Visual assessments of meat surface structure and meat colour to predict beef tenderness between five South African beef breeds

by

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ABSTRACT

Visual assessments of meat surface structure and meat colour to predict beef tenderness between five South African beef breeds

Visual measurements of colour are the ideal standards for the determination of meat quality at the point of sale. Thus far, consumers rely on colour alone to make a purchase decision. The use of visual surface structures to differentiate tender from tough meat could serve as a new technology to assist consumers in predicting meat tenderness at the point of sale. According to the South African Red Meat Carcass Classification system, carcasses are classified according to animal age, fatness class, confirmation class and bruising or damage of the carcass. This system does not clearly indicate to the consumer the physical, compositional and sensory characteristics of the meat, and has limitations in classifying carcasses into very tender, tender, less tender, and slight tender. The use of visual structural measurements as a tool to assist in classifying meat into the categories of tender and tough could be a valuable technology. The objectives of this study were to determine the possibility to predict beef tenderness with experienced vision, determine the possibility of an association between colour, surface structure (morphology), and tenderness; as well as to determine genotypic differences in meat colour, morphological structure, and resulting shelf life. To achieve these aims, the study was conducted in two phases, with Phase 1 being an exploratory phase, after which the findings were implemented from the first phase into the second phase to allow for in-depth analyses. The beef breeds Brahman (Br) (*Bos indicus*), Nguni (N) (Sanga type), Angus (A) (British *Bos Taurus*), Charolais (C) (European *Bos Taurus*) and Bonsmara (Bo) (composite) were used, with 10 steers per genotype per phase. The animals were finished off on a feedlot diet for a period of between 90-110 days at the ARC-API feedlot and were slaughtered at the ARC-API abattoir when they reached a live weight which would produce a carcass of Class A (no permanent incisors), and fat class 2 to 3 (1-≤5 mm) (South African Beef Classification System). After exsanguination, the carcasses were halved. The right sides were electrically stimulated for 20 s (400 V peak, 5 ms pulses at 15 pulses/s) and entered the cold rooms (± 2°C) within 60 min after slaughter

(ES). The left sides were placed in a room with a controlled temperature of 10°C for six hours, thereafter in cold rooms at ±2°C (NS). Temperature and pH and muscle energy samples were taken at 1, 3, 6 and 24 hrs from the *m. longissimus dorsi*. Steaks were sampled at the *m. longissimus dorsi* at 24 hours *post mortem* (pm). One set of steaks were aged for three and nine days pm in polystyrene plates covered with polypropylene cling wrap (PP) at 6°C in a display cabinet; while another set of steaks were aged for 14 and 20 days *pm* in vacuum bags at 1-4°C in a cold room for Phase 1. For Phase 2 of the study, steaks were aged for three days pm in polystyrene plates covered with polypropylene cling wrap at 6°C in a display cabinet and the other steaks were aged for 9, 14 and 20 days pm in vacuum bags at 1-4°C in a cold room. The change of packaging for nine days pm was based on the lipid peroxidation results as was measured by the TBARS assay, the nine days pm steaks had higher TBARS, and the steaks had already started to develop moulds, discolouration and bad odour. The fresh, aged steaks were analysed for visual colour, marbling, fibre separation, texture and structure integrity using a 10 member trained panel. Other analyses included tenderness measurements (e.g. Warner Bratzler shear force, sarcomere length, myofibril fragment length, connective tissue characteristics, and fibre detachment), colour measurements (Minolta CIE. *L**, *a** , *b**, myoglobin derivatives), protein and lipid denaturation, drip loss and water holding capacity. Correlation coefficients were established between visual colour measurements and instrumental colour measurements, visual tenderness measurements, instrumental tenderness measurements; and between visual tenderness measurements and instrumental colour measurements. Results of this study revealed that the evaluation of meat colour by the panel correlated very well with instrumental colour (CIE. *L**, *a**, *b**) and very little with myoglobin and its derivatives (oxymyoglobin, metmyoglobin and deoxymyoglobin). The visual panel was able to differentiate meat colour between the breeds. Good correlations were found between instrumental tenderness measurements (WBSF) and visual tenderness measurements, which included the fibre separation, structure integrity and marbling. Visual texture showed very low correlations with WBSF measurements. The use of visual meat colour to predict meat tenderness showed very low correlations with WBSF. The study also revealed breed differences in meat colour. Nguni breed was found to produce darker meat than Angus, Bonsmara, Brahman and Charolais. The Charolais breed produced lighter meat

during Phase 1 and darker meat during Phase 2. This was due to the harsh weather experienced during Phase 2. In conclusion, visual analysis of colour can accurately be used to evaluate the quality of meat colour. Fibre separation, structure integrity and marbling could be used to visually predict meat tenderness, but only when training is provided. Visual texture did not show any potential as a reliable visual tenderness attribute, therefore cannot be used to predict meat tenderness. Visual analysis of meat colour cannot be used to predict meat tenderness.

DECLARATION

I, Kedibone Yvonne Modika declare that this thesis which I hereby submit for the degree of PhD in Animal Science at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed…….

Date…….

"For I know the plans I have for you," says the Eternal (commander of heavenly armies and God of Israel), plans for peace, not evil, to give you a future and hope—never forget that".

Jeremiah 29:11

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Journal article

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMSA	American Meat Standards Association
ANOVA	Analysis of variance
ARC-AP	Agricultural Research Council- Animal production
ATP	Adenosine triphosphate
CCW	Cold carcass weight
CIE	*Commission International De L' Eclairage*
CP	Creatine phosphate
DCPIP	Dichlorophenol-indophenol
DeoxyMb	Deoxy-myoglobin
DFD	Dark firm dry meat
DP	Dressing percentage
DM	Dry matter
DTNB	5,5′-Dithiobis (2-nitrobenzoic acid)
EDTA	Ethylene diamine tetra acetic acid
EMA	Eye muscle area
FAO	Food and Agriculture Organization
HCl	hydrochloric acid
HO_4	Perchloric acid
G-6-P	Glucose-6-phosphate
GP	Glycolytic potential
KCl	Potassium Chloride
LD	*longissimus dorsi*
KOH	Potassium hydroxide
LW	Live weight
Mb	Myoglobin
MDA	malondialdehyde
MetMb	Metmyoglobin
MFL	Myofibril fragment length

$MgCl_2$	Magnesium Chloride
MSH	Myofibril surface hydrophobicity
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NaN_3	Sodium Nitrite
OxyMb	oxy-myoglobin
PSE	Pale Soft Exudative
pHu	Ultimate pH
SDH	*Succinate dehydrogenase*
RPM	Revolutions per minute
SA	South Africa
SDS	sodium dodecyl sulphate
SEM	Standard error of means
SL	Sarcomere length
SSF	slice shear force method
TBARS	Thiobarbituric acid reactive substances
TPA	texture profile analysis
V	Volts
WBSF	Warner Bratzler shear force
WHC	Water holding capacity

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CHAPTER 1
INTRODUCTION

1.2. Project title:

Visual assessments of meat surface structure and meat colour to predict beef tenderness between five South African beef breeds.

1.1. Project theme:

Meat Science focusing on visual evaluation to predict meat quality (beef).

1.3. Aims:

1.3.1. To determine the possibility of predicting beef tenderness with experienced vision.

1.3.2. To determine whether an association exists between colour, surface structure (morphology), and tenderness;

1.3.3. Determine genotypic differences in meat colour, morphological structure and resulting shelf life.

1.4. Motivation

Meat quality is usually described by the compositional value (lean-fat ratio) and palatability aspects such as appearance, aroma, texture, juiciness, tenderness, and flavour (FAO, 2013). Studies have reported that visual identification of quality meat is usually based on colour, marbling and amount of drip loss. These aspects affect the consumer's decision to make a purchase (Dinh, 2008). Consumers usually associate brighter red colours with freshness. Informed consumers will "know" that dark meat indicates that animals were stressed (suffered) when they were slaughtered. Too much drip in the bag could also indicate bad slaughter practices, and is definitely not value for money. According to Hunt and King (AMSA, 2012), visual evaluations of colour are closely connected to consumer assessments, and these set the standard for assessment of instrumental colour. Assessments of sensory colour using panellists have been reported by fewer studies. Chan *et al*. (1996) conducted a sensory evaluation study where panellists evaluated steaks for the degree of discolouration, visual acceptability and willingness to buy. Panellists also assessed the aroma according to olfactory acceptability, and specified if they would eat the steak. Zanardi *et al*. (1998) used a trained 10-member panel to evaluate the appearance of the brown colour of met-myoglobin (MetMb) on steaks, and conducted

colour stability assessments on pork chops from pigs supplemented with either vitamin E, oleic acid (sunflower oil) or copper.

The biochemical origin of the red colour in meat is due to the concentration and redox state of myoglobin (Mb), haemoglobin and cytochromes, but myoglobin is the primary protein pigment associated with meat colour (Bekhit & Faustman, 2005). Colour change in meat can be attributed to either oxygenation or oxidation of myoglobin. Myoglobin is normally found in three forms: oxy-myoglobin (OxyMb); deoxy-myoglobin (DeoxyMb); and met-myoglobin (MetMb), and the relative proportions of these regulates fresh meat colour. Met-myoglobin is the most undesirable form, because when present on the surface of fresh meat cuts, the meat becomes brownish and this turns consumers away (Renerre, 1990). The rate at which the surface of meat turns from red to brown during display is a characteristic referred to as colour stability, and is believed to be determined by the degree of oxygen diffusion and consumption, the rate of autoxidation of Mb pigment to MetMb, and the rate of MetMb reducing activity. Understanding the physiological and structural components of muscle is essential to comprehending the effects they have on overall colour stability (Sammel *et al*., 2002a).

The role of the MetMb reducing system in preserving meat colour has been debated, and could prove useful to measure the MetMb reducing activity in meat. The manipulation of the MetMb reducing systems has been reported to offer a possible strategic approach to lessen MetMb build-up, and increase fresh meat shelf life (Bekhit & Faustman, 2005). It is therefore necessary to understand the biochemical factors that influence colour stability and the formation of met-myoglobin in beef muscles. Because muscle tissue is a very complex material that depends on many factors, there is no clear evidence as to what contributes to the colour stability of meat. Furthermore, the extent to which breed variations affect muscle oxidative capacity and colour changes during display is unknown, and was thus investigated.

So far, research has yielded inconsistent results regarding the contribution of the MetMb reducing activity to meat colour stability (Calnan *et al*., 2014). O'Keeffe and Hood, (1982), Renerre and Labas (1987) and King *et al*. (2010) have proved that the initial oxygen consumption rate and reducing capacity of beef throughout display showed colour stability variances. Calnan *et al*. (2014) reported that the oxidative capacity of muscle is one of the

most important intrinsic factors that might link meat colour to other carcass characteristics. Muscle oxidative capacity is the amount of oxidative type I, oxidative/glycolytic type IIA and glycolytic type IIX myofibres inside a muscle. Muscles containing higher quantities of oxidative myofibres, such as *musculus semimembranosus*, are darker and redder originally, with higher Mb and iron concentrations. This type of muscle are more oxidative and susceptible to more quicker discolouration after slicing for display than the more glycolytic muscle types, such as *musculus semitendinosus.*

Although the meat colour, marbling, and amount of drip constitute the visual characteristics that consumers use to decide on a purchase, so far it has not been reported whether they can be a direct indication of the meat eating quality attributes, which includes tenderness and texture. It has been reported that consumers rely on meat tenderness to make a repurchase decision (Marino *et al*., 2013; Dinh, 2008; Brooks, 2007; Ellies-Oury *et al*., 2013). Therefore, meat tenderness has been defined as the utmost significant meat quality trait (Ellies-Oury *et al*., 2013).

Texture, "as seen by the eye, is a function of the size of the bundles of fibres into which the perimysial septa of connective tissue divide the muscle longitudinally" (Brooks & Savell, 2004). Several studies have emphasised breed as a significant factor that can have an impact on the characteristics of the raw muscle tissue, and on the final product in beef (Marino *et al*., 2013). Numerous studies have indicated the differences in the tenderness of meat from different breed-groups. Studies have reported an increase in variability and lower meat tenderness related with the presence of *Bos indicus* inheritance. Frylinck and Heinze (2003) and Strydom. (2008) have reported that South African genotypes differ inherently in terms of tenderness characteristics, due to biochemical and physiological factors. Although the *Bos indicus* is frequently discriminated against because of negative perceptions of their carcass value and palatability compared to *Bos taurus* (Wheeler *et al*., 1990), the breed is extensively used in some countries like the United States due to its high productivity, insect resistance and heat tolerance (Muchenje *et al*., 2008a; King *et al*., 2006). Nonetheless, (Koohmaraie *et al*., 2003) reported that variations are observed within each breed than amongst the most different breeds.

The period of ageing is another important factor that can greatly affect the colour, texture, shelf life, tenderness and juiciness of meat. Li *et al*. (2014) reported that the *post*

mortem ageing of beef significantly influences the increase of tenderness and development of flavour characteristics. During ageing, myofibrillar proteins structure (and other related proteins) goes through some alterations, and collagen is weakened, but to a lesser degree (Dransfield, 1994). Shackelford *et al*. (1997) reported that the effect of ageing depends on the potential tenderness of the breed as observed in crossbred steers and heifers representing diverse breed types (0-62.5% *Bos indicus*). Campo *et al*. (1999) reported that breed had a significant effect on the ageing process in cattle slaughtered at 450 kg live weight. The type of packaging used can also affect the period of ageing. Commonly used packaging types include modified atmosphere packaging, PVC wrap and vacuum packaging. Vacuum packaging has an advantage over conventional packaging where it gives an opportunity to make full use of the ageing process to tenderise the meat, with little weight loss and spoilage, but the visible purge loss is thought to be unattractive to consumers. High-oxygen modified atmosphere packaging reduces the shelf life, produces off-flavours, and allow the growth of anaerobic bacteria, due to the presence of oxygen. Of utmost importance is the ageing temperature. Ageing at lower temperatures will delay the met-myoglobin formation by suppressing the residual activity of the oxygen-utilising enzymes, while higher temperatures will promote the formation of myoglobin (Brooks & Savell, 2004).

As mentioned previously, visual attributes (meat colour and visible fat) affect the purchase-decision of consumers, but is not necessary an indication of "non-visual" attributes such as tenderness and juiciness. The visual differences that exist between South African beef genotypes and the impact on structure and therefore tenderness and juiciness have not yet received scientific evaluation. These perceived breed differences could influence the shelf life, pathological susceptibility and consumer perceptions. Morphological differences could indicate tenderness characteristics for the trained eye, and could help predict potential tenderness characteristics. If these differences can be quantified, this may open up opportunities to predict meat characteristics by judging meat appearances by means of experienced vision (similar to that of a trained classifier).

So far, there is no scientific literature published on the surface visual structural properties of meat and its relationship with non-visual attributes. If visual characteristics of meat can be an indication of non-visual attributes, this could be used as an additional

measure by experienced classifiers. If visual characteristics of meat are over-emphasised, consumers can be educated accordingly. *Post mortem* treatments such as ageing and packaging can be adapted accordingly, where monetary benefits will follow. The results can have direct impact on shelf life and present ability of meat, which can have an effect on price and consumer acceptance. A study by O'Sullivan *et al*. (2003) showed that panellist which are not trained are similar to the consumer in the way that they perceive colour changes in meat. Nevertheless, using a trained panellist is beneficial in the assessment of unfamiliar products, where a greater degree of judgement is needed. The authors used both untrained and trained panellists in their study. However, in the present study, only trained panellists were used. In addition to this, variations within each breed were not included in this study.

No precise data is available on the size of structures, which can be perceived by sensory analysis of meat, but it is believed (Hatae *et al*., 1990) that it is perhaps about 100 µm, and therefore might even be visible to the eye in terms of texture and reflectance. Structures, which relate to tenderness that might be visible are approximately the size of two fibres, and would include breaks of the whole fibre, not sarcomeres, and fibre-to-fibre adhesion (Taylor & Frylinck, 2003).

To summarise the objectives of this study: the differences between the five different beef breeds in terms of tenderness related characteristics and meat colour characteristics were studied. The possibility of a trained panel to predict meat tenderness and texture through visualising certain meat surface structural properties was investigated. Then correlations were established between the visual characteristics with the tenderness related measurements to try to establish whether a trained eye can be able to predict meat tenderness by means of visual analysis. Results of this study can assist in improving the current South African carcass classification system. Consumers can also be educated as to what to look for during a meat purchase. If the observed visual differences could have a correlation with the instrumental measurements, this could be used as an additional measure by experienced classifiers. The biochemical *post mortem* processes are key-steps for meat tenderisation (Herrera-Mendez *et al*., 2006) but the mechanism of meat tenderness is highly complex, and not fully understood (Ouali, 1990). Therefore, methods were

developed where complex extracts were evaluated such as multi-component analyses on diode array spectrophotometers, to try to understand the tenderisation process.

The present study was conducted in two phases, to learn from the first phase and implement knowledge learned from the first phase to improve the second phase. Results of Phase 2 of the study will be reported in detail and results of Phase 1 of the study will be reported, to emphasise differences between the two phases. The study will be reported per chapter, with Chapter 2 presenting the literature overview, Chapter 3 describing in detail the materials and methods, Chapter 4 results of the study, and Chapter 5 and 6 discussion, conclusion and recommendations.

1.5. Hypothesis

The tested null hypothesis were:

H_0: Visual evaluation of meat tenderness by using certain visual structural measurements on the surface of the meat can be used to predict meat tenderness.

H_0: Visual evaluation of meat colour characteristics can be used to predict meat tenderness.

CHAPTER 2
LITERATURE REVIEW

Beef of consistent quality is required in order to maintain consumer satisfaction. Meat quality is a broad term used to define measurement of traits that determine the suitability of meat to be consumed as fresh or kept for a realistic period without deterioration (ElMasry *et al*., 2011). Meat quality characteristics could include microbiological attributes, chemical attributes, technological attributes and sensory attributes. But the important attributes assessed by consumers are the sensory attributes of meat, which include tenderness, colour, juiciness, flavour, texture, odour and marbling, as illustrated in Figure 2.1 (Xiong *et al*., 2014); and in this study we will focus on these sensory attributes.

The meat quality characteristics are greatly influenced by pre-slaughter handling, stunning method, and post slaughter treatment, among other factors (Xiong *et al*., 2014).

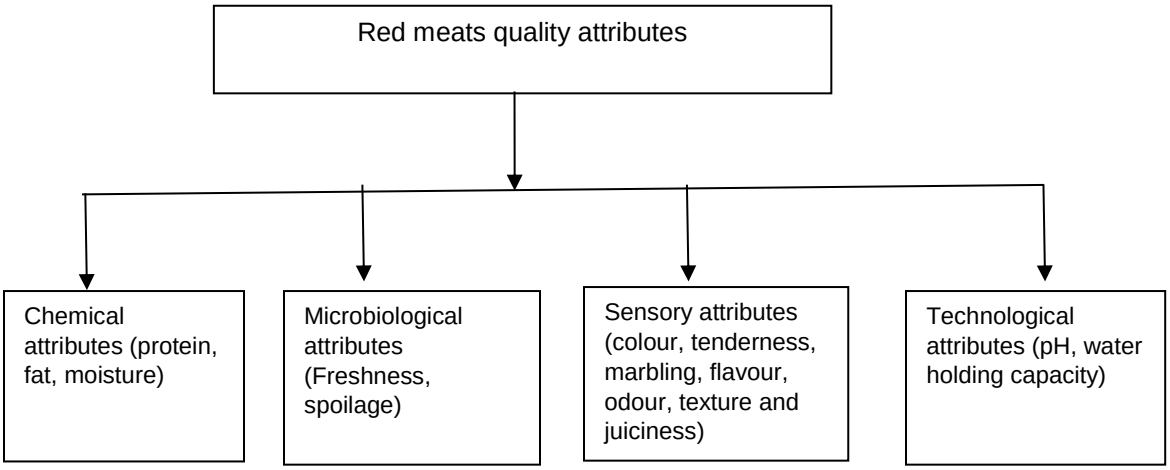


Figure 2.1: Common quality attributes of red meat. Adapted from Xiong *et al*. (2014).

2.1. Meat colour

Meat colour is the most important visual attribute affecting consumer decisions to purchase meat, (Mancini & Hunt, 2005; Bekhit *et al*., 2007) in the case of beef. Hunt *et al*. (1991) reported that consumers usually use meat colour and appearance to accept or reject a product, and therefore suppliers need to create and maintain the preferred colour of the product. Redness and colour in general are due to the concentrations of the deoxymyoglobin (DeoxyMb), metmyoglobin (MetMb) and oxymyoglobin (OxyMb) (Holman *et al*., 2015). Consumers associate the bright cherry red colour of OxyMb to freshness and wholesomeness, while the brown colour of MetMb is considered undesirable (Suman *et al*., 2014; Brugiapaglia & Destefanis, 2010). The visual colour of meat changes during display or storage and can result in loss of sales as increased exposure to oxygen intensifies MetMb levels, thereby negatively influencing consumer acceptance (Holman *et al*., 2015).

2.1.1 Factors influencing meat colour

The colour of beef can vary from deep purplish red to brown subject to the amount and chemical state of myoglobin and by the meat structure, which is linked to its ultimate pH. Fortunately, the colour of meat can be controlled if the numerous factors that affect it are understood (Boles & Pegg, n.d). Some components such as breeding, animal species, genetic composition, age, gender, differences in muscle reducing systems and metabolic type, nutrition, pre-slaughter and handling procedures can influence the colour of meat. In addition to that, some *post mortem* (pm) technological aspects, such as electrical stimulation, period of ageing, cooling rate, muscle temperature, the degree of freezing, and lighting and packaging conditions are also found affecting the colour of meat (Gagaoua *et al*., 2015).

2.1.1.1 Oxidation/reduction state of myoglobin

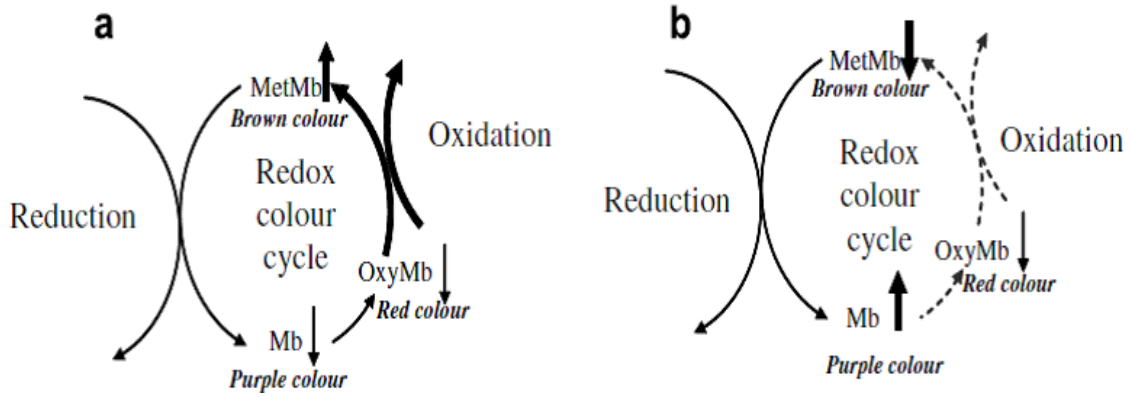
Myoglobin (Mb) is the heme protein accountable for the colour of meat. The oxidation of the central iron atom inside the heme group is accountable for discoloration of meat, a change from red oxymyoglobin (OxyMb) to brownish metmyoglobin (MetMb) (Bekhit & Faustman, 2005), because Mb is commonly found in three forms: OxyMb, deoxymyoglobin (DeoxyMb), and MetMb, the relative proportions of which determine the colour of fresh meat. The type of ligand bound to the heme prosthetic group determines the oxidative forms of Mb. The DeoxyMb state occurs when no O_2 ligand is bound to the myoglobin, resulting in a purplish-red or purplish pink colour. These colours result if meat is vacuum-packaged directly after cutting. Very low oxygen tension is required to maintain myoglobin in a deoxygenated state (Richards *et al*., 2002). Figure 2.2 represents a summary of the oxidation-reduction reactions of myoglobin in aerobic and anaerobic conditions and the formation of OxyMb, DeoxyMb and MetMb.

Metmyoglobin, which is the oxidised form of Mb, is the most stable of the three mentioned, and is the primary cause of deterioration and discount seen in the retail case, as well as consumer rejection of product. The accumulation of MetMb on the surface of meat depends on several mechanisms, which includes the rates of oxygen diffusion and oxygen consumption, the autoxidation of the pigment in the presence of oxygen, and the enzymatic reduction of MetMb. In living muscle, the concentration of MetMb is very low, due to the presence of the enzyme metmyoglobin reductase. The enzyme is very effective in the conversion of Fe^{3+} in the heme prosthetic group of MetMb back to the Fe^{2+} of normal Mb, which is facilitated by the cofactor NADH and the coenzyme cytochrome b_5 (discussed below in detail). In meat, which is dead muscle, the normal processes of removing MetMb are prevented from affecting this repair, or the rate of MetMb formation exceeds their capacity, such that there is a net accumulation of MetMb as the meat ages (Mancini & Hunt, 2005; Abril *et al*., 2001).

