2	5.53	5.59	5.89	0.023	0.173	0.128	0.001	0.005	N.S.
	12	5.58	5.63	5.87						
	20	5.60	5.64	5.90						
Lactate (µmol/g)	2	22.2	22.5	19.5	0.51	2.13	1.70	<0.001	0.012	N.S.
	12	20.3	19.8	17.1						
	20	21.3	22.1	18.6						
TBARS (mg/kg)	2	1.32	1.36	1.53	0.282	0.720	0.674	<0.001	N.S.	N.S.
	12	1.66	1.72	1.48						
	20	2.60	2.70	3.11						

	Light**	
Carcass 1 L	20*	12
Carcass 1 R	2	0
Carcass 2 L	8	16
	cranial	caudal

Figure 1: An example diagram of the allocation of treatments to storage week* (0, 2, 8, 12, 16 and 20) and colour group** (light, medium or dark) to 6 sections of *longissimus lumborum (LL).* Each *LL* was cut into 2 pieces resulting in a cranial or caudal end, and were from 1 ½ carcasses; 2 from one carcass (L;left and R;right sides) and the other from a second carcass. Six half *LL's* were collected for each colour group at each plant within storage time, yielding a total of 54 replicates from 81 carcasses. For this paper, only storage weeks 2, 12 and 20 were of interest, as samples for weeks 0, 8 and 16 were used as part of a larger study.



(a)



(b)

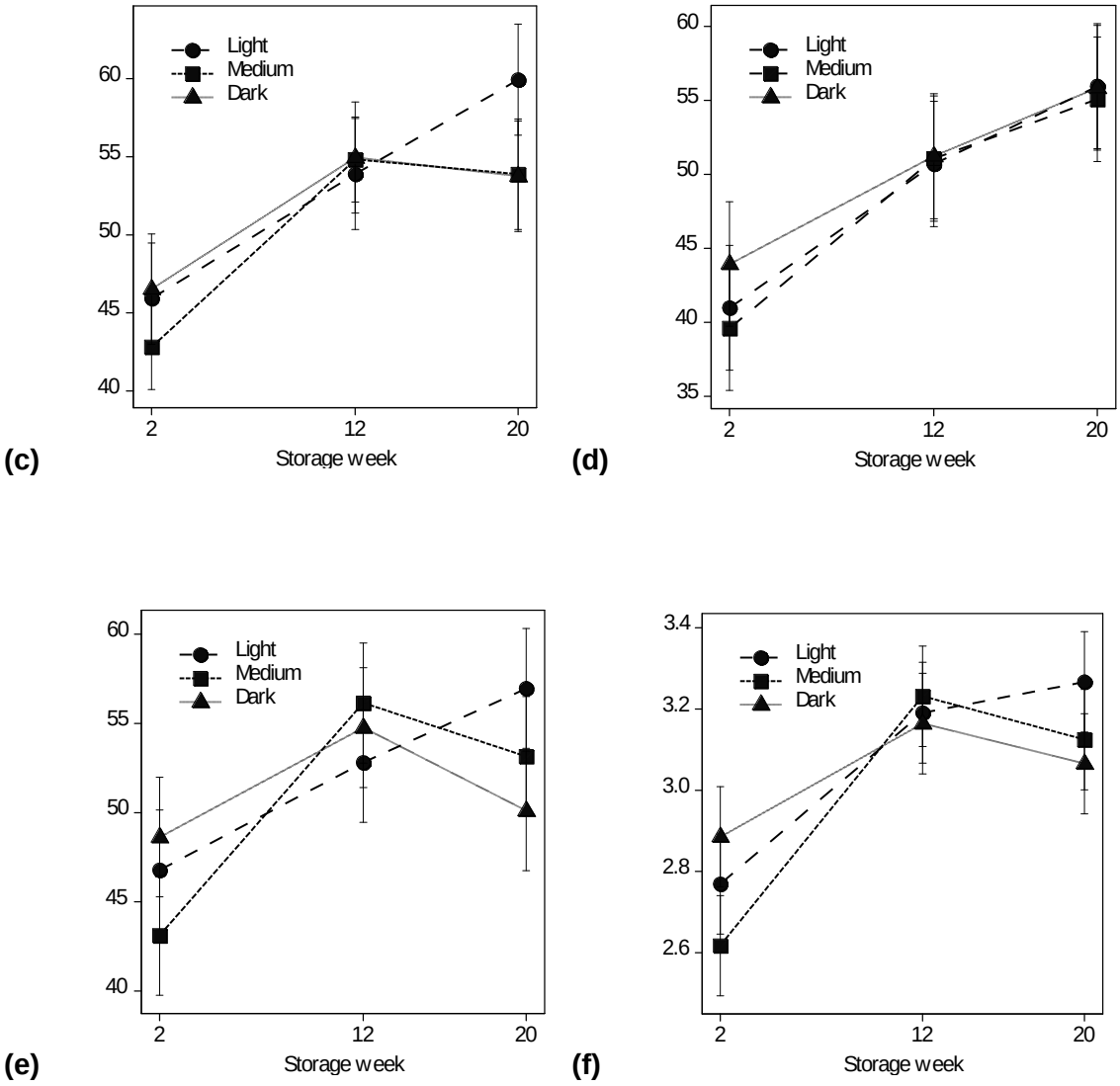


Figure 2: **Effect of colour group (light, medium, dark) and storage week (2, 12, 20) on Meat Standard Australia (MSA) consumer eating quality (a) MQ4 scores (composed of tenderness, juiciness, flavour and overall liking attributes) (b) tenderness (c) overall liking (d) juiciness, (e) flavour and (f) satisfaction scores of beef *longissimus lumborum* muscles. 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. Each point is a mean and the standard error of differences (SED) is shown as a vertical line above and below each mean.**

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