Metmyoglobin has been studied a lot and has been measured as one of the indicators of meat colour stability. Understanding what makes one muscle convert more Mb to MetMb is a very important aspect to evaluate meat colour stability. Apart from the biochemical factors that can affect meat colour, other retail properties also play a critical role in determining the shelf life of meat, including temperature and packaging (Jeremiah

& Gibson, 2001). The authors evaluated the effect of storage period and temperature on colour, reporting that both OxyMb and redness were lost progressively during storage and display and the loss was progressively lower as storage temperatures decreased. The authors also reported that surface discoloration and MetMb content increased progressively during storage and display. Samples displayed at 5°C showed the most surface discoloration, while samples stored at -1.5°C contained the least MetMb and the least surface discoloration. Studies by De Palo *et al*. (2013) compared the effect of cryovac film and weegal film on meat quality and shelf life. The results revealed that colour was influenced by packaging and storage time. Lagerstedt *et al*. (2011) evaluated the influence of vacuum and high-oxygen modified atmosphere packaging (MAP) on beef steaks, and reported that high-oxygen MAP negatively influenced colour stability and induced higher levels of MetMb compared to vacuum ageing.

McKenna *et al*. (2005) reported that discoloration of meat could be muscle- and animal-specific. The authors furthermore reported that discoloration depend on the relative proportions of red fibres, amount of lipid and oxygen consumption rate, muscles that contain greater relative quantities of red fibres, more lipids; and greater oxygen consumption rates appeared to discolour more rapidly.



[Figure 2.2: A diagram representing the chemical state of myoglobin with myoglobin under (a) aerobic and (b) anaerobic conditions. Thickening and direction (upward/downwards) of the arrow shows the degree of the reaction and the change in the products concentration. Under aerobic conditions, the net redox reactions are in favour of oxidation, henceforth the build-up of Metmyoglobin (brown). Under anaerobic conditions, viz. vacuum packaging, the net of the reactions is in favour of reduction; henceforth, the build-up of deoxymyoglobin (purple). Taken from (Bekhit *et al*., 2007).

2.1.1.2. The metmyoglobin reducing system

Metmyoglobin reducing activity (MetMbRA) is the ability of the *post mortem* muscle to regenerate ferrous oxy- or deoxymyoglobin by enzymatic and/or non-enzymatic reactions. This reducing activity of meat is critical to limiting myoglobin oxidation (Elroy *et al*., 2015). Quite a number of studies have demonstrated the importance of the MetMb reductase and oxygen consumption rate in meat colour stability. The enzyme (metmyoglobin reductase) helps to convert MetMb back to myoglobin (Gao *et al*., 2014).

Metmyoglobin reduction systems which includes enzymatic and non-enzymatic reducing systems are summarised in Figure 2.3 (Bekhit & Faustman, 2005). Microsomal NADH-driven electron transfer system which includes NADH-cytochrome b5 reductase and cytochrome b5 the best characterised enzymatic MetMb reducing system is the (Arihara *et al*., 1995). It has been reported that in live animals, the role of the NADH-cytochrome b5 reductase system is to reduce the physiologically inactive MetMb to its natural form, which is myoglobin. In vitro studies by Echevarne *et al*., (1990) using the isolated enzyme indicated that cytochrome b5 reductase activity is reserved in the existence

of adequate quantities of NADH. Nevertheless, NADH quickly deteriorates in muscle/meat *post mortem* owing to the loss of substrates and cofactors, the loss of structural integrity and functional properties of mitochondria and *post mortem* pH decline (Echevarne *et al*., 1990; Renerre & Labas, 1987) and thus, it might be the main limiting factor. If MetMb reduction is to happen in meat, then adequate amounts of NADH must be presented, therefore the potential for NADH regeneration in meat to facilitate MetMb reduction has attracted some interest since the early 1970s (Giddings, 1974).

Several methods have been used for measurement of the MetMbRA. Contradictory results have been reported by several studies on the role of the MetMbRA in the maintenance of fresh meat colour (Reddy & Carpenter, 1991; Echevarne *et al*., 1990; Lanari *et al*., 1995 Sammel *et al*., 2002a). Sammel *et al*. (2002a) reported that the more MRA a muscle retains, the better its colour stability will be. Several methods have been used for the quantification of MetMbRA (Sammel *et al*., 2002a), but only a few studies have actually established a relationship between MRA in meat and its colour stability.

In the mid-60s, Stewart *et al*. (1965) measured Mb reducing ability by oxidising meat with potassium ferricyanide, followed by reduction of the pigment spectrophotometrically. O'Keeffe and Hood (1982) and Renerre and Labas (1987) found differing MRAs among muscles, but were unable to find a significant correlation between MRA and colour stability. Both these methods were criticised for the use of ferricyanide as oxidant, because potassium ferricyanide facilitates electron transfer and forms a complex with Mb when used to oxidise pigments; therefore, true MetMb reduction capacity may not be measured (Faustman & Cassens, 1990). Other methods for determining MRA includes the aerobic reducing ability (ARA), reduction of DCPIP (dichlorophenolindophenol) by MetMb reductase, reduction of nitric oxide MetMb, total reducing activity (TRA), and the reduction of horse and bovine metmyoglobins as reported by Sammel *et al*. (2002a). The authors found the ARA to be best correlated with colour stability over display time, however, the method was criticised in the early 1990s due to the induction of high quantities of MetMb on the surface of meat, which may not relate to natural development of MetMb (Faustman & Cassens 1990). In support of ARA, studies by Sammel *et al*. (2002b), Hutchins, Liu, & Watts, 1967 and Ledward (1972; 1986) have reported a strong

link between MetMbRA and meat colour stability. The reduction of DCPIP was also found to correlate with objectively and instrumentally measured fresh meat colour stability.

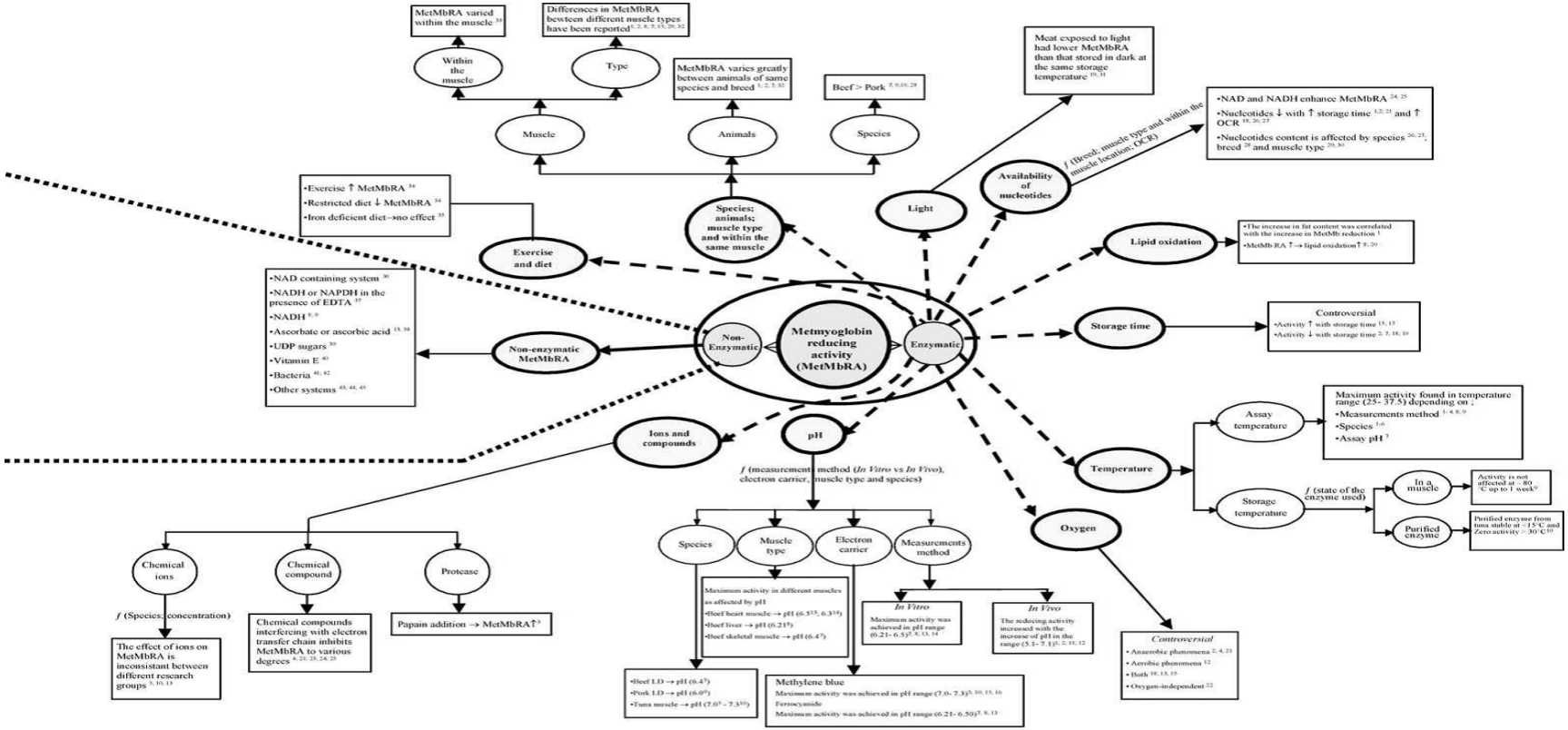
2.1.1.3 Lipid oxidation

Several studies have reported a very good relationship between meat discoloration and lipid oxidation (Faustman *et al*., 2010; Gao *et al*., 2014). It is believed that lipid oxidation occurs because of myoglobin oxidation and that myoglobin oxidation can enhance lipid oxidation and vice versa. This has been reported particularly in beef, because beef has been demonstrated to contain the strongest peroxide forming potential (PFP). The high PFP was reported to be likely due to the meat's high myoglobin levels (Yi *et al*., 2013). Hutchins *et al*. (1967) reported a good positive correlation between lipid oxidation and MetMb reduction. Zakrys *et al*. (2008) studied quality factors in beef packaged under 0%, 10%, 20%, 50% and 80% oxygen (20% CO_2, balance nitrogen). The authors reported that changes in OxyMb and *a** values seemed to be due to lipid oxidation and had a very good relationship with TBARS.

McKenna *et al*. (2005) and Gao *et al*. (2014) reported that the relationships between several endogenous factors that affect beef colour stability were characterised by decreased oxygen consumption rate and less lipid oxidation; these muscles were found to have greater colour stability. Greater concentrations of iron and myoglobin are associated with greater rates of lipid oxidation (Faustman, 1992). Faustman *et al*. (2010) have demonstrated the potential interaction between myoglobin and lipid oxidation reactions in lipid bilayers (Figure 2.4).

A variability of intrinsic factors and processing steps can make meat more prone to lipid oxidation, for example:

- A muscle with abundant red fibres will oxidise more easily and rapidly due to abundant iron and phospholipids; greater amounts of red fibres are susceptible because they contain more iron and phospholipids than muscles with a large amount of white fibres (Wood et al., 2004).



1.Cutaia and Ordal (1964)
2. Stewart *et al.* (1965b)
3. Hutchins *et al.* (1967)
4. Zimmerman and Snyder (1969)
5. Al-Shibani *et al.* (1977)
6. Matsui *et al.* (1975)
7. Reddy and Carpenter (1991)
8. Mikkelsen and Skibsted (1992)
9. Mikkelsen *et al.* (1999)
10. Pong *et al.* (2000)
11. Ledward (1970)
12. Lanier *et al.* (1978)
13. Hagler *et al.* (1979)
14. Faustman *et al.* (1988)
15. Echevarne *et al.* (1990)
16. Taylor and Hochstein (1978)
17. Feldhusen *et al.* (1995)
18. O'Keeffe and Hood (1982)
19. Zhu and Brewer (1998)
20. Hirokata *et al.* (1978)
21. Watts *et al.* (1966)
22. Ledward (1985)
23. Govindarajan *et al.* (1977)
24. Saleh and Watts (1968)
25. Kendrick and Watts (1969)
26. Atkinson *et al.* (1969)
27. Atkinson and Follett (1973)
28. Faustman and Cassens (1991)
29. Renerre (1984)
30. Renerre and Labas (1987)
31. Ledward (1992)
32. Madhavi and Carpenter (1990)
33. Sammel *et al.* (2002a)
34. Hagler *et al.* (1981)
35. Hagler *et al.* (1980)
36. Kajita *et al.* (1970)
37. Brown and Snyder (1969)
38. Fox *et al.* (1975)
39. Hariya *et al.* (1997)
40. Lynch *et al.* (1998)
41. Faustman and Cassens (1989)
42. Arihara *et al.* (1993)
43. Harada *et al.* (1986)
44.Buffinton *et al.* (1988)
45. Romero *et al.* (1992

Figure 2.3: Summary of the aspects influencing metmyoglobin reducing activity (Bekhit and Faustman, 2005)

- Because of increased surface area for oxygen, ground meat will experience greater lipid oxidation more easily than meat that is not ground (Gray et al., 1996).
- The presence of higher concentrations of unsaturated fatty acids with triacylglycerols results in rapid oxidation of fats. For example, meat from non-ruminants contains higher relative concentrations of fatty acids within triacylglycerols than meat from ruminants (Enser, et al., 1996). Campo et al. (2006) evaluated the relationship between human perceptions of lipid oxidation as determined by a taste panel to a chemical measurement of oxidation using TBARS method. The authors reported very good correlations between analytical and sensory characteristics, the TBARS were found to be a good judge of the perception of off-flavours.

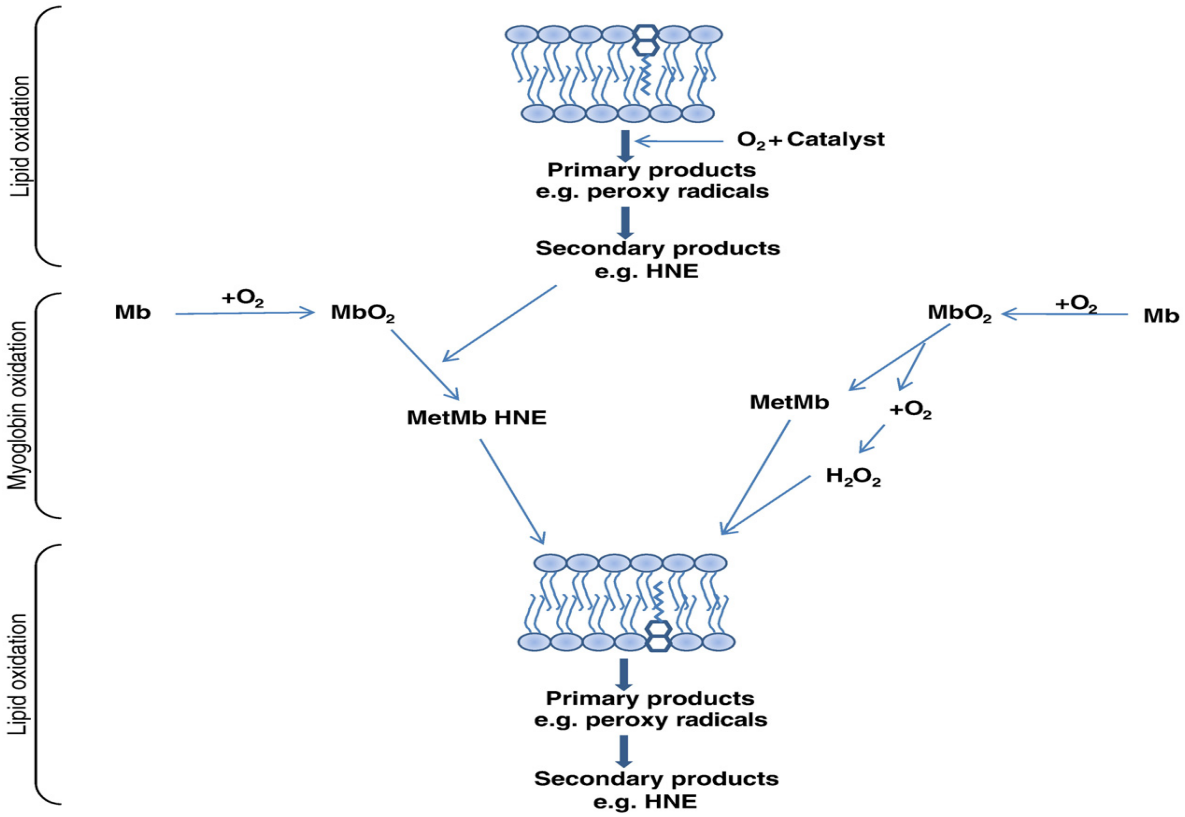


Figure 2.4: Illustrative figure of possible interrelating oxidation reactions between oxymyoglobin and unsaturated fatty acids in bilayers (Faustman *et al.*, 2010).

2.1.1.4 Ultimate pH (pHu)

Meat colour is also dependent on the pH of the meat. If the pHu is higher, there will be greater surviving rate of the cytochrome enzymes. At this point, the proteins will be beyond their iso-electric point (pI 5 iso-electric point of most muscle proteins), and much of the water in the muscle will still be linked with them, fibres will be firmly packed together therefore presenting a barrier for diffusion. As a result, the layer of OxyMb becomes smaller and the meat will become darker (dark cutting beef). The surfaces of this kind of meat will not scatter light to the same degree as will the more open surface of meat of lower pH hence; the meat becomes darker to the eye. Therefore, pH can also influence the colour stability of muscles. Reducing activity, oxygen consumption rate, and oxygen penetration depth are all influenced by changes in pH, where lower pH conditions favour myoglobin oxidation (McKenna *et al.*, 2005).

2.1.2 Measurement of meat colour

Meat colour is a significant indicator of meat quality, because it is one of the most important indicators of the key features influencing the evaluation of meat by the consumers at the point of purchase (Suman *et al*., 2014 León *et al*., 2006; Lu *et al*., 2000; Van Oeckel *et al*., 1999). Indirect and direct methods that can be used as indicators for meat colour include the Mb oxidation/reduction measurements (extraction of the OxyMb, MetMb and DeoxyMb), MetMbRA, Lipid oxidation (using TBARS), and pH measurements. Most commonly used direct methods for colour analysis include the visual analysis of surface meat colour using visual panellists, either trained or untrained. Colour can also be determined by using instruments, popular one being the Minolta CIE (L^*, a^*, b^*). The reflectance of specific wavelengths in the visible spectrum 400-700 nm has also been used and can be more accurate relative to the chemical form of myoglobin (Jacob *et al.*, 2014). The ratio of reflectance of light in the wavelengths of 630 nm and 580 nm, known as "oxy/met" has been utilised to identify chemical variations in meat that resulted from either oxygenation or oxidation of myoglobin (Hunt *et al*., 1991), and has been associated with consumer preference for colour. Nevertheless, calibration with chemical analyses is essential in order to measure the different chemical forms of myoglobin due to light reflectance being affected by other factors such as texture (Hunt *et al*., 1991).

2.1.2.1. Visual analysis

Carpenter *et al*., (2001) have reported a strong relationship between consumer purchase decisions and colour preference and with beef that is not red being discriminated against by consumers (e.g. beef that is purple or brown). Visual assessments are closely related to

consumer evaluations but they are not easy to carry out, because human evaluations may not be replicable from day-to-day, and is affected by individual preference, visual deficiencies of the eye, lighting, and other environmental factors. However, through appropriate panel management, data collection procedures and sample preparation, visual evaluations of colour can provide precise and repeatable data. Panels for colour evaluation studies can be either trained or untrained. Trained panels are descriptive, and are usually used in meat colour research because they can be regarded as objective instruments. On the other hand, consumer panels can be used to rate the acceptance and preference of a product's attributes (AMSA, 2012; Brugiapaglia *et al.*, 2011).

Several studies have reported the use of visual colour assessments of meat using panellists (Vitale *et al*., 2014; Horcada *et al*., 2013; Brugiapaglia *et al*., 2011; Sullivan *et al*., 2002; Boles *et al*., 1998; Zanardi *et al*., 1998). Vitale *et al*. (2014) evaluated the effect of ageing time on colour during display in high oxygen atmosphere packaging using a trained eight member visual panel. The authors reported that visual results correlated with colour data determined instrumentally, indicating that beef aged for extended periods (fourteen and 21 days) affected colour stability negatively when compared with un-aged beef (Day 0), or beef aged for shorter times (3, 6 and 8 days).

Horcada *et al*. (2013) evaluated meat colour in 840 veal carcasses using trained panellists and the spectro-colorimeter (CIE *L**, *a**, *b** colour system). The authors reported the best correlation between visual assessment and *L** values in carcasses of 300-320 kg, then a moderately strong relationship between visual assessment and *a** values in carcasses of 280-300 kg in mass. The association between *b** and visual evaluations was reported to be low.

Brugiapaglia *et al*. (2011) used a visual panel for the assessment of meat colour using photographs in *m. longissimus thoracis* (LT) steaks from different breeds. The authors used consumer panels and reported that consumers were able to discriminate between the samples and they reported that consumers preferred meat with higher lightness and a relatively high yellowness.

Sullivan *et al*. (2002), evaluated pork muscle samples from *m. longissimus dorsi* (LD) using a trained sensory panel. The pigs were fed on three diets supplemented with iron, vitamin E and vitamin E and iron. The authors used polystyrene packaging to age the samples in a display cabinet for five days. Results of their study revealed that the panellists were able to notice differences between the four experimental groups on each day of the study and were more accurate than instrumental evaluations in assessing the colour quality of samples, i.e. the Hunter *L**, *a**, *b** technique.

Boles *et al*. (1998) evaluated the colour of fresh veal sausages after one, three and six days respectively, stored at -1.5, 4.0 and 8.0°C respectively, using a trained sensory panel. The authors evaluated the samples on a 6-point scale for the intensity of the red colour (1-very bright red, 6-not red), brown colour concentration (1-not brown, 6-extremely brown), total colour acceptability (1-very acceptable, 6-very unacceptable). Panellists also had to evaluate if they would buy the sausage (yes/no). Results showed that panellists found sausages stored at -1.5°C suitable for consumption for at least six days, while sausages made from unsalted pre-rigor mince had better colour stability than those made from other meats.

Zanardi *et al*. (1998) also used a trained panel to evaluate the presence of brown colour of MetMb and colour stability three times a week on pork chops from animals supplemented with either vitamin E, oleic acid (sunflower oil), or copper. The authors found no significant differences between sensory scores in the rate of brown appearance in pork chops in oxygen permeable packs. Nevertheless, increased colour stability was detected in modified atmospheres (80% O_2 and 20% CO_2) for groups supplemented with 100 and 200 ppm vitamin E.

2.1.2.2 Instrumental colour

Varieties of instruments for colour analysis are available nowadays and these include calorimeters and spectrophotometers. Researchers can be able to select which instrument is suitable for meeting the objectives of a specific project based on:

- colour systems (Hunter, CIE, and tristimulus);
- illuminants (A, C, D65, and Ultralume);
- observers (2 and 10); and
- aperture sizes (0.64–3.2 cm) (Brewer *et al.*, 2001)

The CIE (*L*a*b**) (Brewer *et al.*, 2001; Von Seggern, 2005) is mostly used by researchers to measure colour. *L** - light vs dark where a low number of 0-50 indicates dark and a high number of 51-100 designates light, *a** - red vs green where a positive number indicates red and a negative number indicates green, and *b** - yellow vs blue where a positive number shows yellow and a negative number shows blue. The time post-cutting that a measurement is taken, also known as bloom time, may also affect instrumental colour values (Tapp *et al*., 2011). The CIE (*L*, a*, b**) colour measurements have been reported to correlate with visual appraisals for meat colour by numerous studies, as discussed above.

2.2 Meat tenderness

The quality of meat most valued by consumers is tenderness, mainly in the case of beef (Boleman *et al*., 1997). It is assured that meat tenderness is an extremely appreciated consumer characteristic, and therefore definition of the multiple processes that effect meat tenderness will offer indications toward improving meat quality and value (Huff-Lonergan *et al*., 2010). Tenderness is a process of meat ripening, which is reached through ageing, through which structural modifications occur in the muscle due to complex mechanisms concerning pH and ionic strength, together with the action of cellular proteolytic enzymes (Ouali, 1990).

2.2.1 Factors affecting meat tenderness and mechanisms determining meat tenderness

2.2.1.1 Breed

The genetic make-up of cattle also influences meat tenderness and thus there is major interest in genetic selection in order to decrease problems with meat tenderness variation. Koohmaraie *et al*. (1996) reported that several breeds produce more tender meat than other breeds. It has been proven in some studies (De Bruyn, 1991) that tenderness decreases as the percentage of *Bos indicus* inheritance increases. Cundiff *et al*. (1990) evaluated the tenderness of loins from feedlot-finished steers, finding that *Bos indicus* crosses were less tender than *Bos taurus* crosses. Further to this, data indicated that as *Bos indicus* content increases from 0% to 75%, the average tenderness decreased and variation in tenderness increased. These differences have been reported to be due to variations in the characteristics of calpain/calpastatin activity and their effects on the myofibrillar properties of the muscle. Ferguson *et al*. (2000) reported that tenderness of *Bos indicus* cattle significantly improved in carcasses that have been electrically stimulated. The process of electrical stimulation has become a common practice in abattoirs to help improve meat quality characteristics such as tenderness (Hwang *et al*., 2003) and drip loss, with no effect on colour (Strydom *et al*., 2005). The process causes an increase in the rate of *post mortem* glycolysis and prevents extreme muscle shortening during rigor, by speeding up the depletion of ATP (energy) and rigor onset, ensuring that full rigor is reached before muscle chill to below 10°C.

2.2.1.2. Connective tissue

Total collagen or collagen solubility also contributes to meat tenderness as collagen are the main protein components of intramuscular connective tissue which constitutes 2-6% of dry matter of muscle and about 40% of dry matter of extracellular matrix (Kotczak *et al*., 2003). The properties of collagen vary with age of the animal, where collagen becomes much less soluble in older animals. Studies by McCormick (1999) reported that collagen concentration and mature crosslinks have an added influence on the toughness of meat and this is referred to

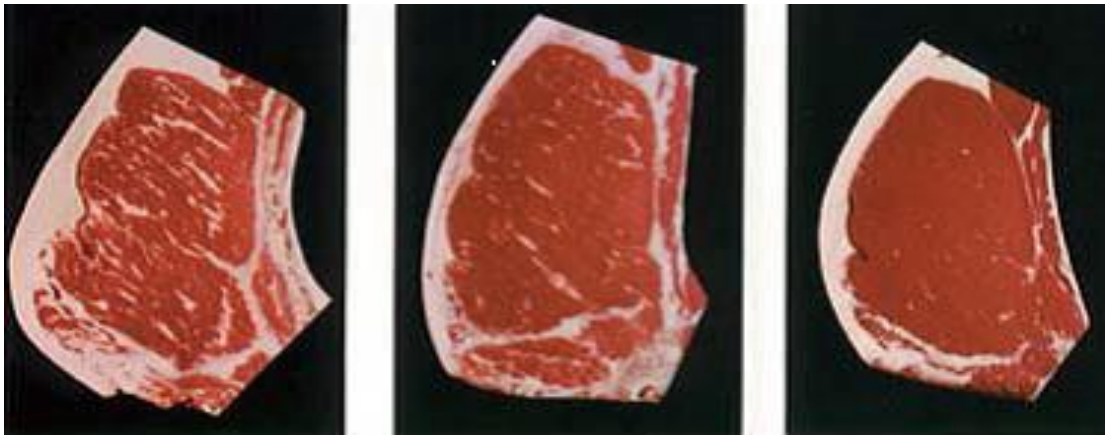
as background toughness. Background toughness is the resistance to shearing of the muscle, which is as a result of the connective tissue component of muscle (Luciano *et al*., 2012).

2.2.1.3. Intramuscular fat (IMF)

Intramuscular fat (IMF), also referred to as marbling, is the deposited fat/adipose tissue between perimysium surrounding muscle bundles. Marbling is visible to the human eye as 'flecks' or spots of fat and can be measured chemically or by giving a score. Hocquette *et al*. (2010) reported that IMF has a direct effect on juiciness and flavour, but influences tenderness indirectly. Other studies have also reported that a close relationship exists between marbling level and the palatability of red meats (Brooks *et al*., 2000; Kim and Lee, 2003). Intramuscular fat appears to separate and dilute perimysial collagen fibres and disorganise the structure of intramuscular connective tissue that contributes to increased meat toughness. It is usually known that an increased level of intramuscular fat has a positive effect on the sensory qualities of meat (Fernandez *et al*., 1999).

Together with tenderness, meat flavour is also a very important meat quality-determining factor. Meat flavour primarily relates to fatty tissue/marbling, where it was reported by Sáenz *et al*., (2008) that "the more marbling a meat cut contains, the more flavourful and tender it is and that marbling has much stronger and more predictable effects on juiciness and flavour than tenderness". Several studies have evaluated marbling in sensory evaluation studies in beef and pork (Corbin *et al*., 2015; Cannata *et al*., 2010; Platter *et al*., 2003). Corbin *et al*. (2015) evaluated varying marbling levels and quality treatments of tender beef strip loin steaks, and reported that marbling was associated with an overall liking ratings as well as acceptability percentages. The authors furthermore reported that overall liking of the steaks was highly correlated with flavour liking, as well as marbling percentage.

Cannata *et al*. (2010), evaluated the effect of visual marbling on sensory properties and quality traits of pork loin, and concluded that visual marbling score has an influence on sensory properties and pork quality. Platter *et al*. (2003) found that marbling score displayed a weak relationship to acceptance of steaks by consumers for beef strip loin steaks. Sensory studies using both trained and untrained personnel usually uses a point scale to rate the amount of marbling on the meat surface. Figure 2.5 illustrates an example of different marbling levels e.g. marbling can be evaluated as moderately abundant, moderate and slight (Dinh, 2008).



Moderately abundant Moderate Slight

Figure 2.5: Different levels of marbling, showing an example of how marbling can be rated in visual evaluation studies.

2.2.1.4 Protein oxidation

One of the most important changes that occur in *post mortem* muscle during ageing is increased oxidation of myofibrillar proteins (Rowe *et al*., 2004). Studies by Huff-Lonergan (Project Progress Report, 2004) showed that protein oxidation greatly slows meat tenderisation by limiting the proteolytic capacity of the meat.

Oxidative processes are the major causes of meat quality deterioration, such as flavour, colour, and nutritional composition (Asghar *et al*., 1988). Metabolic and other processes that occur in muscle tissue result in formation of reactive oxygen species (ROS) and other oxidative compounds, which include hydroxyl radicals, peroxyl radicals, superoxide anions, hydrogen peroxide, and nitric oxide (Burton & Traber, 1990). These reactive oxygen species can influence the quality of meat products due to their interaction with both lipids and proteins in *post mortem* muscle tissue (Rowe *et al*., 2004).

2.2.1.5 Muscle fibre detachment

Muscle is a complex structure of contractile fibres, which are bound together in bundles by thin sheets of connective tissue. There is considerable indication that calpain-mediated proteolysis of vital myofibrillar and related proteins plays a vital role in meat tenderisation (Koohmaraie, 1996; Goll *et al*., 1991). Precisely, proteolysis of these proteins is recognized result in improved fragmentation of myofibrils during ageing of meat, and thus weakening of the firm structure of the muscle results in meat that is more tender (Taylor *et al*., 1995; Ho *et al*., 1997; Taylor and Koohmaraie, 1998). Another structural change that occurs during *post mortem* ageing of muscle is the perimysium and endomysium detachment from the muscle

fibres, possibly due to proteoglycans degradation in the extracellular matrix (Hannesson *et al*., 2003).

Taylor and Frylinck (2003) reported that muscle fibre detachment and breaks across the diameter of the fibre resulted in meat tenderness (Figure 2.6 showing the location of fibres within the muscle). The authors evaluated the muscle fibre detachment and muscle fibre fractures in different cattle breeds and reported that structures which relate to tenderness are the fibre breaks/fractures and fibre detachments and not the sarcomeres. In agreement, Veiseth-Kent *et al*. (2010) evaluated the occurrence of microstructural changes in Norwegian Red cattle, which include: fibre-fibre detachment; contracted muscle fibres; fractured muscle fibres and muscle fibre-perimysium detachment in aged *m. longissimus dorsi* muscle. The authors reported a very good relationship between fractured muscle fibres and the Warner Bratzler shear force. However, no relationship was found between the individual muscle fibres and the muscle fibres and perimysium (the connective tissue network that surrounds the fibre bundles, Figure 2.6). It has been reported that the relationship that exists between the muscle fractures and tenderness are as a result of calpain-mediated proteolysis (Taylor & Frylinck, 2003; Veiseth-Kent *et al*., 2010).

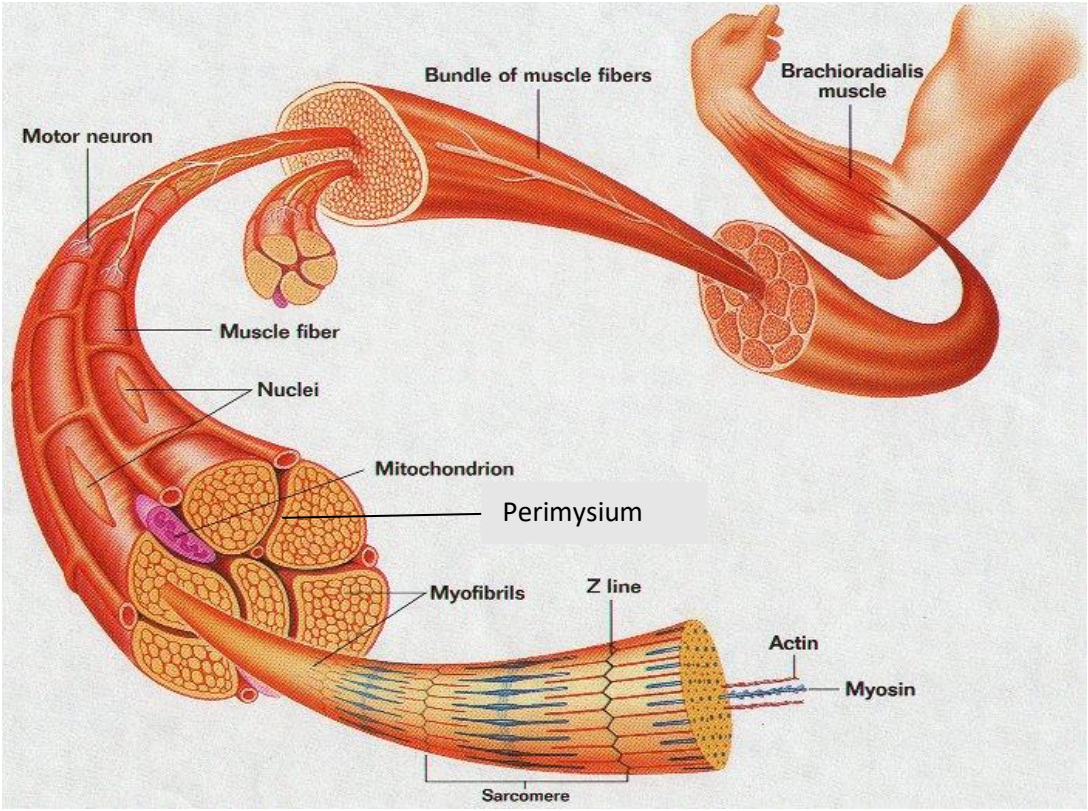


Figure 2.6: Structure of the skeletal muscle showing the different muscle structures (http://163.178.103.176/CasosBerne/3cMusculo/Caso121/HTMLC/CasosB2/ATP/amuscle.html).

2.2.1.6 Other factors that affect meat tenderness

Events prior to slaughter which might induce stress in the animal at, or on the way to lairage can have a negative effect on the ultimate pH of meat, and therefore on its overall quality. Therefore, the rate and extent of pH is one of the most important determinants of meat tenderness. Following exsanguination of the animal, the oxygen within the muscles is quickly exhausted, and the process of converting muscle to meat actually begins. It was reported that *post mortem* conversion of muscle to meat originates from leakage of Ca^{2+} within the muscle and subsequent activation of ATPases, modification of ADP concentrations, and degradation of glycogen to lactic acid through glycolysis. The degradation of glycogen to lactic acid results in acidification of the meat, therefore measurements of pH over time can give an estimate of glycolytic rate in *post mortem* muscle over time. This process is completed when muscles have depleted their energy reserves or have lost the ability to utilise remaining reserves (Lefaucheur, 2010).

ATP production is essential to keep the muscle in the relaxed state, and the permanent bonds between actin and myosin form when the level of ATP decreases, leading to the process of *rigor mortis*. During this process the muscle shortens, and there is increase in isometric tension (Lepetit *et al*., 2000). Certain studies have reported contradictory results on the relationship between muscle shortening and meat tenderness (shear force/sensory analysis). Smulders *et al*. (1990) reported that a relationship exists between the rate of *rigor* onset, sarcomere length and shear force (tenderness). Strydom (2008) and Wheeler *et al*. (2000) reported a strong negative relationship between sarcomere length and meat toughness, where shorter sarcomeres result in tougher meat. Contradictory results have been reported by Shackelford *et al*. (1994), who found a weak relationship between sarcomere length and tenderness. Sarcomere length differs between muscle fibres, because each muscle fibre goes into *rigor* at different times. Minimal shortening of sarcomere can be seen at intermediate rates of temperature decline, and if the temperature decline is too rapid and glycolysis too slow, cold shortening arises, resulting in tougher meat. In such case where the temperature fall is too low and glycolysis faster, then a process called heat shortening occurs, and also results in tougher meat (Van Oeckel *et al*., 1999;Warner *et al*., 2010).

Maltin *et al*. (2003) reported that meat with low ultimate pH (pHu) has poor tenderness because the enzymes associated with *post mortem* tenderisation are repressed by acidification, and low pHu is related to increased drip loss resulting in lower acceptable meat. The rate of glycolysis *post mortem* affect the extent of myofibrillar contraction and the rate and degree of proteolysis throughout ageing (Ferguson *et al*., 2001; Koohmaraie and Geesink, 2006), which

ultimately result in myofibrillar tenderness. The pH of the meat is therefore also significant in determining the water-holding capacity of meat, which is the ability of meat to hold its water during application of external forces such as cutting, heating, grinding, or pressing.

2.2.1.7 Water holding capacity (WHC) and drip/ juiciness

Water holding capacity (WHC) is the ability of the meat to hold its water during application of external forces such as cooking, cutting, grinding etc. The quality of fresh meat depends to a large extent on its water holding capacity (WHC), which is very important for consumers as an important attribute when making a purchase decision (Prevolnik *et al*., 2010).

Factors that can affect the WHC of meat at *post mortem* includes pH decline, proteolysis and protein oxidation, as already mentioned in the previous sections above. Much of the water in the muscle is held in structures of the cell, including the extra- and intra-myofibrillar spaces; thus, crucial changes in the intracellular structure can influence the amount of drip lost. During rigor, when the myofibrils shrink, fluid is forced in to extra-myofibrillar spaces where it is easily lost as drip. The majority of water in muscle is held either within the myofibrils, amongst the myofibrils and amongst the myofibrils and the cell membrane (sarcolemma), amongst muscle cells and amongst muscle bundles (groups of muscle cells). Once muscle is collected from the animal, the water can change in volume and location depending on a number of factors related to the tissue itself and how the product is handled (Honikel, 2004). Faster pH decline and low ultimate pH result in the development of low water-binding capacity. Faster pH decline resulting in ultimate or near-ultimate pH, while the muscle is still warm, resulting in heat shortening of the muscle, and causing denaturation of numerous proteins, including those involved in binding water.

Apart from the meat pH, numerous biochemical and physical processes that take place during the *post mortem* period have been reported to affect meat WHC e.g chilling and ageing (Bertram *et al*., 2004). Figure 2.7 illustrates the events of early *post mortem* mechanisms of importance for changes in water distribution within muscles. The initial event that takes place is a swelling of the cells, caused by the increased intracellular osmolality *post mortem*, whereby the membrane structures are rendered nearly intact, resulting in increased intracellular water volume. Throughout the second water relocation phase, water will be ejected from the myofibrillar matrix by combined longitudinal shrinkage of the myofilament lattice, due to the continuing rigor process and lateral reduction in myofilament spacing caused by falling pH. The decreased pH forces intra-myofibrillar water into the extra-cellular space, where it becomes potential drip.

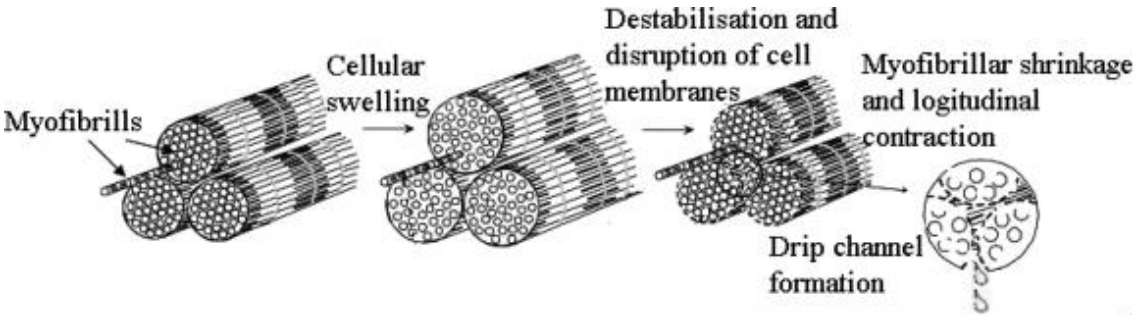


Figure 2.7: Diagram showing the processes of early *post mortem* events of significance for variations in water distribution inside muscles (Bertram *et al*., 2004).

2.2.2 Methods to determine meat tenderness

2.2.2.1 Use of objective measurements to assess meat tenderness

Objective methods for measuring meat tenderness or meat hardness include amongst others the Warner Bratzler shear force (WBSF) and the texture profile analysis (TPA) (Choe *et al*., 2011). The WBSF measurement is the most popular and accurate measurement of meat quality and yields the best correlations with sensory evaluations on cooked meat according to certain studies (Destefanis *et al*., 2008; Schönfeldt & Strydom, 2011), TPA was also found to correlate with the texture of food (Choe *et al*., 2011). Both of these methods mechanically measure the chewiness, gumminess, cohesiveness and firmness of meat.

The WBSF involves cooking meat samples and cutting cylindrical cores from the cooked meat and measuring the shear force using an Instron instrument (Destefanis *et al*., 2008; Schönfeldt & Strydom, 2011). The tests mimic how tough it is to cut through the meat with your teeth (Thompson, 2002). Shear force can be measured as the maximum force in kg required to shear a core of meat; thus, the tougher the meat, the larger the force required (Frylinck *et al*., 2009; Marais, 2007), while for TPA, the meat is cooked the same way as for WBSF and analysed using a Tetrometer (Choe *et al*., 2011). The sample is then compressed at least twice, using a small flat-faced cylinder and quantifying the mechanical parameters from the force distortion curves generated (Szczesniak, 2002). Both these tests have the advantage of being cheaper, but they are one-dimensional measurements, which can be done on cooked meat. External factors that may affect the accuracy of the test include the ‘doneness’ of the meat; the uniformity of the cylindrical sample size; the, amount of connective tissue; the final temperature of the sample; and the speed of shearing (Marais, 2007). Shackelford *et al*. (1999) developed the slice “shear” force method (SSF) and reported that it can also be used to determine beef tenderness. The SSF could be used as a rapid method to guarantee tenderness in the industry using a cooked steak (Derington *et al*., 2011) however; it has not been implemented due to cost implications.

The sarcomere length and myofibril fragment length can also be used to assess the tenderness of meat indirectly. Stolowski *et al*. (2006) reported that meat with shorter sarcomeres becomes tougher than meat with longer sarcomeres. Starkey *et al*. (2016) reported that sarcomere length accounted for some of the variation in shear force/tenderness. According to Sañudo *et al*. (2004), the increase in myofibrillar fragmentation is an indication of the amount of tenderisation that has taken place. Frylinck and Heinze (2003) found a relationship between myofibril fragment length with sensory tenderness and shear force measurements. The authors reported that proteolysis of myofibrillar proteins by collagenase leads to increased breaking down of myofibrillar proteins and decreased shear force during ageing (i.e. shorter MFLs, or higher MFI are usually related with a higher degree of proteolysis and allegedly meat tenderness) (Zhang *et al*., 2005). In contrast, Devine *et al*. (1999) reported a lack of correspondent between MFL and tenderness differences under non-electrical stimulation conditions. King *et al*. (2004) found no effect of low or high voltage electrical stimulation on MFL during extended ageing (over 14 days), although stimulated meat was more tender at one and three days *post mortem*. In contrast, Strydom *et al*. (2005) reported that MFL was a good indicator of the change in tenderness during extended ageing, but not for the early *post mortem* differences in tenderness for both electrical stimulation and non-stimulation conditions.

2.2.2.2 Use of objective methods to assess meat tenderness

Sensory evaluation is a common method for objective evaluation of meat tenderness. Sensory analysis can be evaluated by tasting of cooked meat and can be performed using trained or untrained personnel. Compared to trained personnel, untrained personnel can have a large variance in the results but the results are unbiased (Thompson, 2002). Sensory analysis methods can be very expensive, and are challenging to conduct when compared to mechanical measurements, as they involve humans (Peachey *et al*., 2002).

2.2.2.3. Relationship between instrumental and objective measurements of meat tenderness

Quite a number of studies have reported on the relationship between instrumental and objective measurements of meat tenderness. Peachey *et al*. (2002) reported the correlations between instrumental and objective measurements of meat tenderness. The authors reported that the sensory attributes such as hardness, cohesiveness and toughness correlate with meat mechanical measurements. Table 2.1 summarises results from different studies, which determined meat tenderness using the sensory and mechanical measurement (WBSF). There is variability in correlations between instrumental measures of meat tenderness and sensory evaluation from different studies, where this could be due to: the use of various types of sensory panels (either trained, semi-trained or consumer); various cooking methods (temperature, time,

and final temperature); the use of different muscles and differences in sample preparation. Recently, Colle *et al*. (2015) evaluated the influence of extended ageing on beef quality and sensory perception of steaks from *m. gluteus medius* and *m. longissimus lumborum*. Their study showed that longer ageing periods increases tenderness in both muscles. The results further showed that longer ageing periods decreased retail colour stability of the muscles but had positive effects on consumer perception for tenderness. However, inconsistent results were reported by Brewer and Novakofski (2008), who reported that consumers perceived the majority of change in tenderness occurred during the first seven days of ageing, and the change in WBSF was also similar during the first seven days of ageing.

Table 2.1: A summary of correlations between sensory analysis and mechanical analysis from different studies (Adapted from Peachey *et al.*, 2002).

	Brady and Hunecke (1985)	Crouse et al. (1985)	Dransfield et al. (1984)	Otremba et al. (1999)	Hovenir et al. (1993)	Van Oeckel et al. (1999)
Muscle source and number	Beef semitendinosis roasts (choice grade) from 16 animals	*Longissimus* muscle removed from entire (87) and castrated (75) Angus and Simmental cattle	*M. longissimus dorsi* used from 71 bulls and 84 steers	The *longissimus lumborum* and semitendinosis were used from 18 beef animals	64 *Longissimus* muscles	120 *M. longissimus thoracis* samples
Treatment	Four muscles randomly assigned to each of 60°, 70° or 80°C end point temperatures	Cattle fed either high or low energy diets slaughtered at 8, 12, 16 or 17 mo of age	Animals reared at 4 farms under intensive or semi-intensive conditions	12 Choice and 6 Select grade carcasses selected to represent a quality grade mix found in the industry (US)	From both barrows and gilts of Dutch Yorkshire and Duroc breeds	From barrows and gilts (pork) of three diverse genotypes
Sensory Evaluation:						
Level of training of panel	Trained over 9 one hour sessions	Trained	Trained and consumer	Trained following AMSA guidelines	Trained over 2 weeks	Trained
Size of panel	9 panelists	10	12	10	20	6
Size of samples	2.5 cm thick	1.25 cm × 5 cm long	3×21×2 cm	2.54×1.26 cm cubes	Not reported	
Internal temperature reached	60, 70 or 80°C	70°C	74°C	71°C	> 74°C	Grilled to 74°C
Scale used	15 cm unstructured scale	1–8 hedonic scales	Triangular and 8 category scale	8-point number scale	Unstructured line scale 0 (tough) – 100 (tender)	Hedonic scale 1–8
Instrumental: Instrument(s) used	Modified Warner–Bratzler shear blade	Warner–Bratzler shear	Volodkevich-type compression	Warner–Bratzler shear	Warner–Bratzler shear	Warner–Bratzler shear
Size of samples	Cores 2.5 cm diameter	1.25 cm × 5 cm long	1 cm × 1 cm cross section	1.27 cm diameter	1.25 cm diameter	1.25 cm diameter
Internal temperature	60°, 70° or 80°C	70°C	75°C	71°C	Cooked at 75°C for 1 h	75°C for 50 min
Correlation coefficents: sensory toughness versus …	Shear firmness: 0.668*[a]	Shear force : 0.58*	Instron compression: 0.76***	Longissimus cores parallel to fibre orientation with flat blade: 0.54* semitendinosis as above: 0.56*	Shear force: 0.50** (phenotypic) Corrected for measurement errors: 0.74**	Shear force (grilled): 0.31* Shear force (waterbath): 0.25*

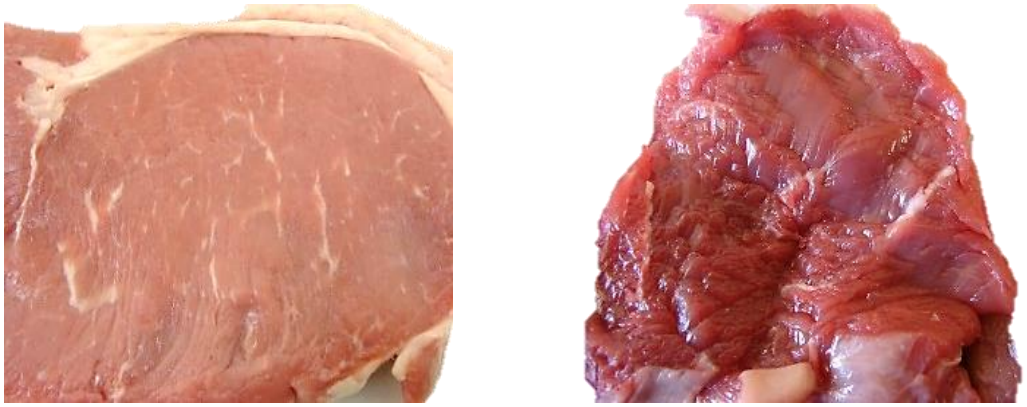
2.2.2.4 The need for other methods to determine meat tenderness

According to Belk *et al*. (1997), as cited by Howard (2010), an ideal system for prediction of beef tenderness would involve an objective, non-invasive accurate technology. These includes objective colour measurements, near infrared reflectance (NIR) (Ripoll *et al*., 2008; Andrés *et al*., 2008; Liu *et al*., 2003; Park *et al*., 1998). However, the accuracy of these technologies still remain unacceptable for overwhelming industry acceptance (Wheeler., 2000), particularly given the contradicting reports that exist regarding which values of these measurements best correlate to beef tenderness (Vote *et al*., 2003). So far, consumers rely on the visual attributes of meat, such as colour, visible fat (marbling) and odour to decide on a purchase. These visual characteristics do not indicate anything about the meat eating quality. It is therefore necessary to develop an objective, efficient, simple and rapid inspection method for beef tenderness using visual surface structural properties of meat.

The visual structural properties of meat to predict tenderness could include texture, fibre separation, and possibly the integrity of the meat structure. Texture can be described as the degree of meat fineness or coarseness, and can be measured visually, touched or chewed (Suharyanto, 2011). Although this is rather a difficult determination, it can be predicted visually, as the texture results from specified structure of muscle fibres. So far, no scientific data has been published on the visual assessment of meat texture, but studies have only reported on instrumental assessment of texture. It is therefore necessary to look in to this important meat quality attribute.

Another method, which is called the “finger method”, has been reported to be used in some abattoirs. This method uses a sense of touch to feel the meat texture, or rather, the integrity of the meat structure (Xiong *et al*., 2014).

Separation of the muscle fibres can also account for much of the tenderness differences, as illustrated in examples from Figure 2.8, where differences can be observed visually between the two steaks for the amount of fibre separation. Studies by Thiel *et al*. (1997), whereby the authors compared round and square cuts in cooked meat to measure meat tenderness, has revealed that the higher tenderness found in round cores were due to the fibre separation. So far, no studies have reported on the relationship between visual separation of the fibres and meat tenderness in raw meat. There would be an added advantage if these methods could be established for the objective analysis of meat tenderness. This can be an added advantage to the industry, abattoirs, consumers etc. as this technology would be cost effective and not time consuming.



A. No fibre separation B. Abundant fibre separation

Figure 2.8: Illustration of the amount of fibre separation between two steaks. A. As can be seen there is very little or no separation of the fibres and B, with abundant fibre separation.

The use of colour as a palatability predictor has been investigated by a number of studies (Sun *et al*., 2012;Wulf and Page 2000; Hilton *et al*., 1998;Wulf *et al*., 1997; Hodges *et al*., 1992). Some studies have reported conflicting results in the usage of colour to predict ultimate beef tenderness. Wulf and Page (2000) conducted a study to determine whether objective measures of muscle colour are useful in differentiating tender beef from tough beef. The authors reported that steaks from darker beef muscles were less tender than those from lighter beef muscles. Wulf *et al*. (1997) demonstrated that correlations of colour measurements with tenderness measurements were higher than tenderness measurements with marbling measurements. Within the CIE *L**, *a**, *b** colour space, the authors reported higher correlation measurements between *b** values with shear force measurements (r=0.38), and taste panel tenderness ratings (r=0.37). Sun *et al*. (2012) used a support vector machine to predict beef tenderness using colour image features. The authors reported that the support vector machine classifier correctly predicted the tenderness of 100% of the tender m. *biceps femoris* (BF) and *m. longissimus thoracis* (LT) steaks and 90% of the tender *m. supraspinatus* (SP) and *m. semimembranosus* (SM) steaks. However, the model was reported not to be effective for prediction of steaks classified as tough. Hodges *et al*. (1992) and Hilton *et al*. (1998) found that objective lean and fat colour scores for mature cow carcasses had a good correlation with cooked beef palatability. Objective meat colour assessments are especially effective in classifying the least palatable carcasses from palatable ones. Therefore, according to Woerner and Belk (2008), objective colour measurements are the basis in other technologies aimed to predicting beef tenderness in a non-invasive manner. Based on these reviewed studies, no study

has linked objective colour and instrumental meat tenderness. This study is necessary to close this gap.

2.3. Chapter summary

Meat colour is a very important meat attribute, as it affects meat sales and consumer purchase decisions (Jacob *et al*., 2014; Bekhit *et al*., 2007; Mancini & Hunt, 2005; Faustman & Cassens, 1991;Hunt *et al*., 1991). The colour of meat depends on the redox state of the myoglobin. Meat discoloration/colour stability depends on the accumulation of MetMb on the surface of meat (Holman *et al*., 2015). Quite a number of studies have reported on the importance of metmyoglobin reductase in maintaining the colour of meat (Gao *et al*., 2014; Sammel *et al*., 2002b). Although several methods have been used to establish the relationship between colour stability and metmyoglobin reductase, conflicting results have been reported on the role of metmyoglobin reductase in the maintenance of fresh meat colour (Reddy & Carpenter, 1991; Echevarne *et al*., 1990; Lanari *et al*., 1995; Sammel *et al*., 2002a). Therefore, there is a need to evaluate an accurate method for assessing the colour stability of meat.

Studies have reported that among all the factors that affect colour of meat, breed has a direct influence on the colour (Gagaoua *et al*., 2015). Dark meat could be attributed to species, and breed characteristics, rather than pre-and post-slaughter factors. For instance, it was observed that South African breeds like the Nguni produced darker meat than other beef breeds. On the other hand, Brahman meat may be lighter, and differ structurally from other breeds. These visual differences between South African beef genotypes and the impact on structure and therefore tenderness and juiciness have not yet received formal scientific study.

It would be an added advantage if the structural differences could be assessed visually on the surface of raw meat, and if the visual structural differences could have a relationship with objective measurements of cooked meat tenderness. So far, studies have only reported a good relationship between visual meat tenderness and the WBSF measurements on cooked meat (Brewer & Novakofski, 2008).

The use of CIE L^*, a^*, b^* colour measurements has proven to be a rapid and non-invasive technology. There are contradicting reports as to which values of these measurements best correlate to beef tenderness. The inability of an accurate colour measurement to explain differences in the factors influencing muscle tenderness demonstrate the need for a precise technology that can assess structural traits in order to enhance the accuracy of tenderness prediction.

Quite a few studies have reported on the relationship between fibre-fibre detachments and meat tenderness. Some studies have reported a good relationship (Taylor & Frylinck,

2003), while others have reported no relationship (Veiseth-Kent *et al*., 2010). Most of the measureable variations in muscle structure defined before by a lot of studies have been associated with changes in the muscle structures at the ultrastructural level (e.g., fractured myofibrils and degradation of costameres). According to Hatae *et al*. (1990), the maximum size for distinguishing structures in meat is about 100 µm. Therefore, variations that occur at the microstructural level of meat are more likely to be perceived by sensory analysis than changes at the ultrastructural level. In depth studies of these structures would help unravel the contradicting information around these structures, and assist in determining their relationship with tenderness perception.

CHAPTER 3
MATERIALS AND METHODS

The project was conducted at the feedlot and the Meat Science Centre of the Agricultural Research Council Animal Production Institute (ARC-API) at Irene, South Africa. The study was conducted in two phases, with Phase 1 being an exploratory phase and to implement knowledge gleamed from first phase to improve the second phase. The following cattle genotypes were studied: *Bos indicus* (Brahman), Sanga type (Nguni), British *Bos taurus* (Angus), European *Bos taurus* (Charolais) and the composite (Bonsmara). A total number of 50 animals were included in each phase of the study, which included between 9 to 11 replicates per genotype. All experimental animals were the progeny of registered bulls and pure bred cows, which were purchased from stud cattle breeders registered at the respective breeders' associations.

3.1 Raising of animals and feedlot procedures:

All the animals received a Revalor® H growth implant in accordance with general feedlot practices in South Africa. The animals were housed in pens holding 10 animals per pen, feed and clean fresh water was available at all times. Each animal was provided 10 m^2 and 50 cm of feed-bunk space. A standard type of high concentrate diet was supplied to the animals (12 MJ/kg DM, 13.5% protein), to which they were adapted to in a three-week period, from high levels of hay (15%) to low levels of hay (6%). Animals were weighed at two-weekly intervals and daily health observations about animal morbidity, consistent breathing and manure consistency were made. All these actions were necessary to comply with the rules of the Ethics Committee of the ARC (ARC AEC-I 2010 001). The weaners were fed a feedlot diet for a period of between 90-110 days depending on their readiness for slaughter. The weight gain and feed intake of the animals were monitored. As soon as the animals reached a visually optimum degree of carcass fatness, they were slaughtered at the ARC-API research abattoir over a period of 10 weeks to accommodate all the tests in the given time *post mortem*, and not to overload the sensory panel capacity.

3.2 Procedures on slaughter day (SL-D)

Animals arrived at the abattoir the day before they were slaughtered. Clean water was provided at all times. The animals were slaughtered at an age of 10 to 12 months, so as to produce carcasses in the A-age class and fatness class two to three of the current South African Beef Carcass Classification System. The animals were slaughtered over a period of 10 weeks, according to the experimental layout highlighted in Table 3.1.

After stunning and exsanguination, the carcasses were dressed and split in half and the left sides were electrically stimulated (ES), (400 V peak, 5 ms pulses at 15 pulses per second, for 15 seconds) within 30 minutes post-stunning. These carcass halves were then chilled at 0-5°C within one hour post-stunning, for at least 18 hours, thereby simulating ideal commercial slaughter conditions. The right sides were placed in a 10°C controlled cold room until the loin pH reached about six, after which the carcasses were place in a chiller at 0-5°C to prevent cold toughening (NS carcasses). Temperature and pH were measured at one, three, and six hours from the *m. longissimus dorsi* muscle until the pH reached at least 5.8, and then at 20 hours *post mortem* (pH_u). Samples for analysis of muscle energy were taken at one hour and 20 hours for Phase 1. From results obtained in Phase 1, it was observed that there were larger differences between muscle energy status at one hour and 20 hours, respectively. Therefore, for Phase 2, muscle energy samples were taken at one, three, six and 24 hours. Samples for other analysis were taken from the *m. longissimus dorsi* at 20 hours *post mortem*.

3.3 Sampling and ageing

Sampling was done at 20 hours *post mortem*. Both the left and right side carcasses were sampled at the *m. longissimus dorsi* (Figure 3.1) and the muscle was cut in to steaks as shown in Figure 3.2. Two retail procedures were simulated for ageing of the steaks. The steaks were aged for three and nine days *post mortem* on polystyrene plates, covered with polypropylene cling wrap (PP) at 6°C in a display cabinet (Figure 3.3), and the other steaks were aged for 14 and 20 days *post mortem* in vacuum bags at 1-4°C in a cold room (Figure 3.4) for Phase 1. Based on the lipid peroxidation results as measured by the TBARS assay, the day nine steaks had higher TBARS and the steaks had already started to develop moulds, discolouration and a bad odour. Therefore, for Phase 2, the steaks were aged for three days *post mortem* on polystyrene plates covered with polypropylene cling wrap (PP) at 6°C in a display cabinet and the other steaks were aged for nine, 14 and 20 days *post mortem* in vacuum bags at 1-4°C in a cold room. All the analyses were conducted on all packaged and aged steaks (left/electrically stimulated and right side/non-electrically stimulated).

Table 3.1: Experimental layout for Phase 1 and Phase 2

Week no.	Sunday		Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1				SL-D1	1 d pm	2d pm	3d pm	4d pm
					SAMP1		SEN11	
2	5d pm		6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
			SEN12		SEN13			
				SL-D2	1 d pm	2d pm	3d pm	4d pm
					SAMP2		SEN21	
3	12d pm		13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
				SEN14		SEN15		
	5d pm		6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
			SEN22		SEN23			
				SL-D3	1 d pm	2d pm	3d pm	4d pm
					SAMP3		SEN31	
4	19d pm		20d pm					
			SEN16					
	12d pm		13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
				SEN24		SEN25		
	5d pm		6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
			SEN32		SEN33			
				SL-D4	1 d pm	2d pm	3d pm	4d pm
					SAMP4		SEN41	
5	19d pm		20d pm					
			SEN26					
	12d pm		13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
				SEN34		SEN35		
	5d pm		6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
			SEN42		SEN43			
				SL-D5	1 d pm	2d pm	3d pm	4d pm
					SAMP5		SEN51	
6	19d pm		20d pm					
			SEN36					
	12d pm		13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
				SEN44		SEN45		
	5d pm		6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
			SEN52		SEN53			
				SL-D6	1 d pm	2d pm	3d pm	4d pm
					SAMP6		SEN61	
7	19d pm		20d pm					
			SEN46					
	12d pm		13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
				SEN54		SEN55		
	5d pm		6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
			SEN62		SEN63			
				SL-D7	1 d pm	2d pm	3d pm	4d pm
					SAMP7		SEN71	

8	19d pm	20d pm					
		SEN56					
	12d pm	13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
			SEN64		SEN65		
	5d pm	6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
		SEN72		SEN73			
			SL-D8	1 d pm	2d pm	3d pm	4d pm
				SAMP8		SEN81	
9	19d pm	20d pm					
		SEN66					
	12d pm	13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
			SEN74		SEN75		
	5d pm	6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
		SEN82		SEN83			
			SL-D9	1 d pm	2d pm	3d pm	4d pm
				SAMP9		SEN91	
10	19d pm	20d pm					
		SEN76					
	12d pm	13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
			SEN84		SEN85		
	5d pm	6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
		SEN92		SEN93			
			SL-D10	1 d pm	2d pm	3d pm	4d pm
				SAMP10		SEN101	
11	19d pm	20d pm					
		SEN86					
	12d pm	13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
			SEN94		SEN95		
	5d pm	6d pm	7d pm	8d pm	9d pm	10d pm	11d pm
		SEN102		Sen103			
12	19d pm	20d pm					
		SEN96					
	12d pm	13d pm	14d pm	15d pm	16d pm	17d pm	18d pm
			SEN104		SEN105		
13	19d pm	20d pm					
		SEN106					

SL-D= slaughter days (10 over 10 weeks);
Five animals per week – one of each genotype, ES and NES treatments = 10 units that must be judged per sensory analyses session (SEN);
Six ageing periods to study (d pm= days *post mortem*) = SEN1 to SEN6;
SEN1=3 d pm – PP wrap, SEN2=6 d pm – PP wrap, SEN3=8 d pm – PP wrap;
SEN4=14 d pm – VP, SEN5=16 d pm – VP, SEN6=20 d pm.
Two presentation treatments: PP = polystyrene plates covered with polypropylene cling wrap and VP = vacuum packaged, then displayed normally.

Figure 3.1: Picture of carcasses showing the *m. longissimus dorsi* muscle where pH, temperature and samples were taken. The green circle indicates the spot of pH and temperature measurements (Photo by Dr Lorinda Frylinck, © ARC, 2012).

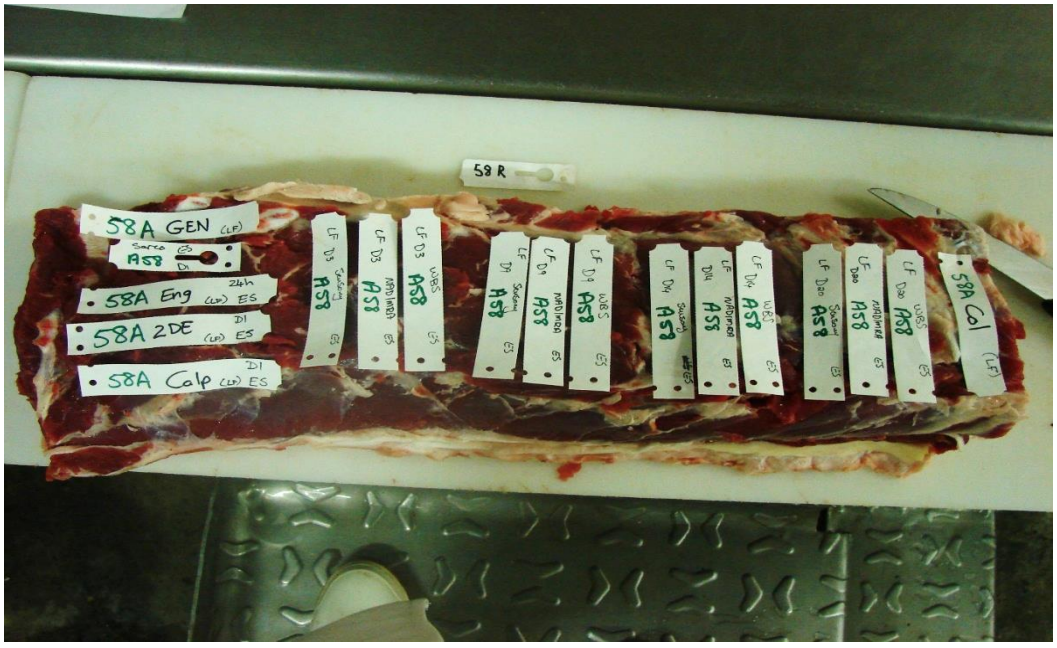


Figure 3.2: The *m. Longissimus dorsi* muscle, cut from the left side carcass (electrically stimulated) and indication of how the different steaks for analysis were taken (Photo by Dr Lorinda Frylinck, © ARC, 2012).

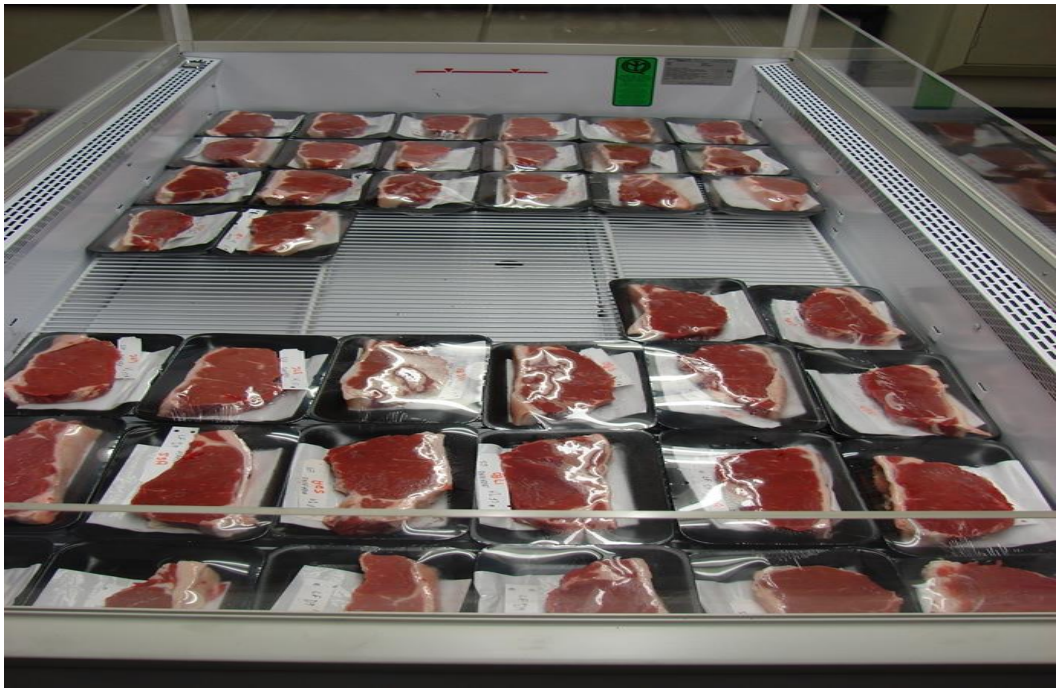


Figure 3.3: Steaks that were aged in display cabinet at 3 days *post mortem* at 6°C (Photo by Dr Lorinda Frylinck, © ARC, 2012).

Figure 3.4: Steaks that were aged in vacuum bags at 9, 14 and 20 days *post mortem* at 4°C (Photo by Dr. Lorinda Frylinck, © ARC, 2012).

3.4 Sensory panel analyses

In order for the sensory analysis and other analysis of fresh meat samples not to overlap with the slaughter days, an experimental layout was used (Table 3.1). The samples were evaluated according to the methods described in the Annual book of ASTM Standards (ASTM, 1989). The sensory analysis facilities were constructed with all the elements necessary for an efficient sensory program according to ASTM design guidelines for sensory facilities.

Visual analysis was evaluated by a 10 member trained sensory panel at the ARC-Animal Production Institute, Meat Science laboratory using fresh samples (Figure 3.5 shows example of two members of the sensory panel during evaluation). A visual analysis form was developed internally and it was used for visual analysis of the different attributes (Table 3.2 and 3.3). The Phase 1 visual analysis form was adjusted for Phase 2 to make it easier for the panel by removing observations that were deemed unnecessary in the first phase. Posters (reference guides – © ARC 2012) were developed internally to assist the panel during analysis of meat colour (Figure 3.6), marbling (Figure 3.7), fibre separation (Figure 3.8) and structural integrity (Figure 3.9). The steaks were allowed to bloom for one hour prior to visual observations, after which they were evaluated for colour using the "Guidelines for Meat Colour Evaluation" (Hunt *et al*., 1991). The colour was not evaluated on a scale, but according to different defined colours as follows for Phase 1:

- 1 = Brownish-orange
- 2 = Greyish-brown
- 3 = Pink-brown
- 4 = Pale-pink
- 5 = Pink
- 6 = Light cherry red
- 7 = Cherry red
- 8 = Dark red
- 9 = Very dark red to purple
- 10 = Green

Due to the modifications, Phase 2 steaks were evaluated for colour as follows:

- 1 = Brown-orange
- 2 = Brown
- 3 = Pale pink
- 4 = Light pink

- 5 = Pink
- 6 = Light cherry red
- 7 = Red
- 8 = Dark red
- 9 = Purple red

The other visual attributes were evaluated on different scales. Marbling was evaluated as follows for Phase 1:

- 1 = Practically devoid of marbling
- 2 = Very slight amount
- 3 = Slight amount
- 4 = Moderate amount
- 5 = Fair amount
- 6 = Fairly abundant
- 7 = Very abundant

Modifications were made for this attribute for Phase 2 as follows:

- 1 = Practically devoid of marbling
- 2 = Slight
- 3 = Small
- 4 = Modest
- 5 = Moderate
- 6 = Slight abundant
- 7 = Moderately abundant
- 8 = Abundant

Surface texture was evaluated as follows for both Phase 1 and Phase 2:

- 1 = Very smooth (can hardly distinguish fibre bundles)
- 2 = Smooth can distinguish fibre bundles
- 3 = Slightly coarse (fibre bundles well distinguished)
- 4 = Moderately coarse (distinguish fibres within bundles)
- 5 = Coarse (can see fibres within bundles well)
- 6 = Very coarse (rough)

Figure 3.10 shows examples of how steaks were analysed by the sensory panel for fibre separation. The figures show the different levels of fibre separation. Figure 3.10 A shows that the fibres have slight separation, whereas Figure 3.10 B shows the fibres have pulled apart. Fibre separation was evaluated as follows for both Phase 1 and Phase 2:

- 1 = No separation (fibres fit tightly together
- 2 = Slight separation (can just see separation between fibre bundles)
- 3 = Moderate separation (separation of fibres and bundles more noticeable)
- 4 = Slightly abundant separation (Fibres are starting to pull apart)
- 5 = Abundant separation (fibres structure is pulling apart)

Structural integrity was evaluated by giving the steak a slight finger press, and scored as follows, for both Phase 1 and Phase 2:

- 1 = Firm
- 2 = Compressible
- 3 = Soft
- 4 = Very soft

Figure 3.5: Some of the sensory panel members during analysis of fresh steaks with some of the reference posters hung on the wall (Photo by Dr Lorinda Frylinck (© ARC), 2012).

Table 3.2: Form (© ARC, 2012) used for Phase 2 visual analysis of the different attributes (Phase 2)

Please evaluate the following samples of beef for the designated characteristics (please take your time, especially to identify small differences).

Characteristics	Rating scale	Sample code									
Meat/muscle colour – there is no real ideal – maybe cherry red, but it is seldom achieved.	1 = Brownish-orange (frozen meat) 2 = Brown 3 = Pale pink 4 = Light pink 5 = Pink 6 = Light cherry red 7 = Red 8 = Dark red 9 = Purple red In the case of two-toning, please indicate: most dominant colour using above scale secondary colour using above scale										
Marbling (Visible intra-muscular fat)	1 = Practically devoid of marbling 2 = Slight 3 = Small 4 = Modest 5 = Moderate 6 = Slightly abundant 7 = Moderately abundant 8 = Abundant										

Characteristics	Rating scale	Sample code									
Visual surface texture **Tip: if you look at the meat from an angle, would you see more shade?**	1 = Very smooth (can hardly distinguish fibre bundles) 2 = Smooth (can distinguish fibre bundles) 3 = Slightly coarse (fibre bundles well distinguished) 4 = Moderately coarse (distinguish fibres within bundles) 5 = Coarse (can see fibres within bundles well) 6 = Very coarse (rough)										
Fibre separation	1 = No separation (fibres fit tightly together) 2 = Slight separation (can just see separation between fibre bundles) 3 = Moderate separation (separation of fibres and bundles more noticeable) 4 = Slightly abundant separation (Fibres are starting to pull apart) 5 = Abundant separation (fibre structure is pulling apart) 6 = Fibre structure is falling apart										
Structural integrity	1 = Stiff/hard 2 = Compressible 3 = Soft 4 = Very soft										

Table 3.3. Form (©ARC 2012) used for Phase 1 visual analysis of the different attributes (Phase 1).

Please evaluate the following samples of beef for the designated characteristics. Please take your time especially to identify small differences.

Characteristics	Rating scale	Sample code									
Meat/muscle colour – there is no real ideal – maybe cherry red, but it is seldom achieved.	1 = Brownish-orange 2 = Greyish-brown 3 = Pink-brown 4 = Pale pink 5 = Pink 6 = Light cherry red 7 = Cherry red 8 = Dark red 9 = Very dark red to Purple 10= Green (If patches of discoloration-give more than one number)										
Marbling (Visible intra muscular fat)	1 = Practically devoid of marbling 2 = Very slight 3 = Slight amount 4 = Moderate amount 5 = Fair amount 6 = Fairly abundant 7 = Very abundant										

Characteristics	Rating scale	Sample code									
Visual surface texture **Tip: If you should look at the meat from an angle, would you see more shade?**	1 = Very smooth (can hardly distinguish fibre bundles) 2 = Smooth (can distinguish fibre bundles) 3 = Slightly coarse (fibre bundles well distinguished) 4 = Moderately coarse (distinguish fibres within bundles) 5 = Coarse (can see fibres within bundles well) 6 = Very coarse (rough)										
Fibre separation	1 = No separation (fibres fit tightly together) 2 = Slight separation (can just see separation between fibre bundles) 3 = Moderate separation (separation of fibres and bundles more noticeable) 4 = Slightly abundant separation (Fibres are starting to pull apart) 5 = Abundant separation (fibre structure is pulling apart) 6 = Fibre structure is falling apart										
Structural integrity	1 = Firm 2 = Compressible 3 = Soft 4 = Very soft										

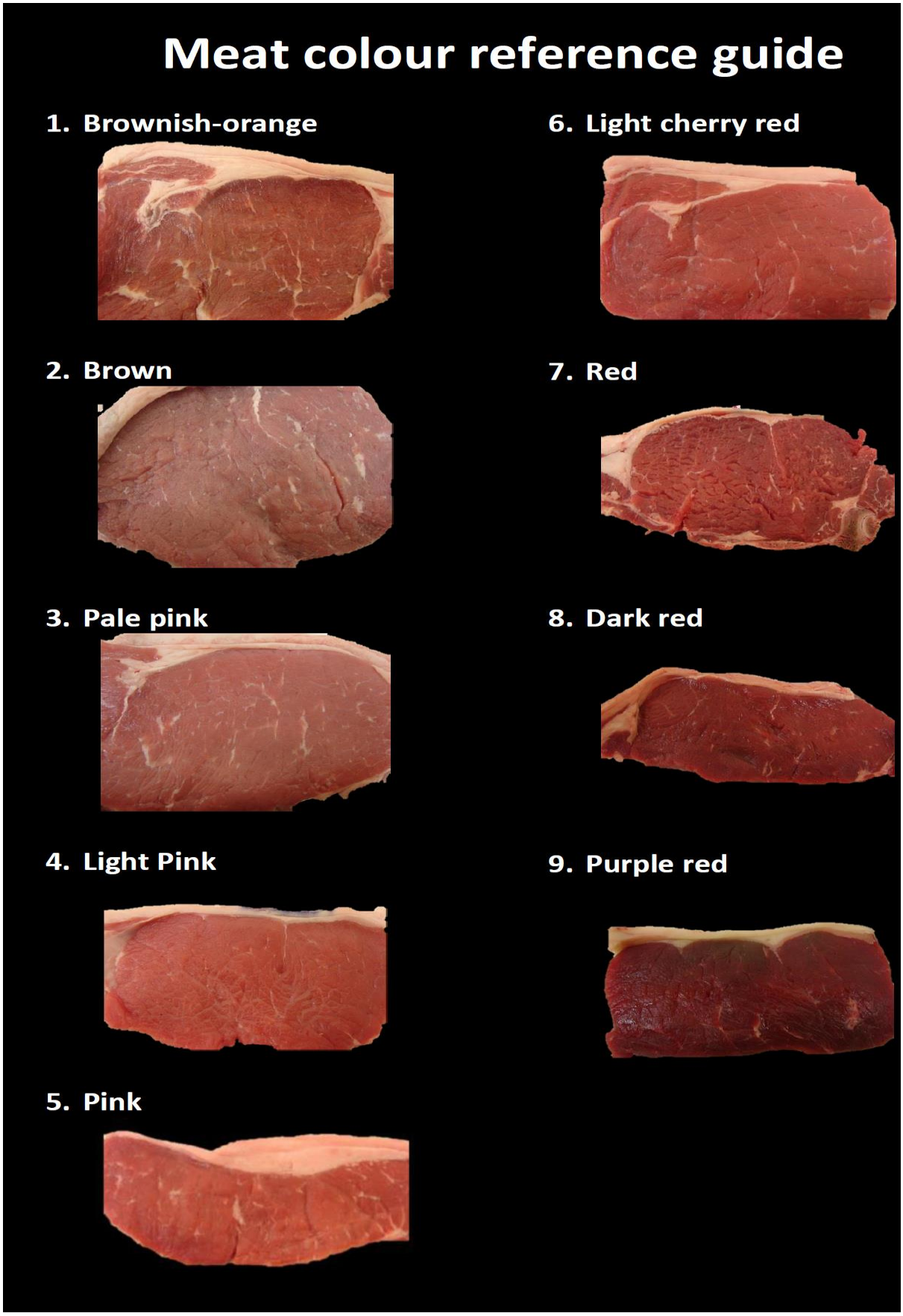


Figure 3.6: Meat colour reference guide - © ARC 2012

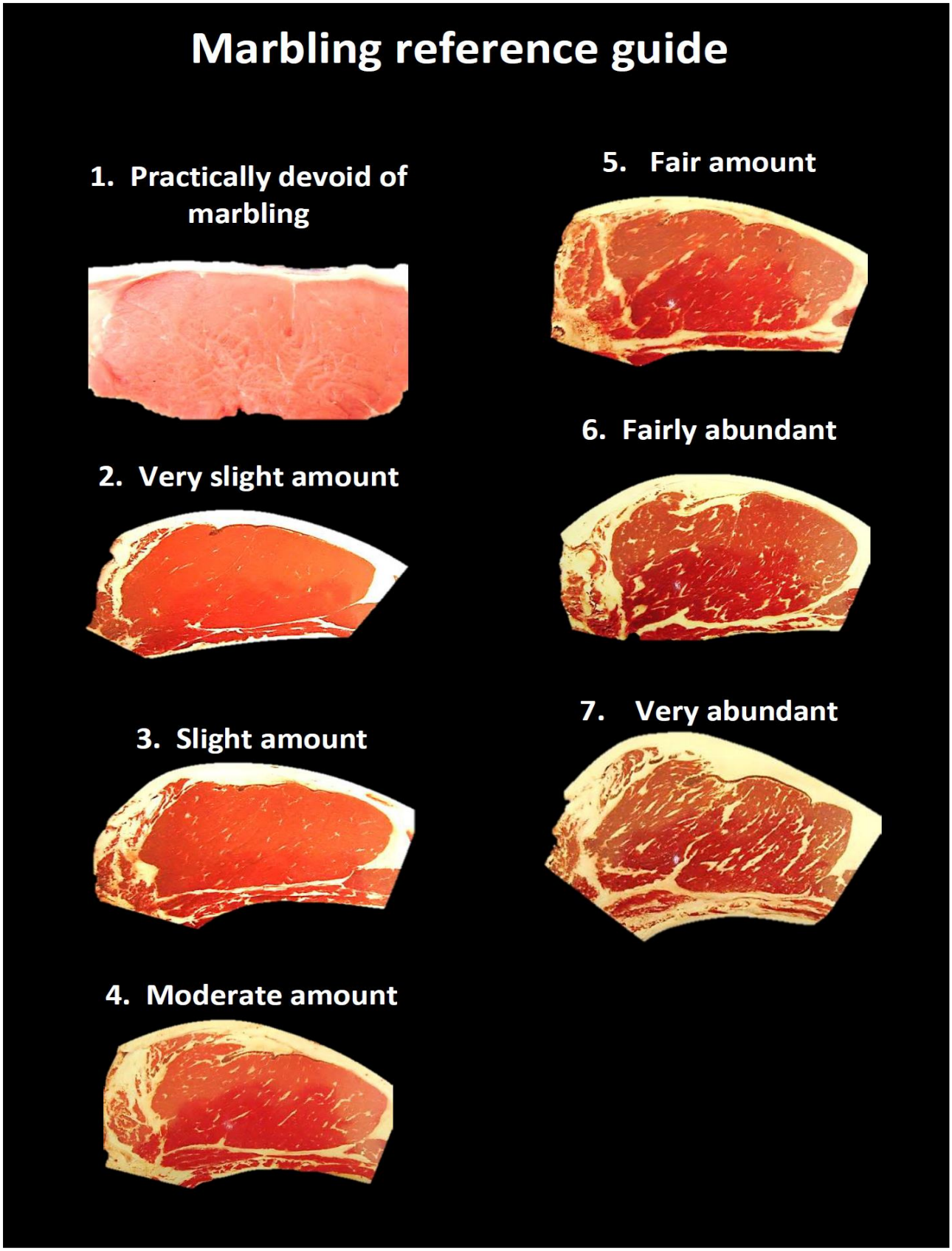


Figure 3.7: Marbling reference guide - © ARC 2012

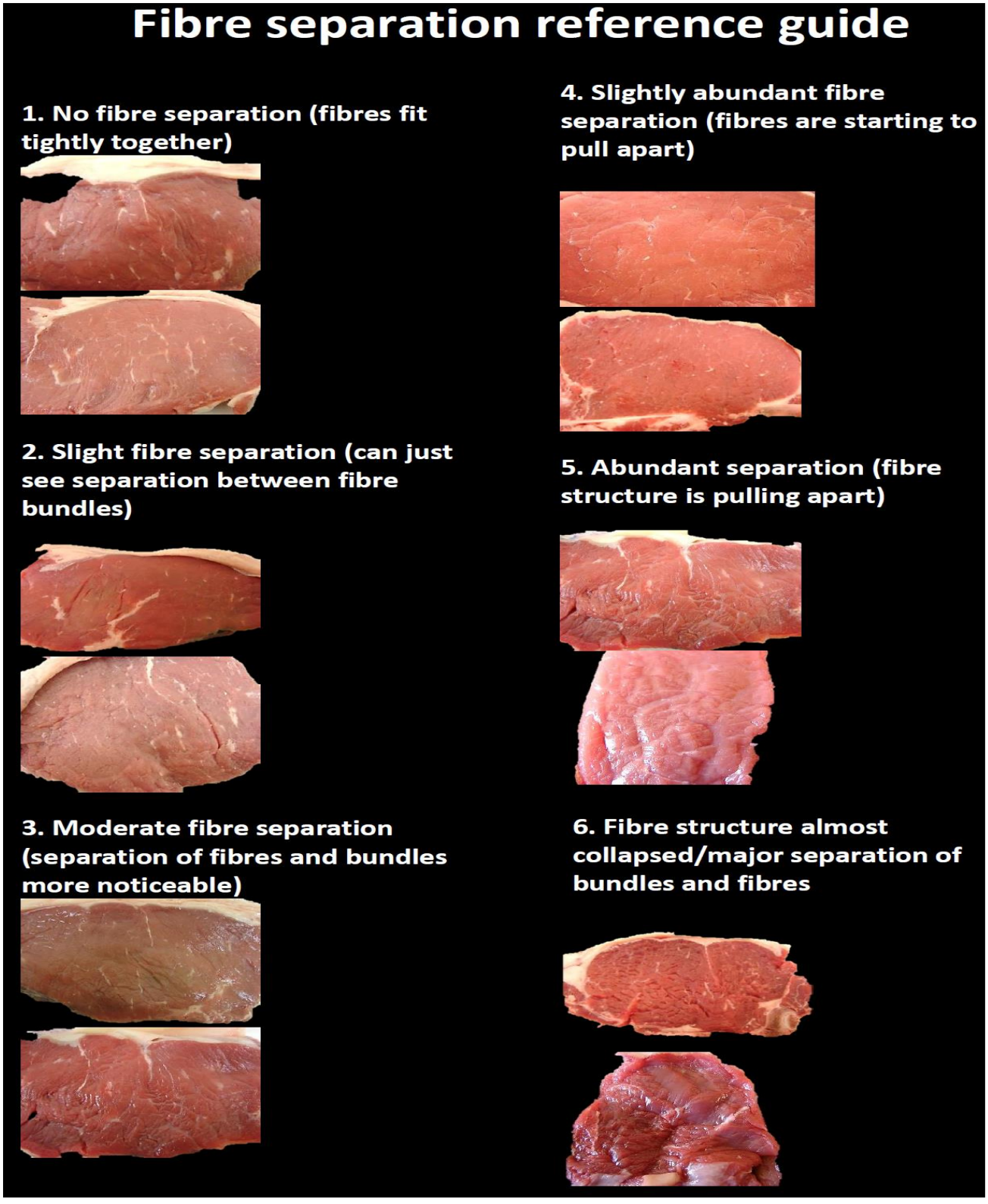


Figure 3.8: Fibre separation reference guide – © ARC 2012

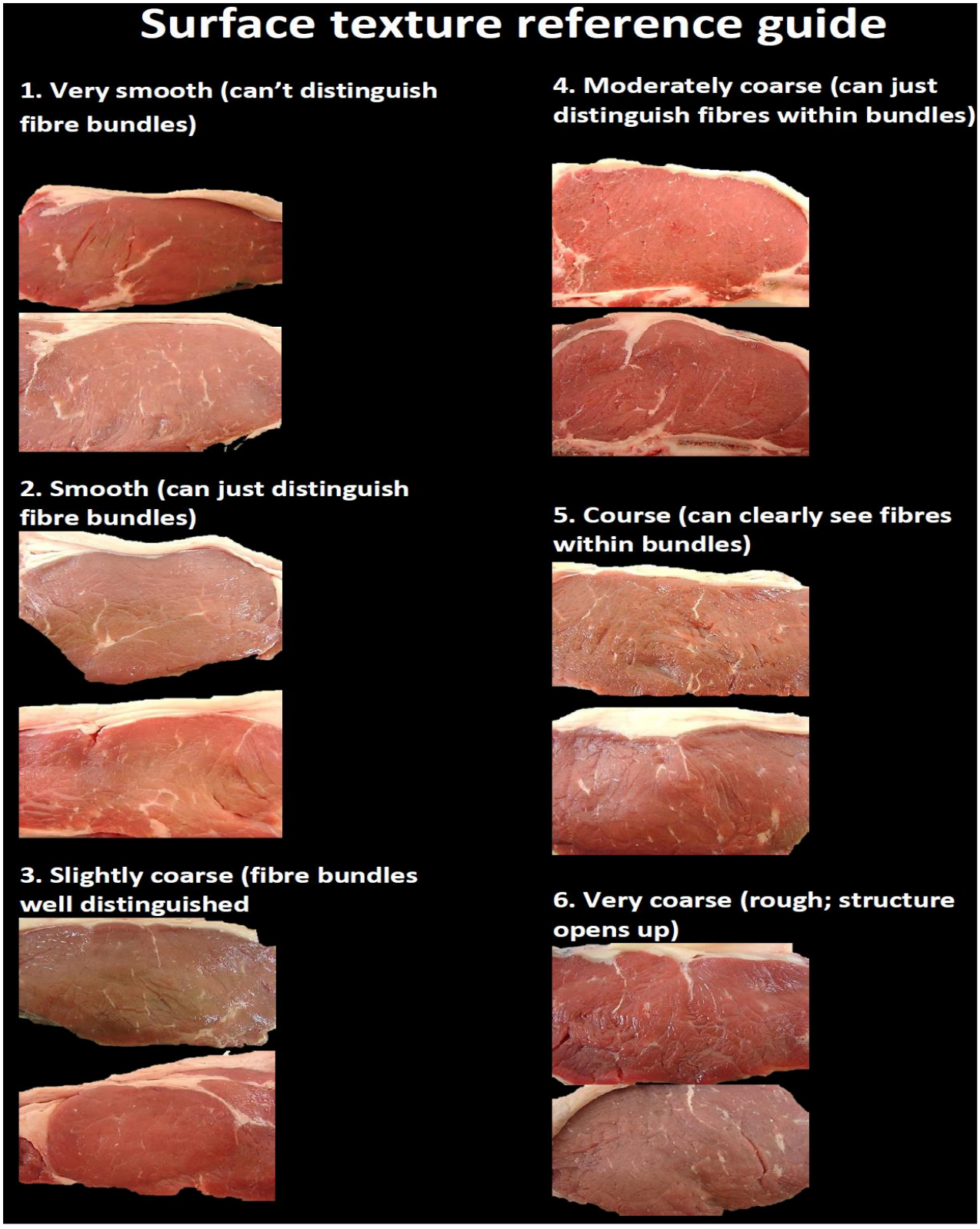


Figure 3.9: Surface texture reference guide – © ARC 2012

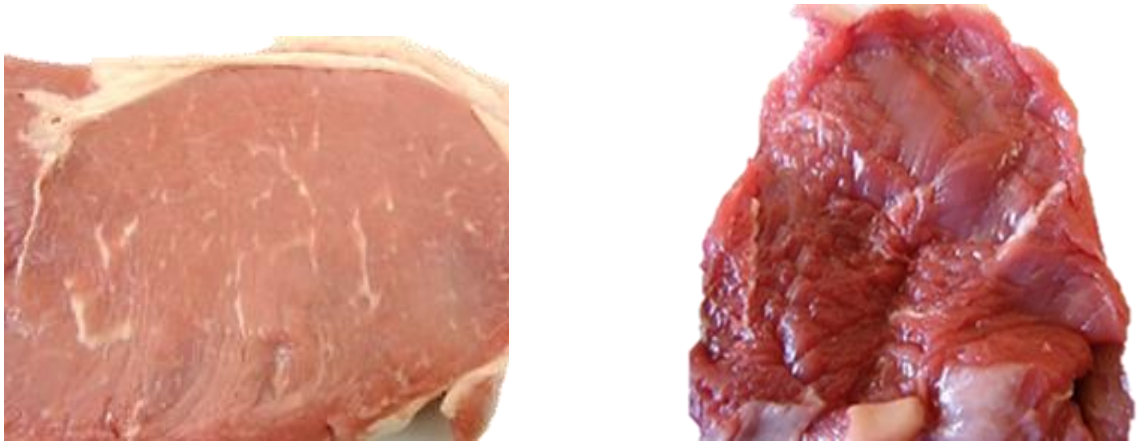


Figure 3.10: Demonstration of typical samples to evaluate: A. No fibre separation (fibres fit tightly together); B. Fibre structure almost collapsed/major separation of bundles and fibres (Photos by Dr. Lorinda Frylinck -© ARC, 2012).

3.5 Determination of protein and lipid denaturation and water holding capacity

3.5.1 Drip loss

Drip loss was determined in fresh steaks for each ageing period. Cubes of 10 mm × 10 mm × 20 mm were cut from steaks to determine drip loss. The cubes were suspended on a pin inside a sample bottle (200 ml) taking care that the meat did not touch the sides of the bottle and stored for 72 hours at 2 ± 1°C. The amount of drip was measured as the difference between the sample mass at 24 and 72 hours *post mortem* and was expressed as a percentage of the starting mass.

3.5.2 Water holding capacity

The water holding capacity (WHC) was determined by calculating the ratio of meat area divided by meat plus expressed juice area (total area) after pressing a 400-600 mg fresh meat sample on a filter paper (Whatman 4) sandwiched between two Perspex plates, and pressed at a constant pressure of 300 psi for 60 seconds according to the method described by Irie *et al*. (1996). The areas were measured by means of video image analyses (VIA) using Olympus video photo adapter at magnification of two. Image processing and calculations were done by means of Analysis Life Science software package (Soft Imaging Systems Gmbh, Münster, Germany).

3.5.3 Myofibril hydrophobicity

Myofibrillar proteins are important structural proteins associated with tenderness and water holding capacity of meat. Hydrophobicity can be an appropriate factor to estimate protein denaturation (Wallace & Dirr, 1999). Hydrophobicity of myofibrils was thus determined by a three-step procedure, which included the isolation, heat treatment, and determination using bromophenol blue (BPB).

3.5.3.1 Isolation of myofibrils

Myofibrils were prepared according to the method of Ouali and Talmant (1990), with some modifications as outlined by Chelh *et al*. (2006). About 0.5 g of frozen muscle was homogenised in 5 mL of a solution at pH 6.5 containing 150 mM NaCl, 25 mM KCl, 3 mM $MgCl_2$, 4 mM EDTA, to which two protease inhibitors (1 mM PMSF and 1 mM E-64) had been added. The homogenate was filtered with a cheesecloth to eliminate collagen. After 30 minutes stirring in ice, the extract was centrifuged at 2000 x g for 15 minutes at 4°C. The pellet was washed twice with 5 mL of a 50 mM KCl solution at pH 6.4, and once with 5 mL of 20 mM phosphate buffer at pH 6. The pellet was finally resuspended in the same phosphate buffer, and the protein concentration was adjusted to 5 mg/mL, as determined by the Biuret method (Gornall *et al*., 1949).

3.5.3.2 Heat treatment of myofibrils

One millilitre of myofibril suspension was heated, under agitation, in a temperature-controlled water bath set at a constant temperature of 70°C for 60 minutes. Heating was performed in closed tubes to avoid evaporation. After heating the myofibril suspensions were immediately cooled for 10 minutes in ice to stop further denaturation, and all samples were then analysed for hydrophobicity.

3.5.3.3 Determination of protein surface hydrophobicity

Hydrophobicity of non-solubilised myofibrils was determined, using bromophenol blue sodium salt (electrophoresis grade). To 1 mL of myofibril suspension, 200 µL of 1 mg/mL BPB (in distilled water) was added, and mixed well. Addition of the weak acid BPB slightly decreased the pH of the solution from 6 to 5.8. A control, without myofibrils, consisted of the addition of 200 µL of 1 mg/mL BPB (in distilled water) to 1 mL of 20 mM phosphate buffer at pH 6. Samples and control were kept under agitation at room temperature for 10 minutes and then centrifuged for 15 minutes at 2000 x g. The absorbance of the supernatant (diluted 1/10) was measured at 595 nm against a blank of phosphate buffer.

The amount of BPB bound to the hydrophobic myofibrillar proteins was calculated using the formula:

BPB bound protein (µg) = 200 µg protein X (A control- A_{595} nm sample) / A control

With A_{595} nm = absorbance at 595 nm

3.5.4 Protein solubility (total, myofibrillar and sarcoplasmic)

The solubility of sarcoplasmic and myofibrillar proteins has been reported as a better indicator for muscle quality (Sayre and Briskey, 1963). To determine the solubility of the sarcoplasmic and total (sarcoplasmic plus myofibrillar) proteins, two extractions were conducted according to the method of Joo *et al*. (1999). Sarcoplasmic proteins were extracted from 0.75 g muscle using 5 mL of ice-cold 0.025 M potassium phosphate buffer (pH 7.2). The samples were homogenised, then left on a shaker at 4°C overnight. Samples were centrifuged at 1500 x g for 20 minutes, and protein concentration in the supernatants was determined by the Biuret method. Total protein was extracted from 0.5 g muscle using 5 mL of ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2). The same procedures for homogenisation, shaking, centrifugation, and protein determination were used as described above. Myofibrillar protein concentrations were obtained by difference between total and sarcoplasmic protein solubility.

3.5.5 Oxidative status of meat by means of the following methods:

3.5.5.1 Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) are formed as a by-product of lipid peroxidation (i.e., as degradation products of fats), which can be detected by the TBARS assay using thiobarbituric acid (TBA) as a reagent. Assay of TBARS measures malondialdehyde (MDA) present in the sample. The MDA as lipid oxidation was evaluated using 2-Thiobarbituric acid (TBA; 4,6-dihydroxy-2-mercapto-pyrimidin), as described by Raharjo *et al*. (1992). About 4 g of sample was sliced from the middle of the steak; the top, middle and bottom layers of the steak were included. The sample was homogenised in 15 mL 5.0% (w/v) aqueous solution of trichloroacetic acid for one minute. The homogenate was centrifuged at 10,000 x g for 10 minutes and the supernatant was filtered through a paper filter. An amount of 2.0 mL of filtrate was mixed with 2.0 mL of 40 mM TBA and incubated at 94 ± 1°C in a water bath for 10 minutes. The absorbance of the red pigment formed was scanned from 400 to 600 nm. Results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde per kg meat, using a standard curve prepared from 1,1,3,3-

tetraethoxypropane (malonaldehyde). A 78% recovery value of malondialdehyde-TBA complex was used in the final calculations.

Calculations: concentration (µM/L) x end point (25 mL) ÷ (mass sample x 1000) x 100 ÷ recovery value.

3.5.5.2 Amount of free thiol groups in proteins

Protein oxidation was measured as the amount of free thiol groups in proteins determined as µM thiol per mg protein (high values indicate low oxidation). The amounts of free thiol groups were determined by the method of Lund *et al*. (2008), where 2.5 g of minced sample was homogenised for 20 seconds at 13500 rpm in 15 mL of 5% SDS (sodium dodecyl-sulphate buffer) and 0.10 M Tris-HCL buffer, pH 8.0. The homogenate was heated in a water bath at 80 °C for 30 minutes. After 30 minutes, the homogenate was allowed to cool for 10 minutes. The sample was diluted 30X (100 µL sample + 2900 µL SDS buffer), and protein concentration was determined at 280 nm against a BSA standard curve.

For determination of free thiols, the supernatants were diluted to a concentration of 1.5 mg/mL with 5% SDS in 0.10 M Tris-HCL buffer. Then the assay was prepared by mixing 0.50 mL diluted filtrate, 2.0 ml Tris-HCL buffer (0.10 M, pH 8.0) and 0.5 ml of 10 mM DTNB (5,5′-Dithiobis (2-nitrobenzoic acid)) in 0.10 M Tris-HCL buffer. After 30 minutes, the absorbance of the samples was measured at 412 nm against an aqueous reference solution of 0.50 ml 5% SDS in 0.10 M Tris-HCL buffer, 2.0 ml Tris-HCL buffer. The amount of free thiol groups was expressed as µM thiol per mg protein.

3.6 Determination of colour related parameters

3.6.1 Determination of myoglobin derivatives

Relative concentrations of myoglobin derivatives were determined in fresh meat sample extracts according to the method described by Viriyarattanasak *et al*. (2011), with some modifications. About 2 g sample of the upper 1/3 of a 25 mm steak sample was homogenised in 10 ml of 2 mM pH 7 phosphate buffer for 30 sec using an ultra-turrax T25. The steaks that were aged up to 3 days were packaged in polystyrene plates and the rest were packaged in vacuum bags. The homogenate was centrifuged at 10 000 g (4°C) for 30 minutes, and filtered through Whatman No. 134 filter paper. The spectra of the filtrates were recorded from 400-700 nm using Agilent 8453 diode-array spectrophotometer. The concentrations of metmyoglobin (MetMb), deoxy-myoglobin (DeoxyMb) and oxy-myoglobin (OxyMb) were calculated using absorbance at 503 nm, 544 nm and 581 nm respectively, 525 nm was taken as the isobestic

absorbance according to previously published methods (Viriyarattanasak *et al*., 2011; Krzywicki, 1982; Tang *et al*., 2004).

The following equations were used to calculate the relative concentrations of myoglobin derivatives as modified by (Tang *et al*., 2004) from the original equations of Krzywicki, (1982)

Calculations:

$[DeoxyMb] = C_{DeoxyMb}/ C_{Mb} = -0.543R_1 + 1.594R_2 + 0.553R_3 - 1.329$

$[OxyMb] = C_{OxyMb}/ C_{Mb} = 0.722R_1 - 1.432R_2 - 1.659R_3 + 2.599$

$[MetMb] = C_{MetMb}/ C_{Mb} = -0.159R_1 - 0.085R_2 + 1.262R_3 - 0.520$

Where $R_1 = A_{581}/A_{525}$, $R_2 = A_{544}/A_{525}$, $R_3 = A_{503}/A_{525}$

3.6.2 Metmyoglobin reducing activity in meat

The accumulation of metmyoglobin (MetMb) on the surface of meat leads to meat colour deterioration; this is a concern for producers as it results in consumer discrimination against the product. The enzyme metmyoglobin reductase (MRA) helps in the reduction of MetMb back to myoglobin (Mb). The activity of this enzyme is an important factor to examine in studies on colour stability (Behrends, 2000). The MRA was determined by the method of Sammel *et al*. (2002a) using the reduction of dichlorophenol-indophenol (DCPIP) method. A 2 g meat sample was taken from the upper 1/3 of a 25 mm thick steak and homogenised in 10 ml of 0.2 mM sodium phosphate buffer (pH 5.6) using an ultra-turrax T25 The homogenate was centrifuged at 1500 x g for 30 minutes at 4°C. The supernatant was filtered using a filter paper. Reduction of dichloro-phenolindophenol (DCPIP) was measured by a change in absorbance at 600 nm. The DCPIP reagent consisted of 0.56 mg 2,6-DCPIP and 3.7 mg disodium EDTA in 20 ml of 50 mM Tris buffer (pH 8.1). To a plastic cuvette, 2.4 ml of DCPIP reagent was added, the reaction was initiated by simultaneously adding 200 µL NADH, and 400 µL of muscle extract and absorbance was measured at 600nm after 3 minutes at 37°C. As DCPIP was reduced by the muscle extract, absorbance decreased. Reducing activity was calculated from the linear phase of the assay using Beer's law as nM reduced/min/g.

3.6.3 Muscle colour measured with Minolta

Meat colour was measured on each steak for each ageing period with a Minolta colour meter (Model CR200, Osaka, Japan; 8 mm diameter measuring area, diffuse illumination and

0° viewing angle) (CIE, 1976) at three different locations on each steak, after blooming the meat for exactly one hour at 20°C. Three recordings were performed on each steak. The steak colours were obtained as three components; luminance or lightness, *L** (dark to light), and two chromatic components; *a** (green to red) and *b** (blue to yellow) values (CIE colour model). Chroma (intensity of the red colour/ saturation index) (S) = $(a^2+b^2)^{1/2}$) and hue angle (the meat discoloration= $\tan^{-1}(b^*/a^*)$) were calculated.

3.6.4 Fibre typing and fibre bundles

Muscle fibre typing was performed on 100 g of a sample that was frozen in liquid nitrogen. For histochemical demonstration of succinic dehydrogenase situated in the mitochondria, the nitro-blue tetrazolium technique of Malaty and Bourne (1953) was used. Fibres were classified under 100X magnification by means of VIA (Soft Imaging System, Olympus, Japan) into red, intermediate, and white, according to the intensity of the staining reaction. Fibre cross-sectional areas were also determined by VIA.

3.7 Tests related to meat tenderness according to established methods in the ARC-API Meat Science Laboratories.

3.7.1 Warner-Bratzler shear force (WBSF) on cooked meat

Steaks that were frozen at -20°C were thawed at 4°C for overnight. The steaks were prepared according to an oven broiling method, using direct radiant heat (AMSA, 1978). The steaks were cooked at 260°C until reaching an internal temperature of 70°C. Each steak was allowed to cool to an internal temperature of 18°C. Six cylindrical cores were removed with a core diameter of 12.5 mm parallel to the grain of the meat. Using a Warner Bratzler shear device, the cores were sheared perpendicular to the fibre direction. The mean value of the six recordings was used as a shear value.

3.7.2 Sarcomere length at three days *post mortem*

Sarcomere lengths were determined by using a video image analyser after preparation of fresh sample, according to the method of Hegarty and Naudé (1970) by using distilled water instead of Riger Locke solution (Dreyer *et al*., 1979). A meat sample was scratched along the grain of the fibres; the sample was homogenised in 5 mL distilled water, with an ultra turrax. After homogenising, a drop of the sample was transferred onto a slide and covered with a coverslip. Excess water was dried and the slide cleaned with a paper towel. Five sarcomere lengths were measured at a time from the bottom of the first sarcomere length with an Olympus BX40 system microscope at a 1000 X magnification. About 100 readings were taken and the averages were calculated.

3.7.3 Myofibril fragmentation

Myofibrillar fragmentation is the extent of fragmentation of myofibrils caused by proteolytic activity during ageing. Myofibril fragment lengths (MFL) has been shown to be highly correlated with shear force and sensory panel tenderness of meat tenderness (Moller *et al*., 1973; Olson *et al*., 1976).

Myofibrillar fragmentation were determined as described by Culler *et al*. (1978) with some modifications by (Heinze *et al*., 1994). Sample slices were cut from a frozen muscle sample with a knife, and any visible fat and connective tissue were removed. The sample was then finely minced with scissors and 3 g was weighed in to 50 mL Bühler glass; 30 mL of the MFL extraction buffer (0.02 M Potassium phosphate buffer containing:100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 1 mM NaN_3) at 4°C was added. The sample was allowed to thaw for 60 seconds and homogenised for exactly 30 seconds in a Bühler HO_4 homogeniser at 20,000 rpm while chilled in ice water. The blade was turned around in order to fragment the myofibrils rather than to cut them. The samples were subsequently transferred into centrifuge tubes and centrifuged at 4°C at 3000 rpm for 15 minutes. The pellet was washed once with 30 ml MFL extraction buffer and centrifuged at 3000 rpm. The supernatant was then discarded and pellet was suspended in 10 mL MFL extraction buffer. The suspension was then filtered under vacuum through a 1000 µm polyethylene strainer, additional 5 mL MFL buffer was used to facilitate the passage of the myofibrils through the strainer. The samples were transferred on to a slide and covered with a slip. The excess water on the slide was dried with a paper towel. Fifty single myofibril fragments were measured by means of a video image analyser (Olympus BX40 system microscope at a 1000X magnification).

3.7.4 Fibre detachments

At the light microscopic level, the evident changes includes fibre contraction, fibre detachment, and partial and full breaks in the fibres, these were analysed from muscle samples according to method developed by Taylor and Frylinck, (2003).

For measurement of fibre detachment, fibre breaks, percentage fibre separation score, fat cell score and fibre diameter, samples were frozen in liquid nitrogen. Sub-samples of 7 mm X 4 mm were mounted on a cryotome disk. Two sections of 15 µm thickness were cut along the grain of the fibre with a Shandon Cryotome E (Thermo Fisher Scientific, Pittsburgh, USA) and fixed on a microscope slide. The sections were stained with Amaranth (Sigma A 1016-100G) and examined with an Olympus BX41 system microscope at 100X magnification (Olympus, Tokyo, Japan). The AnalySIS Life Science software package (Soft Imaging Systems Gmbh,

Münster, Germany) was used to measure the area of muscle fibres and total area of white space between the fibres (detachments) in a field of 0.57mm^2. The number of clear breaks in fibres in the specified area was counted. This procedure was repeated at 16 different locations. The average tenderness score (this is the score taking all the above measurements in to consideration) was perceived by the analyst on a scale of 1-5. Figure 3.11 and 3.12 illustrates examples of pictures that were taken for analysis of these measurements. The figures show the differences in fibre breaks as ageing progresses with the breaks being more prominent at extended ageing periods (Taken at 3 and 20 days *post mortem*).

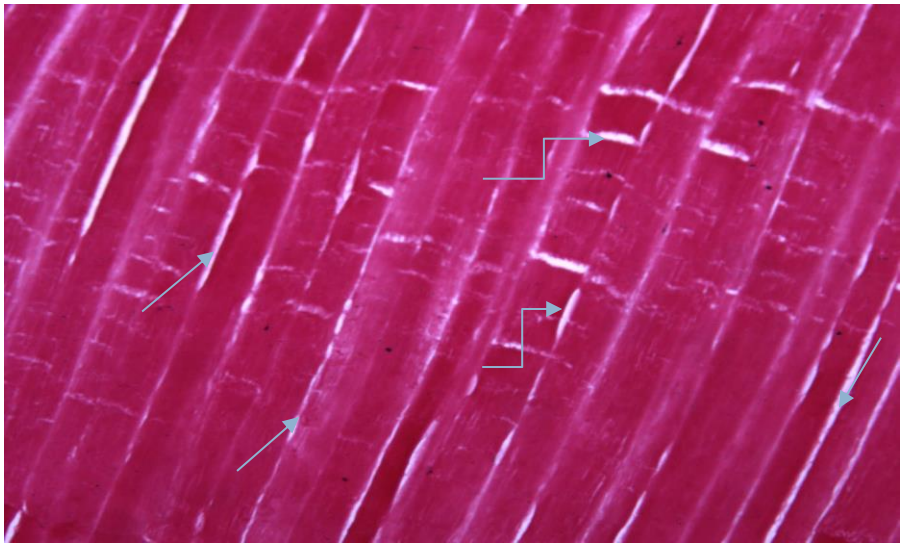


Figure 3.11: A Longitudinal sections of the *m. longissimus dorsi* at early days *post mortem* (3 days), fractured muscle fibres are indicated with an elbow arrow. The straight arrow indicates the breaks within the fibres (Photo by Ms. JD Snyman, © ARC, 2014).

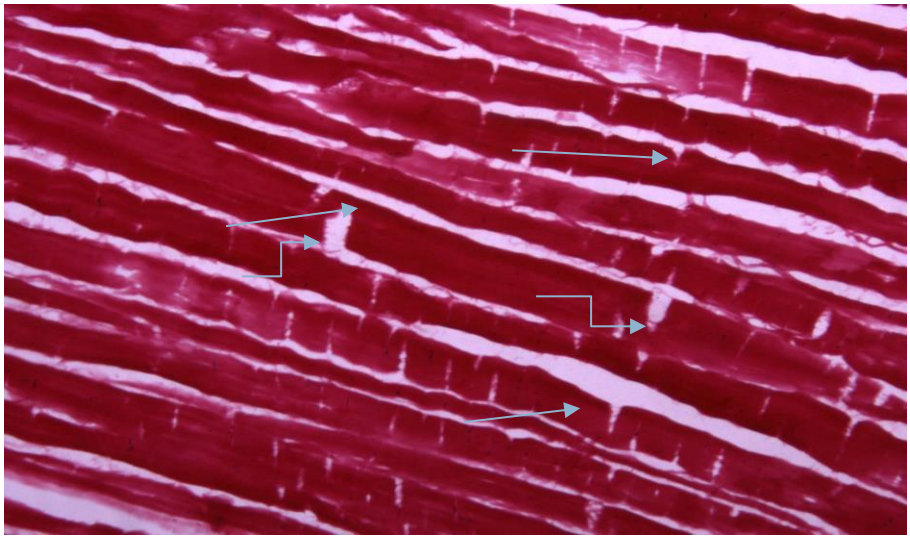


Figure 3.12: A Longitudinal sections of the *m. longissimus dorsi* at extended days *post mortem* (14 days), fractured muscle fibres are indicated with an elbow arrow. The straight arrow indicates the breaks within the fibres (Photo by Ms. JD Snyman, © ARC, 2012).

3.7.5 Connective tissue characteristics (total collagen and solubility)

Collagen is the main structural protein of the various connective tissues in animals. As the main component of connective tissue, it is the most abundant protein in mammals, making up from 25% to 35% of the whole-body protein content. Collagen is a contributing factor to variation in meat tenderness and texture. Soluble, insoluble and total collagen were determined in minced samples that were freeze dried according to the method of Weber (1973).

3.7.5.1 Soluble and insoluble collagen extractions

One gram of freeze dried sample was stirred in 12 ml of 1% NaCl. The samples were heated in a shaking water bath at 78°C for 60 minutes and thereafter, they were allowed to cool for 15 minutes and centrifuged at 6000 x g for 10 minutes. The supernatant was poured in to a hydrolysing tube, marked as soluble. The pellet was poured in to another hydrolysing tube and marked insoluble. To each tube, 30 mL of 6 N HCl was added and hydrolysed overnight at 110°C. The next day, the tubes were allowed to cool, 0.5 g of active carbon was added, stirred, and the homogenate was filtered in to 100 mL volumetric flask. The flasks were filled up to the mark with distilled water. An aliquot of 50 mL was use for determination of both soluble and insoluble collagen.

3.7.5.2 Total collagen extraction

About 0.5 g of freeze dried sample was weighed in to a hydrolysed tube and mixed with 30 ml of 6 N HCl. The samples were hydrolysed at 110°C for overnight. The next day, the tubes were allowed to cool and 0.5 g active carbon was added to each tube, stirred and filtered

using Whatman 4 filter paper. The aliquots were collected in to a 100 ml volumetric flask and filled up to a volume with distilled water. An aliquot of 50 ml was used for the determination of total collagen.

3.7.5.3 Procedure for determination of soluble, insoluble and total collagen

About 1 ml of the final sample was added in to test tubes, where 1 ml of 10% KOH solution was added (to neutralise the acid in the sample, this is always a 2 X dilution that must be included in all sample calculations). A blank consisting of 2 ml distilled water was prepared. Standard solutions were made and consisted of 0.1 to 7.5 µg/mL and 2 mL of each standard was added in to a test tube.

To each test tube (including standards and blanks), 1.0 mL of the oxidant solution (1.41 g Chloramine-T in a 100 ml, pH 6.8 buffer solution consisting of: 26 g citric acid monohydrate, 14 g sodium hydroxide, 78 g Anhydrous sodium acetate and 250 mL propan-1-ol) was added. The tubes were vortexed for five seconds each, and left for 20 minutes at room temperature. After 20 minutes, 1.0 mL of the colour reagent (10 g para-dimethylaminobenzaldehyde, 35 mL perchloric acid solution (60%), 65 mL propan-2-ol, prepared fresh) was added and the tubes were vortexed. The tubes were placed in a water bath heated to 62°C ± 5°C for exactly 30 minutes, then vortexed. Thereafter, they were cooled to room temperature (a strong aromatic pink liquid with a white salt residue forms in the tubes). The top transparent pink liquid was pipetted in to disposal micro-cuvettes and read on a spectrophotometer at 558 nm (± 2 nm). Analysis was done between 480 nm and 620 nm.

Total collagen content was calculated as the ratio of hydroxyproline nitrogen relative to the total nitrogen content, expressed as a numeric value multiplied by 1000 (Boccard *et al*., 1979). Collagen solubility was determined according to a combination of the methods of Hill (1966) and Bergman and Loxley (1963), being expressed as the hydroxyproline content of the filtrate as a percentage of total hydroxyproline (filtrate plus residue).

3.8 Muscle energy metabolism

The concentrations of lactate, glycogen, creatine phosphate, ATP (adenosine triphosphate) and glucose-6-phosphate, were determined according to the method of Dalrymple and Hamm (1973). About 2 g of meat sample (frozen in liquid nitrogen) was homogenised in 10 mL of 0.6 N perchloric acid and centrifuged at 10000 rpm for 15 minutes. The supernatant was poured in to clean tubes. About 100 µL of the homogenate was poured in to glass test tubes, 100 µL of 0.6 N perchloric acid + 50 µL of 1 N KOH + 1 mL AGS solution were added to the test tubes. The test tubes were then incubated in a water bath at 40°C for two

hours. The reaction was stopped by addition of 1 mL of 0.6 N perchloric acid to each glass tube. The foaming supernatants were aspirated and the fluid was used for glycogen determination.

With the remaining homogenised sample left in the test tubes, a few drops of methyl orange indicator was added to each test tube. Drops of 5.4 N KOH were slowly added to neutralise the pH (colour turned from pink to yellow). The samples were placed in the fridge for 20 minutes to precipitate out. Pieces of filter paper (Whatman no. 4) the size of the glass filter tubes were cut and using the glass filters, filter paper, clamps, measuring tubes and pump: the samples were filtered into the measuring tubes and the volumes were noted. The samples were poured in clean tubes and stored in the fridge until spectrophotometer determination of lactate, blank for glycogen, creatine phosphate, ATP and glucose-6-phosphate.

3.8.1 Spectrophotometric analyses of L-lactate

The lactate concentration was determined according to the method of Gutmann and Wahlefeld (1974). About 2.5 ml lactate buffer, 200 µL NAD solution was added to cuvettes with 20 µL 0.6 N perchloric acid, 20 µL of the sample was added, and absorbance was read at 37°C at 340 nm [E0]. To each cuvette, 20 µL of L-lactate dehydrogenase was added and absorbance [E1] was read after 30 minutes. A blank consisting of 2.5 mL lactate buffer, 200 µL NAD solution and 20 µL of 0.6 N perchloric acid was used.

Calculations:

L-lactate = Total volume ÷ sample volume × ΔE ÷ 6.33 × extraction volume

Extraction volume= muscle mass/muscle extracted

ΔE: E_1-E_0 = E_2

E_2-E blank = ΔE

3.8.2 Spectrophotometric analyses of glycogen

The glycogen concentration was determined as glycolysis units after hydrolysis with amyloglucosidase according to Keppler and Decker (1974). About 1 mL of the glycogen buffer (0.012 g ATP, 0.016 g NADP, 20 ml triethanolamine buffer, 100 µL. Glucose -6-phosphate) was added to cuvettes and 50 µL of the sample was added, the absorbance [E0] was read at 340 nm and 37°C after five minutes' incubation. Hexokinase (5 µL) was added and the absorbance [E1] was read after 10 minutes. The blank consisted of 1 mL glycogen buffer, 50 µL sample and 5 µL hexokinase.

Calculations:

Glycogen= Total volume ÷ sample volume × ΔE ÷ 6.33 × extraction volume

Extraction volume = muscle mass/muscle extracted

ΔE: E_1-E_0 = E_2

E_2-E blank = ΔE

3.8.3 Spectrophotometric analyses of creatine phosphate; ATP; glucose-6-phosphate

The creatine phosphate, ATP and Glucose-6-phosphate concentrations were determined by the method of Lamprecht *et al*. (1974). About 2.5 ml of triethanolamine buffer, 100 µL NADP, 100 µL $MgCl_2$, 20 µL ADP and 50 µL sample were added in to cuvettes and incubated for five minutes at 37°C, absorbance [E0] was read at 340 nm. Absorbance ([E1], [E2], [E3]) were read after addition of 5 µL G-6-P, 100 µL glucose and 5 µL Hexokinase, after addition of each solution, the samples were incubated for five minutes before reading the absorbance. The samples were incubated for a further 20 minutes after addition of 5 µL hexokinase and absorbance [E4] was read, 10 µL creatine kinase was then added and absorbance [E5] was read after 10 minutes' incubation.

Calculations:

Creatine phosphate; ATP; Glucose-6-phosphate

= Total volume÷ sample volume × ΔE ÷ 6.33 × extraction volume

Extraction volume= muscle mass/muscle extracted

ΔE: E_1-E_0 = E_2

E_2-E blank = ΔE

3.9 Carcass weight and eye muscle area

The following back up data were collected (the weight of the carcass and the area of the eye muscle could have an influence on the physiological characteristics and chilling rate of the meat and were kept into consideration (low cost measurements):

3.9.1 Warm and cold carcass weight

3.9.2 Eye-muscle area (EMA) (m. *longissimus dorsi*), which was measured between 9th and 10th rib, by tracing the eye muscle area of an animal on a paper. The EMA was determined using external Olympus video photo adapter mounted on to an Olympus camera.

3.10 Statistical evaluation of results

All the data was recorded using Excel 2016. The recorded data was subjected to a 5 x 2 factorial ANOVA with repeated measurements over time with breed (Angus, Bonsmara, Brahman, Charolais and Nguni) as whole plots and the four ageing periods (3, 9, 14 and 20 days *post mortem*) and treatments combinations (electrical stimulation, followed by direct chilling at 0-5ºC and non-electrical stimulation, delayed chilling for 6 hrs at 10ºC followed by 12 hrs of chilling at 0-5ºC) as sub-plot factors. Means for the interactions between sub-plot and whole-plot were separated using Fisher's protected t-test least significant difference (LSD) at 5% level of probability. The Shapiro-Wilk's test was performed on the standardised residuals to test for deviations from normality (Shapiro & Wilk, 1965). In cases where significant deviations from normality were observed due to skewness, outliers were removed until it was normal or symmetric distributed (Glass *et.al.* 1972). Student's t-LSD (Least significant difference) was calculated at a 5 % level of significance to compare means of significant source effects. All the above data analysis was performed with SAS version 9.3 statistical software (SAS, 2011). Correlations were measured using Pearson correlation coefficients (SAS, 2011).

Preamble to Results

The study was carried out in two phases, with Phase 1 being an exploratory phase and implement the findings from Phase 1 into Phase 2, to allow for an ore in-depth analysis in the second phase. Upon analysis of the results, it was observed that most of the results from Phase 1 were similar to that of Phase 2, with minor differences due to climate differences between years, seasonal differences in feed quality and composition, differences in management (stress, transportation, climatic factors); and due to certain changes, which were made to the methods during Phase 2. Therefore, the results obtained in Phase 1 will be reported in Chapter 4; and the results of Phase 2 will be reported in Chapter 5. Possible reasons for the differences between the two phases will be discussed in Chapter 6 (discussion).

Aspects of results for this study were peer reviewed and published in the South African Journal of Animal Science.

Modika, K.Y., Frylinck, L., Moloto, K.W., Strydom, P.E., Heinze, P.H., Webb, E.C., 2015. Visual evaluation of beef tenderness by using surface structural observations and its relationship to meat colour. S. Afr. J. Anim. Sci. 45 (3), 255-262.

CHAPTER 4
RESULTS OF PHASE 1

4.1 Effect of breed on carcass characteristics, visual sensory panel evaluation of meat characteristics, meat colour measurements/evaluations, muscle energy metabolism, protein denaturation, lipid oxidation and meat tenderness.

Table 4.1: ANOVA table to summarise *P*-values for Phase 1 results.

TREATMENTS				
	Breed	**Ageing**	**Treatment**	**Breed X ageing**
Carcass characteristics	**p69**		**p85**	
Live animal (kg)	<0.0001			
Warm carcass mass (kg)	<0.0001		0.801	
Cold carcass mass	<0.0001		0.056	
Dressing %	0.378			
Eye Muscle Area (mm^2)	<0.0001		0.385	
Ultimate pH (pHu)	0.382			
Visual appraisals	**p71**	**p71**	**p86**	**p91**
Colour[2]	<0.0001	<0.0001	0.106	0.009
Marbling[2]	<0.0001	<0.0001	0.379	0.014
Fibre separation[2]	<0.0001	<0.0001	0.002	0.001
Surface texture[2]	<0.0001	0.035	0.166	0.012
Structural integrity[2]	0.001	<0.0001	0.890	0.006
Protein and lipid denaturation	**p71**	**p79**	**p87**	**p93**
Drip-loss	0.286	<0.0001	0.026	0.513
Water holding capacity	<0.0001	0.001	0.160	0.566
Total protein solubility (mg/g)	<0.0001	<0.0001	<0.0001	0.0001
Myofibril protein solubility (mg/g)	<0.0001	<0.0001	0.001	<0.0001
Sarcoplasmic protein solubility (mg/g)	0.013	<0.0001	0.009	0.382
Myofibril surface hydrophobicity (µg)	0.002	<0.0001	0.001	0.069
TBARS (mg/kg)	0.352	<0.0001	0.004	0.390
Thiols (µM/mg)	0.008	<0.0001	0.984	0.992
Instrumental colour	**p73**	**p80**	**p88**	**p95**
Deoxymyoglobin %	0.575	<0.0001	0.016	0.468
Oxymyoglobin %	0.282	<0.0001	0.309	0.463
Metmyoglobin %	0.227	<0.0001	0.073	0.361
Metmyoglobin reductase activity	<0.0001	<0.0001	0.850	0.003
L*	<0.0001	<0.0001	0.014	0.319
a*	<0.0001	<0.0001	0.043	0.582
b*	0.014	<0.0001	0.020	0.440
Chroma	0.659	<0.0001	0.027	0.359
Hue angle	<0.0001	<0.0001	0.256	0.726
Fibre Typing	**p74**			
Red Type 1	0.913			
Intermediate type IIA	0.717			

White type IIB	0.477			
% Fibre type				
Red	0.200			
Intermediate	0.018			
White	0.001			
Muscle energy				**p97**
Lactate 1 hr (µM/g)				0.320
Lactate 20 hrs (µM/g)				0.008
Glucose 1 hr (µM/g)				0.030
Glucose 20 hrs (µM/g)				0.389
Glycogen 1 hr (µM/g)				0.476
Glycogen 20 hrs (µM/g)				0.352
Glucose-6-Phosphate 1 hr (µM/g)				0.028
Glucose-6-Phosphate 20 hrs (µM/g)				0.176
ATP 1 hr (µM/g)				0.788
ATP 20 hrs				0.274
Creatine Phosphate 1 hr (µM/g)				0.854
Creatine Phosphate 20 hrs (µM/g)				0.064
Tenderness related measurements	**p76**	**p82**	**p89**	**p98**
Warner Bratzler shear force (N)	<0.0001	<0.0001	<0.0001	<0.0001
Sarcomere length (µm)	0.245		0.007	
Myofibril fragment length (µm)	0.0004	<0.0001	0.565	0.0001
Total collagen (mg/g)	0.016			
Insoluble collagen (mg/g)	0.245			
Collagen solubility (%)	0.013			
Intra muscular fat (%)	0.097			
VIA muscle fibre structure measurements	**p74**	**p98**		**p98**
Fibre detachment (%White to red area)	0.502	<0.0001	0.207	0.018
Fibre breaks score (1-5)	0.727	<0.0001	0.206	0.302
% Fibre separation score	0.946	<0.0001	0.732	0.921
Fat area score (1-5)	0.117	0.558	0.945	0.943
Average analyst tenderness score (1-5)	0.635	<0.0001	0.122	0.002

Table 4.1 summarises the *P*-values for all the analysis for Phase 1 results. Values with $P<0.05$ indicates significant differences between variables as highlighted in green and $P>0.05$ indicates non-significant differences between variables.

4.1.1 Carcass characteristics

All carcasses assessed in this study were of A-age class, fatness Class 2 and confirmation Code 3. Results of carcass characteristics are summarised in Table 4.1. Breed had a significant effect on carcass characteristics like live animal weight, warm carcass mass; cold carcass mass and eye muscle area, as shown in Table 4.1. The Charolais breed had the highest live weight, warm carcass mass and cold carcass mass compared to Angus, Bonsmara, Brahman and Nguni. This was followed by the Angus and Brahman, which had no differences in live weight and then the Bonsmara. Warm and cold carcass masses were lower for the Brahman compared to

Angus and Bonsmara, which had no significant differences. Nguni had the lowest live weight, warm carcass mass, cold carcass mass, and eye muscle area. Dressing % did not differ between any of the breeds studied. Table 4.2 also presents ultimate pH-values (pHu) between breeds, but statistical analyses indicated that there were no differences between breeds (P=0.382). Figure 4.1 indicates that there were no differences for pH and temperature data for all the breeds from 1, 3, 6 and 20 hours *post mortem*.

Table 4.2: Effect of beef cattle breed on carcass characteristics of *m. longissimus dorsi* (LD).

	Beef Breed						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM**	***P*-Value**
Live animal (kg)	395.8[b]	338.3[c]	380.6[b]	428.6[a]	303.3[d]	10.54	<0.0001
Warm carcass mass(kg)	221.91[b]	215.68[b]	195.30[c]	241.83[a]	170.03[d]	6.29	<0.0001
Cold carcass mass	216.44[b]	210.29[b]	188.46[c]	235.87[a]	165.58[d]	6.15	<0.0001
Dressing %	55.4	56.4	55.9	55.8	55.3	0.76	0.378
Eye Muscle Area (mm^2)	5770[b]	5665[b]	6478[a]	6931[a]	5366[b]	273.96	<0.0001
pHu	5.48	5.43	5.41	5.49	5.50	0.0922	0.382

[1] Standard error of means; *post mortem* = *post mortem*
[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05).

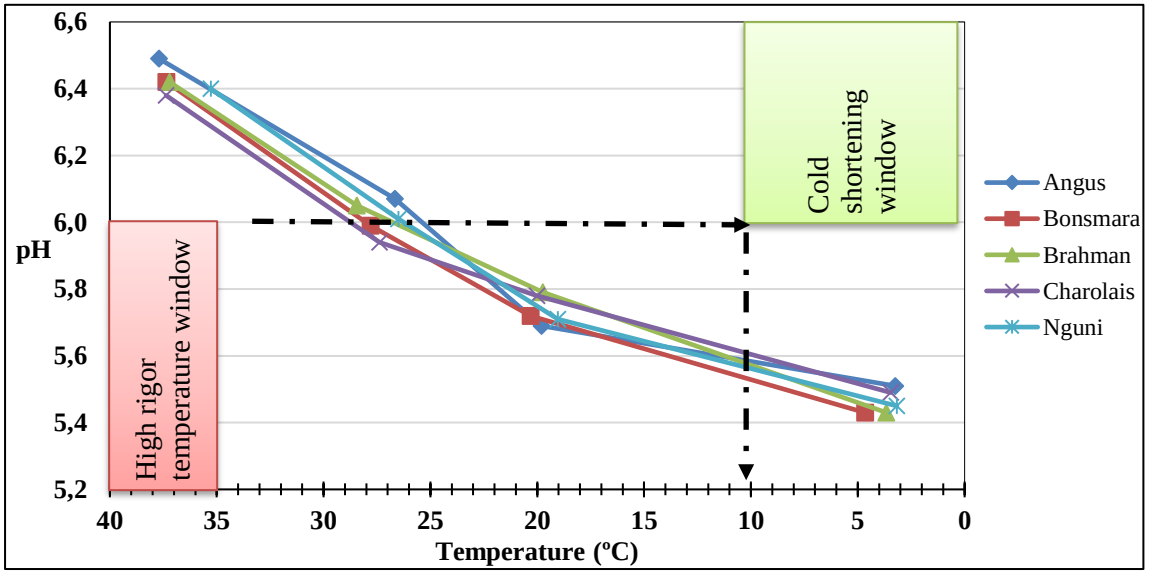


Figure 4.1: Temperature and pH decline profiles at 1, 3, 6 and 20 hours *post mortem* of five beef breeds (Angus, Bonsmara, Brahman, Charolais and Nguni) of *m. longissimus dorsi* (LD). Cold shortening and high rigor temperature windows according to Pearson and Young (1989) and as discussed in the review of Thompson, (2002).

4.1.2 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form, Table 3.2, and visual reference guides, Figures 3.6-3.9)

Visual assessment of meat colour is the "essential standard" of colour measurement in meat, because it closely relates to consumer evaluations, and sets the benchmark for instrumental measurement comparisons. Visual evaluations of meat colour were conducted using a 10-member trained panel, by using posters as references. The panel evaluated differences in visual colour between the five different cattle breeds. Results of the effect of breed on meat visual surface structural properties are summarised in Table 4.3. Breed had an effect on means of evaluated sensory attributes, including visual colour ($P<0.0001$), marbling ($P<0.0001$), fibre separation ($P=0.003$), surface texture ($P=0.0001$), and structural integrity ($P<0.0001$).

Steaks from Nguni carcasses had the darkest colour rating, Bonsmara and Angus were not significantly different, and Brahman and Charolais were the lightest, rated as light pink to pink as evaluated using the Reference Guide, ARC-AP, Meat Science, 2013, Figure 3.6.

Steaks from Angus had more marbling compared to that of other breeds, rated as slight to small. Steaks from Bonsmara, Charolais and Nguni did not show significant differences in the amount of marbling and were rated as practically devoid of or had slight marbling. Brahman steaks had the least amount of marbling, but steaks from this breed were rated as being devoid of or having slight marbling as evaluated using the Reference Guide, ARC-AP, Meat Science, 2013, Figure 3.7.

The sensory panel also observed that Brahman steaks had the least fibre separation followed by steaks from Bonsmara. Steaks from Angus, Charolais and Nguni carcasses had the most fibre separation, as evaluated using the Reference Guide, ARC-AP, Meat Science, 2013, Figure 3.8.

Surface texture was rated as smooth to slightly coarse for steaks from all breeds, according to Visual Surface Texture Reference Guide, ARC-AP, Meat Science, 2013, Figure 3.9. Angus and Nguni steaks had slightly coarse visual texture (fibre bundles well distinguished), followed by steaks from Charolais, then Bonsmara, Brahman had smooth visual surface texture (can distinguish fibre bundles).

Steaks from Charolais were more compressible, followed by Angus and Bonsmara. Steaks from Brahman were less compressible. In summary, steaks from Brahman were found

to have less marbling, less fibre separation, smooth surface texture, and less compressible structure.

Table 4.3: Effect of breed on visual sensory panel evaluations of meat characteristics as judged by the sensory panel on *m. longissimus dorsi* (LD).

	Beef Cattle Breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Colour[2]	5.3[b]	5.3[b]	4.6[c]	4.51[c]	6.3[a]	0.117	<0.0001
Marbling[2]	2.2[a]	1.8[b]	1.4[c]	1.9[b]	1.8[b]	0.070	<0.0001
Fibre separation[2]	2.3[a]	2.2[b]	2.0[c]	2.4[a]	2.3[ab]	0.0531	<0.0001
Surface texture[2]	2.5[ab]	2.3[c]	2.2[d]	2.5[bc]	2.6[a]	0.0538	<0.0001
Structural integrity[2]	2.2[b]	2.2[b]	2.1[c]	2.4[a]	2.2[cb]	0.048	0.001

[1] Standard error of means

[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05).

[2] Average of panel ratings as define under methods

4.1.3 Protein denaturation and lipid oxidation.

Table 4.4: Effect of breed on protein denaturation and lipid oxidation of *m longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM**	***P*-Value**
Drip-loss	0.78[b]	1.01[ab]	1.09[ab]	1.15[a]	0.86[ab]	1.230	0.286
WHC	0.34[b]	0.37[a]	0.35[b]	0.34[b]	0.37[a]	0.051	<0.0001
TPS (mg/g)	144.9[c]	149.6[b]	150.8[ab]	143.8[c]	152.9[a]	10.620	<0.0001
MPS (mg/g)	84.52[b]	86.36[b]	91.52[a]	83.27[b]	90.63[a]	12.783	<0.0001
SPS (mg/g)	60.42[bc]	63.22[a]	59.26[c]	60.53[bc]	62.30[ab]	7.756	0.013
MSH (µg)	87.51[bc]	84.75[c]	90.91[b]	99.22[a]	82.74[c]	14.70	0.002
TBARS (mg/kg)	0.07	0.10	0.09	0.08	0.07	0.131	0.352
Thiol (µM/mg)	47.36[b]	47.74[ab]	48.83[a]	48.26[ab]	47.88[ab]	4.463	0.008

[1] Standard error of means

[a,b,c,] Means in the same row with a different superscript letter differ (P<0.05)

WHC- Water holding capacity

TPS- Total protein solubility

MPS- Myofibril protein hydrophobicity

SPS- Sarcoplasmic protein solubility

TBARS- Thiobarbituric acid reactive substances

MSH- Myofibril surface hydrophobicity

Lipid oxidation is a major cause of deterioration in the quality of meat and meat products. The quality of the secondary products of lipid oxidation is measured using the thiobarbituric acid reactive substances (TBARS) method. These secondary products cause rancid, fatty, pungent and other off-flavours (Min and Ahn, 2005). Protein oxidation is also considered as an important oxidative process that can cause changes in meat quality. Oxidation of meat

proteins affects digestibility, decreases nutritional value due to oxidation of essential amino acids, and increases the risk of some diseases, these were measured using free thiol groups. Another important factor of meat quality is the water-holding capacity (ability of meat to hold intrinsic water). This is an important factor of fresh meat as it affects both the yield and the quality of the end product. This characteristic can often be described as drip or purge loss in fresh products that have not been extensively processed.

The results of breed comparisons showed that breed had an effect on drip loss (P=0.028), water holding capacity (P<0.0001); total protein solubility (P<0.0001); myofibril protein solubility (P<0.0001); sarcoplasmic protein solubility (P=0.013); myofibril surface hydrophobicity (P=0.002); and Thiols (P=0.008), as shown in Table 4.4. Charolais breed had higher drip loss and Angus the lowest drip loss. Bonsmara, Brahman and Nguni were not different to both Angus and Charolais (P>0.05). Nguni and Bonsmara had higher drip loss and lower water holding capacity. Angus, Brahman and Charolais had lower water holding capacity, and there was no significant differences between them. Total protein solubility was lower for Charolais and Angus, followed by Bonsmara. Nguni had higher total protein solubility. Myofibril protein solubility was higher for Nguni and Brahman and lower for Charolais, Bonsmara and Angus. Sarcoplasmic protein solubility was lower for Brahman and higher for Bonsmara. Myofibril surface hydrophobicity was lower for Bonsmara and Nguni and higher for Charolais, followed by Brahman and Angus. The amount of free thiol groups were higher for Brahman and lower for Angus, Bonsmara, Charolais and Nguni were did not differ from Angus and Brahman. There were no differences between breeds for thiobarbituric acid reactive substances (P=0.352).

4.1.4 Minolta measured colour and related parameters

Table 4.5: Effect of breed on colour related parameters of *m. longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM**	***P*-Value**
DeoxyMb %	80.35	79.30	78.92	78.58	81.02	1.731	0.575
OxyMb %	16.47	17.50	17.20	15.51	16.87	0.101	0.282
MetMb %	3.177	4.256	5.388	3.776	3.385	0.056	0.227
MRA (nM reduced/min/g)	8.60×10^{-8b}	8.48×10^{-8b}	8.57×10^{-8b}	8.79×10^{-8a}	8.79×10^{-8a}	1.73×10^{-17}	<0.0001
Meat colour characteristics (CIE)							
L*	39.2^{c}	38.9^{c}	41.4^{b}	42.6^{a}	36.9^{d}	1.502	<0.0001
a*	12.3^{a}	11.8^{ab}	11.8^{ab}	11.3^{b}	11.9^{ab}	0.558	<0.0001
b*	8.3^{abc}	8.09^{c}	8.88^{a}	8.65^{ab}	7.9^{c}	0.003	0.014
Chroma	15	14.5	14.9	14.4	14.4	0.045	0.659
Hue angle	34.1^{b}	34.3^{b}	37.1^{a}	37.4^{a}	33.4^{b}	0.372	<0.0001

[1] Standard error of means, d *post mortem*-days *post mortem*
[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05)
DeoxyMb- Deoxymyoglobin
OxyMb- Oxymyoglobin
MetMb-Metmyoglobin
MRA- Metmyoglobin reductase activity

Colour is of utmost important, because it influences consumer decisions, as it is the first impression consumers have on any meat product. Meat colour depends on myoglobin oxidation rate (Boles *et al*., 1998). Colour related parameters, which include myoglobin derivatives (deoxymyoglobin, oxymyoglobin and metmyoglobin), metmyoglobin reductase activity and meat colour characteristics (*L**, *a**, *b**, Chroma and Hue angle) were evaluated using both extraction methods and Minolta meter (CIE colour measurements).

Concentrations of myoglobin derivatives showed that there were no differences between breeds for deoxymyoglobin (P=0.575), oxymyoglobin (P=0.282) and metmyoglobin (P=0.227). Differences were observed for metmyoglobin reductase activity (P<0.001). Nguni and Charolais had higher metmyoglobin reductase activity followed by other breeds, which did not differ significantly (Table 4.5).

Instrumental colour results showed differences for *L** (P<0.0001), *a** (P<0.0001, *b** (P=0.014) and Hue angle (P 0.0001). Nguni produced the darkest steaks, followed by Angus and Bonsmara, which were not significantly different. Charolais produced the lightest steaks. Steaks from Angus were brighter red, than steaks from Charolais breed. Steaks from Bonsmara, Brahman and Nguni were not different to steaks from both Angus and Charolais breeds. Steaks from Brahman produced higher *b** values whereas Bonsmara and Nguni produced steaks with the lowest *b** values. Charolais was not significantly different to Brahman and Angus. Hue

angle showed that steaks from Brahman and Charolais had more discoloration than steaks from Angus, Bonsmara and Nguni. There were no differences in Chroma between the breeds (*P*=0.568).

4.1.5 Muscle fibre typing

Table 4.6: Effect of breed on muscle fibre typing of *m. longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM**	***P*-Value**
Fibre areas (µm²):							
Red Type 1	2489	2473	2413	2299	2324	55.3	0.913
Intermediate type IIA	3579	3149	3356	3433	3292	97.2	0.717
White type IIB	5954	5543	6330	5831	6181	78	0.477
% Fibre type:							
Red	38.00	34.71	39.23	37.00	37.71	2.228	0.200
Intermediate	29.59[a]	27.30[a]	27.19[ab]	25.16[b]	29.06[a]	0.028	0.018
White	32.42[b]	37.99[a]	33.58[b]	37.84[a]	33.23[b]	0.633	0.001

[1] Standard error of means

[a,b] Means in the same row with a different superscript letter differ (*P*<0.05).

Muscle fibre type composition is an important source of variation in meat quality (Listrat *et al*., 2016; Geesink *et al*., 2001). Fibre type composition has been reported to influence colour stability and tenderness in beef, and water holding capacity, colour and eating quality in pork (Klont *et al*., 1998). Fibre type composition was determined using VIA according to the method of Malaty and Bourne (1953). The effect of breed on fibre typing is illustrated in Table 4.6. No differences were observed between breeds for red fibre areas (Type 1) (*P*=0.913), intermediate fibre areas (Type IIA) (*P*=0.712), white fibre areas (Type IIB) (*P*=0.477) and percentage red fibres (*P*=0.201). Differences were observed between breeds for percentage intermediate fibres (*P*=0.018), and percentage white fibres, (*P*=0.001). Angus, Bonsmara and Nguni breeds had the highest intermediate fibre percentage, Charolais had the lowest intermediate fibre percentage, and Brahman did not differ significantly in intermediate fibre percentage to Angus, Bonsmara, Charolais and Nguni. White fibre percentage was higher for Bonsmara and Charolais, and lower for Angus, Brahman and Nguni.

4.1.7 Tenderness related measurements of *m. longissimus dorsi* (LD).

Tenderness is considered the most important quality trait of beef by consumers (Hollung *et al*., 2011). Tenderness depends on a number of factors including the meat grain, the amount of connective tissue, and the amount of fat (Troy and Kerry, 2010). There are several methods to determine tenderness, as will be discussed below. Results that represent mechanisms related

to tenderness are summarised in Table 4.7. There were differences between breeds for Warner Bratzler shear force (P<0.0001), MFL (P= 0.0004), total collagen, (P=0.016) and Collagen solubility (P=0.013). There were no significant differences between breeds for sarcomere length, and percentage IMF. The results showed that Bonsmara and Charolais were more tender, Brahman was the least tender and Angus and Nguni were intermediate. Charolais had higher MFLs and Nguni had the lowest MFLs. Angus, Bonsmara and Brahman had intermediate MFLs. Brahman, Charolais and Nguni had the highest total collagen, Angus was intermediate and Bonsmara had the lowest total collagen. The table also summarises the effect of breed on VIA muscle fibre structure measurements. There were no significant differences between breeds for VIA muscle fibre structure measurements which included fibre detachment.

Table 4.7: Effect of breed on tenderness related measurements of *m. longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM**	***P*-Value**
WBSF (N)	48.03^{ab}	42.95^{c}	56.61^{a}	43.78^{c}	51.04^{ab}	0.24	<0.0001
SL (µm)	1.95	1.92	1.97	2.01	1.90	0.161	0.245
MFL (µm)	27.23^{ab}	21.98^{c}	27.86^{a}	24.41^{bc}	25.96^{ab}	0.026	0.0004
Total collagen (mg/g)	1.64^{ab}	1.47^{b}	1.78^{a}	1.73^{a}	1.85^{a}	0.248	0.016
Insoluble collagen (mg/g)	1.95	1.92	1.97	2.01	1.90	0.161	0.245
Collagen solubility (%)	14.41 ^{a}	13.93 ^{b}	11.82 ^{a}	13.77 ^{a}	15.27 ^{a}	2.123	0.013
IMF (%)	1.76^{a}	1.17^{ab}	1.03^{b}	1.68^{a}	1.41^{ab}	0.691	0.097
VIA muscle fibre structure measurements							
Fibre detachment (%White to red area)	21.59	20.08	20.42	20.47	20.40	5.644	0.502
Fibre breaks score (1-5)	2.93	2.71	2.71	2.63	2.64	1.486	0.727
% Fibre separation score	21.93	19.67	20.96	20.82	21.69	1.783	0.946
Fat area score (1-5)	2.63	2.18	2.11	2.69	2.69	1.406	0.117
Average analyst tenderness score (1-5)	3.61	3.40	3.49	3.54	3.34	1.218	0.635

[1] Standard error of means
[a,b,c] Means in the same row with a different superscript letter differ (P<0.05)
WBSF-Warner Bratzler shear force
SL- Sarcomere length
MFL- Myofibrillar fragment length
IMF- Intramuscular fat
VIA – video image analyses

4.2 Effect of ageing on visual sensory panel evaluation of meat characteristics, on meat visual characteristics, meat colour measurements/evaluations, muscle energy metabolism, protein denaturation, and lipid denaturation, drip-loss and water holding capacity and meat tenderness.

4.2.1 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form, Table 3.2 and 3.3, and visual reference guides, Figures 3.6-3.9).

The effect of ageing and packaging on the visual analysis of meat surface structure is summarised in Table 4.8. The results show that there were differences between ageing period/packaging and meat colour, marbling, fibre separation, structural integrity ($P<0.0001$) and surface texture ($P=0.035$). Meat colour became lighter with ageing between day 3 and day 9, and became darker with ageing from day 9 to day 20, but the day 14 and 20 *post mortem* which were aged in vacuum bags, were not significantly different. The day 3 aged steaks were rated as pink, the day 9 aged steaks as pale pink to pink and the day 14 and 20 steaks were rated as pink to light cherry red.

Marbling which can be seen as flecks or thin strips of fat evenly distributed in a cut of meat and can also be referred to as intramuscular fat. Marbling seemed to become more noticeable and with ageing. The day 3 aged steaks were rated as practically devoid to slight marbling. There were no significant differences between day 9 and 14 vacuum bag aged steaks; the steaks were rated as having slight marbling. The day 20 vacuum bag aged steaks were rated as having slight marbling but were significantly different to other ageing periods.

Fibre separation was rated as no separation (fibres fit tightly together) to slight separation (can just see separation between fibres) for day 3 aged steaks. Days 9, 14 and 20 aged steaks were rated as slight separation (can just see separation between fibres). The day 3 aged steaks had lower separation, followed by the day 9 aged steaks, the days 14 and 20 aged steaks did not differ significantly in fibre separation.

Surface texture for day 3 aged steaks was rated as smooth (can just distinguish between fibres) and days 9, 14 and 20 aged steaks were rated as smooth (but can distinguish fibres) to slightly coarse (fibres well distinguished); there were no significant differences between the days 9, 14 and 20 ageing periods. The structure of the meat became more compressible, or softer with ageing. At day 3 *post mortem* the structural integrity was rated as stiff or hard. Days 9, 14 and 20 were rated as compressible to soft, but there were significant differences between means for ageing periods with the structure becoming softer with ageing.

Table 4.8: Effect of ageing on visual meat characteristics of *m. longissimus dorsi* (LD).

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM**	***P*-Value**
Colour[2]	4.83[b]	4.60[c]	5.77[a]	5.61[a]	0.062	<0.0001
Marbling[2]	1.50[c]	1.84[b]	1.84[b]	2.07[a]	0.745	<0.0001
Fibre separation[2]	1.88[c]	2.11[b]	2.52[a]	2.43[a]	0.033	<0.0001
Surface texture[2]	2.37[b]	2.42[ab]	2.49[a]	2.41[ab]	0.029	0.035
Structural integrity[2]	1.75[d]	2.03[c]	2.40[b]	2.60[a]	0.033	<0.0001

[1] Standard error of means
[2] Visual/sensory measurements
[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05)

4.2.2 Protein, lipid denaturation, drip-loss and water holding capacity

The effect of ageing on protein and lipid denaturation and water binding is summarised in Table 4.9. There were differences between ageing periods for drip-loss, water holding capacity, total protein solubility, myofibril protein solubility, sarcoplasmic protein solubility, TBARS, Thiols and myofibril surface hydrophobicity (P<0.0001). Drip loss increased with ageing, with day 20 *post mortem* as the highest, followed by day 14 and lower for days 3 and 9 *post mortem*, which were not significantly different. Water holding capacity was higher for days 3, 9, and 20; these days were not different and significantly different to the day14, which had lower water holding capacity. Total protein solubility increased with ageing, but day 14 was higher than day 20. The myofibril protein solubility increased with ageing, while sarcoplasmic protein solubility was high on day 20, followed by days 9 and 14, which were not significantly different. The day 3 *post mortem* had the lowest sarcoplasmic protein solubility. TBARS were high on day 9 and days 3, 14 and 20 were lower and not significantly different. Thiols were higher for day 3 *post mortem*; there were no significant differences between days 9 and 14, and between days 14 and 20. Myofibril hydrophobicity increased with ageing, but day 14 was not significantly different to day 20.

Table 4.9: Effect of ageing on protein denaturation and lipid oxidation of *m. Longissimus dorsi* (LD).

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM[1]**	***P*-Value**
Drip-loss	0.24[c]	0.37[c]	1.49[b]	1.75[a]	0.707	<0.0001
WHC	0.36[a]	0.36[a]	0.34[b]	0.35[a]	0.026	0.001
TPS (mg/g)	117.6[d]	145.2[c]	167.3[a]	163.5[b]	5.379	<0.0001
MPS (mg/g)	64.30[d]	80.18[c]	106.20[a]	98.36[b]	1.735	<0.0001
SPS (mg/g)	53.29[c]	65.05[b]	61.13[b]	65.10[a]	5.126	<0.0001
MSH (µg)	73.68[c]	81.55[b]	100.3[a]	112.3[a]	4.098	<0.0001
TBARS (mg/kg)	0.048[b]	0.210[a]	0.036[b]	0.029[b]	0.106	<0.0001
Thiols (µM/mg)	49.76[a]	48.17[b]	47.34[bc]	46.78[c]	4.138	<0.0001

[1] Standard error of means, d *post mortem*=days *post mortem*,
[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05)
WHC- Water holding capacity
TPS- Total protein solubility
MPS- Myofibril protein hydrophobicity
SPS- Sarcoplasmic protein solubility
TBARS- Thiobarbituric acid reactive substances
MSH- Myofibril surface hydrophobicity

4.2.3 Minolta measured colour and related parameters

There were differences between ageing periods for the deoxymyoglobin, oxymyoglobin, Metmyoglobin, metmyoglobin reducing activity, *L**, *a**, *b**, Chroma and Hue angle (P<0.0001), as shown in Table 4.10. There was a higher concentration of deoxymyoglobin for day 3 *post mortem,* followed by the days 9, then 14 and day 20 *post mortem,* which had higher concentrations of deoxymyoglobin. The concentration of oxymyoglobin was higher for day 3, followed by day 9 *post mortem*. Days 14 and 20 had the lowest concentration of oxymyoglobin, and were not significantly different. Metmyoglobin concentration was very high for day 9 *post mortem,* followed by day 14 and 20 that were not significantly different, and day 3 *post mortem* had lower concentration. Metmyoglobin reductase activity was low for day 3 *post mortem* and increased with ageing from day 9 to day 20 *post mortem*.

Instrumental colour results showed that days 3, 14 and 20 *post mortem* steaks were lighter and not significantly different, and day 9 *post mortem* were darker. The day 9 *post mortem* steaks were less red, followed by day 3 *post mortem,* and days 14 and 20 *post mortem* were more red. The *b** values were higher for day 3 *post mortem,* followed by day 9; and days 14 and 20 *post mortem* had lower *b** values. Chroma was higher for days 14 and 20 *post mortem* and lower for day 9 *post mortem*. Days 14 and 20 *post mortem* had higher Chroma values. The Hue angle was higher for day 9 *post mortem,* followed by day 3 *post mortem*. Days 14 and 20 had lower Hue angle values that did not differ significantly.

Table 4.10: Effect of ageing on colour related parameters of *m. longissimus dorsi* (LD).

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM[1]**	***P*-Value**
DeoxyMb %	73.90[a]	80.94[d]	91.43[c]	95.06[b]	4.882	<0.0001
OxyMb %	7.35[c]	2.02[b]	2.21[a]	3.31[a]	0.491	<0.0001
MetMb %	1.84[c]	15.83[a]	2.63[b]	0.63[c]	0.003	<0.0001
MRA (nM reduced/min/g)	8.44 x 10^{-8d}	8.87 x 10^{-8a}	8.69 x 10^{-8b}	8.59 x 10^{-8c}	3.82 x 10^{-9}	<0.0001
Meat colour characteristics (CIE)						
L*	40.2[a]	38.5[b]	40.1[a]	40.4[a]	1.417	<0.0001
a*	11.8[b]	8.3[c]	13.7[a]	13.5[a]	0.417	<0.0001
b*	9.4[a]	8.76[b]	7.6[c]	7.7[c]	0.095	<0.0001
Chroma	15.1[b]	12.2[c]	15.7[a]	15.6[a]	1.517	<0.0001
Hue	38.5[b]	46.7[a]	29[c]	29.8[c]	0.836	<0.0001

[1] Standard error of means, d *post mortem*-days *post mortem*
[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05)
DeoxyMb - Deoxymyoglobin
OxyMb - Oxymyoglobin
MetMb - Metmyoglobin
MRA - Metmyoglobin reductase activity

4.2.4 Tenderness related measurements of *m. longissimus dorsi* (LD).

Figure 4.3 and Table 4.11 indicates the effect of ageing on myofibrillar fragment length (MFL) and Warner Bratzler shear force (WBSF). There were differences between ageing periods for both myofibrillar fragment length and Warner Bratzler shear force (P< 0.0001). Shear force decreased with ageing, but day 14 was not significantly different to day 20 *post mortem*. Myofibril fragment length also decreased with ageing. The day 3 *post mortem* had longer MFLs than day 9, which was followed by day 14, where day 20 *post mortem* had the shortest MFLs.

Table 4.11 also shows that there were differences between ageing periods for the percentage white to red area (P<0.0001), fibre breaks score (P<0.0001), percentage fibre separation score (P<0.0001), and fibre diameter (P<0.0001). There were no significant differences between ageing periods for the average analyst tenderness score, as shown in Table 4.11. The percentage white to red area increased with ageing, but days 3 and 9 were not significantly different, and days 14 and 20 were also not significantly different. The fibre breaks score was higher for day 20 *post mortem* and lower for the day 9 *post mortem*. The percentage fibre separation score was lower for days 3 and 9 *post mortem* and higher for days 14 and 20 *post mortem*. The average analyst tenderness score increased with ageing, but day 3

was not significantly different to day 9. There were no significant differences between fat area score for all ageing periods.

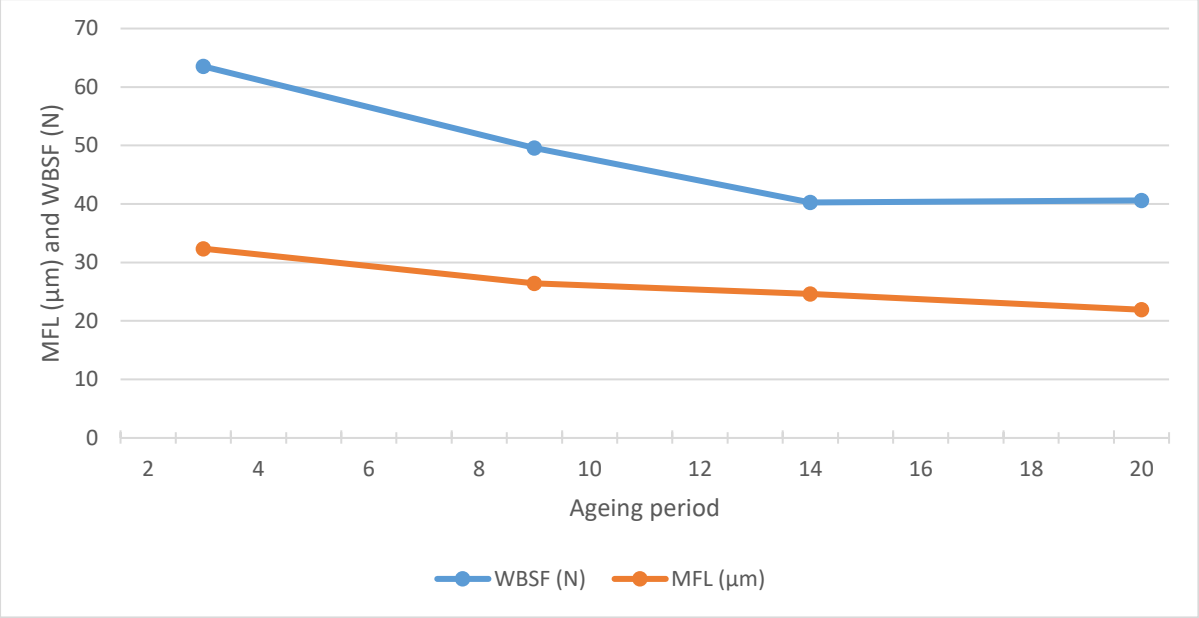


Figure 4.2: Effect of ageing on myofibril fragment length (MFL) and Warner Bratzler shear force (WBSF) of *m. longissimus dorsi* (LD).

Table 4.11: Effect of ageing on tenderness related measurements of *m. longissimus dorsi* (LD).

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM[1]**	***P*-Value**
WBSF (N)	63.53^{a}	49.56^{b}	40.26^{c}	40.58^{c}	0.445	<0.0001
MFL (µm)	34.71^{a}	24.81^{b}	23.09^{c}	21.28^{d}	3.975	<0.0001
VIA muscle fibre structure measurements						
Fibre detachment (%White to red area)	18.14^{b}	18.72^{b}	22.31^{a}	23.21^{a}	0.151	<0.0001
Fibre breaks score (1-5)	2.53^{bc}	2.38^{c}	2.81^{b}	3.16^{a}	1.235	<0.0001
%Fibre separation score	17.135^{b}	15.94^{b}	25.95^{a}	24.83^{a}	1.420	<0.0001
Fat area score (1-5)	2.45	2.33	2.57	2.53	1.498	0.558
Average analyst tenderness score (1-5)	0.25^{c}	0.37^{c}	1.49^{b}	1.75^{a}	0.707	<0.0001

[1] Standard error of means, d *post mortem*- days *post mortem.*
[a,b,c] Means in the same row with a different superscript letter differ (P<0.05).

4.3 Effect of *post mortem* treatment (electrical stimulation and delayed chilling) on carcass characteristics, meat visual characteristics related physiological characteristics, colour measurements/evaluations, muscle energy metabolism, protein denaturation, lipid oxidation and meat tenderness.

4.3.1 Carcass characteristics

The effect of electrical stimulation (ES) on temperature and pH decline at 1, 3, 6 and 20 hours *post mortem* is illustrated in Figure 4.3. Carcasses that were not electrically stimulated (NS) experienced higher pH values as compared to those that were electrically stimulated. pH decline for ES carcasses was faster than the pH decline for NS carcasses. Although ES pH decline was faster, at 20 hours *post mortem*, the pH for both ES and NS was not different (5.50 and 5.41 respectively). Temperature decline for both ES and NS were not significantly different except at three and six hours, where NS was higher than ES.

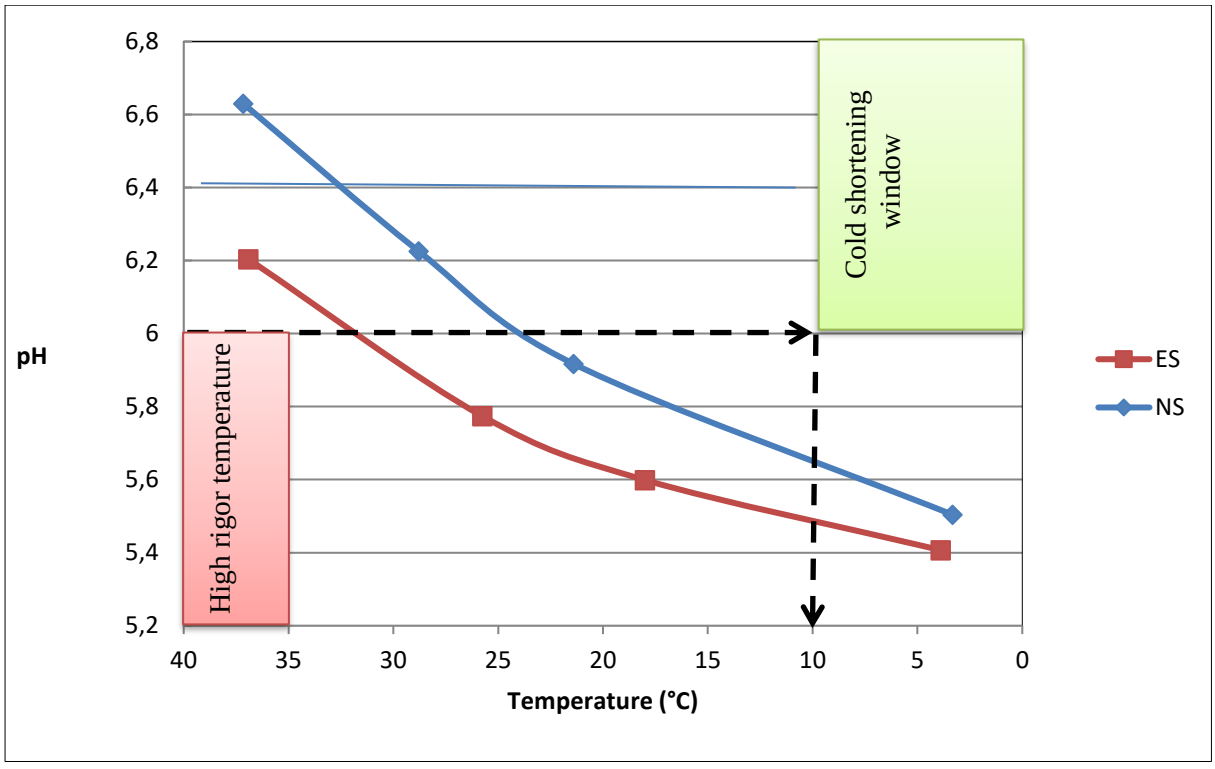


Figure 4.3: Effect of *post mortem* treatment (ES and NS) on temperature and pH decline profiles at 1, 3, 6 and 20 hours *post mortem* of *m. longissimus dorsi* (LD). Cold shortening and high rigor temperature windows according to Pearson and Young (1989) and as discussed in the review of Thompson (2002).

Table 4.12: Effect of treatment (ES and NS) on carcass characteristics of *m. longissimus dorsi* (LD)

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
Warm carcass mass (kg)	104	104.5	14.11	0.801
Cold carcass mass (kg)	101.4	102	13.51	0.056
Eye Muscle Area (mm^2)	6205	5825	75.10	0.385

[1] Standard error of means.

Table 4.12 shows the effect of electrical stimulation on carcass characteristics. There were no significant differences in carcass characteristics between electrically stimulated and non-stimulated carcasses. These included warm carcass mass, cold carcass mass and eye muscle area.

4.3.2 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form (Tables 3.2 and 3.3) and visual reference guides, Figures 3.6-3.9).

Table 4.13: Effect of treatment (ES and NS) on visual meat characteristics (colour, marbling, fibre separation, surface texture and structural integrity) of m. *longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
Colour[2]	5.13	5.28	0.074	0.106
Marbling[2]	1.84	1.79	0.033	0.379
Fibre separation[2]	2.16^{b}	2.31^{a}	0.034	0.002
Surface texture[2]	2.39	2.45	0.034	0.166
Structural integrity[2]	2.19	2.20	0.030	0.890

[1] Standard error of means

[2] Visual/sensory measurements

[a,b] Means in the same row with a different superscript letter differ ($P<0.05$)

The effect of treatment on visual meat characteristics is summarised in Table 4.13. There were no significant differences between steaks from electrically stimulated carcasses and those from carcasses that were not electrically stimulated for the visual analysis measurements, which included the meat colour, marbling, fibre separation, surface texture, and structural integrity. Differences were observed between ES and NS for fibre separation ($P= 0.002$), with ES steaks having less fibre separation than NS steaks.

4.3.3 Protein, lipid denaturation and water holding capacity

Table 4.14 summarises the effect of treatment (ES and NS) on protein and lipid denaturation and water binding. There were differences between electrically stimulated and non-stimulated samples from carcasses, which were analysed for drip loss (P=0.026), total protein solubility (P<0.0001), myofibril protein solubility (P= 0.001), sarcoplasmic protein solubility (P=0.009), TBARS (P=0.004), and myofibril surface hydrophobicity (P=0.001). Drip loss, TBARS and myofibril surface hydrophobicity were high in samples from electrically stimulated carcasses and low in samples from carcasses, which were not electrically stimulated. Total protein solubility, myofibril protein solubility and TBARS were high in non-stimulated carcasses and low in stimulated carcasses. Water holding capacity showed no significant differences between stimulation and non-stimulation.

Table 4.14: Effect of treatment (ES and NS) on protein denaturation and lipid oxidation of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
Drip-loss	1.10[a]	0.82[b]	1.230	0.026
WHC	0.35	0.36	0.051	0.160
TPS (mg/g)	145.5[b]	151.4[a]	6.617	<0.0001
MPS (mg/g)	85.34[b]	89.18[a]	4.783	0.001
SPS (mg/g	60.10[b]	62.19[a]	2.756	0.009
MSH (µg)	91.84[a]	86.19[b]	1.698	0.001
TBARS (mg/kg)	0.100[a]	0.061[b]	0.131	0.004
Thiol (µM/mg)	48.01	48.02	1.951	0.984

[1] Standard error of means.

[a,b] Means in the same row with a different superscript letter differ (P<0.05).

WHC- Water holding capacity

TPS- Total protein solubility

MPS- Myofibril protein hydrophobicity

SPS- Sarcoplasmic protein solubility

TBARS- Thiobarbituric acid reactive substances

MSH- Myofibril surface hydrophobicity

4.3.4 Colour related parameters

The effect of treatment (ES and NS) on colour related parameters is summarised in Table 4.15. There were differences (P>0.05) between treatments for deoxymyoglobin concentration (P=0.016), metmyoglobin concentration (P=0.073), L^*(P= 0.014), a^*(P=0.043), b^* (P=0.020), and Chroma (P=0.027). The L^*, a^*, b^*, and Chroma were higher for samples from electrically stimulated carcasses and lower for samples from non-stimulated carcasses. There were no significant differences between ES and NS for oxymyoglobin, metmyoglobin concentration and metmyoglobin reductase activity.

Table 4.15: Effect of treatment (ES and NS) on colour related parameters of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
DeoxyMb %	79.58[a]	76.95[b]	1.731	0.016
OxyMb %	17.02	16.40	2.101	0.309
MetMb %	3.40	4.59	0.558	0.073
MRA (nM reduced/min/g)	8.64 x 10^8	8.65 x 10^8	4.123 x 10^{17}	0.850
Meat colour characteristics (CIE)				
L*	40.2[a]	39.4[b]	3.502	0.014
a*	12.1[a]	11.6[b]	0.613	0.043
b*	8.6[a]	8.1[b]	0.003	0.020
Chroma	15[a]	14.3[b]	0.045	0.027
Hue angle	35.5	35.1	0.372	0.256

[1] Standard error of means.

[a,b] Means in the same row with a different superscript letter differ (P<0.05).

DeoxyMb- Deoxymyoglobin
OxyMb- Oxymyoglobin
MetMb-Metmyoglobin
MRA- Metmyoglobin reductase activity

4.3.5 Tenderness related parameters

Table 4.16 shows the effect of treatment on tenderness related measurements which are Warner Bratzler shear force, myofibril fragment length, and muscle fibre related VIA measurements (percentage white to red area, fibre breaks score (1-5), percentage fibre separation score, fat area score (1-5), fibre diameter, and overall tenderness score). There were no significant differences between treatments for Warner Bratzler Shear force, sarcomere length and myofibril fragment length. Electrical stimulation showed a different effect on WBSF and sarcomere length. There were differences between ES and NS for WBSF (P<0.0001)) and SL (P=0.007). The shear force for steaks from electrically stimulated carcasses was lower than shear force for steaks, which were from carcasses not electrically stimulated.

Table 4.16: Effect of treatment on tenderness related measurements of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
WBSF (N)	41.94[b]	55.02[a]	1.071	<0.0001
SL (µm)	1.91[b]	1.99[a]	0.161	0.007
MFL (µm)	25.71	26.23	2.014	0.565
VIA muscle fibre structure measurements				
Fibre detachment (%White to red area)	20.95	20.24	5.644	0.207
Fibre breaks score (1-5)	2.82	2.63	1.486	0.206
% Fibre separation score	20.73	21.32	0.782	0.732
Fat area score (1-5)	2.48	2.46	0.041	0.945
Analyst tenderness score (1-5)	3.57	3.38	0.483	0.122

[1] Standard error of means
[a,b] Means in the same row with a different superscript letter differ ($P<0.05$)
WBSF-Warner Bratzler shear force
SL- Sarcomere length
MFL- Myofibrillar fragment length

There were no significant differences between ES and NS for percentage white to red area, fibre breaks score, percentage fibre separation score, fibre diameter and tenderness score as shown in Table 4.16.

4.4 Effect of breed and ageing interaction on visual sensory panel evaluation of meat characteristics, meat colour measurements/evaluations, muscle energy metabolism, protein denaturation, lipid oxidation and meat tenderness.

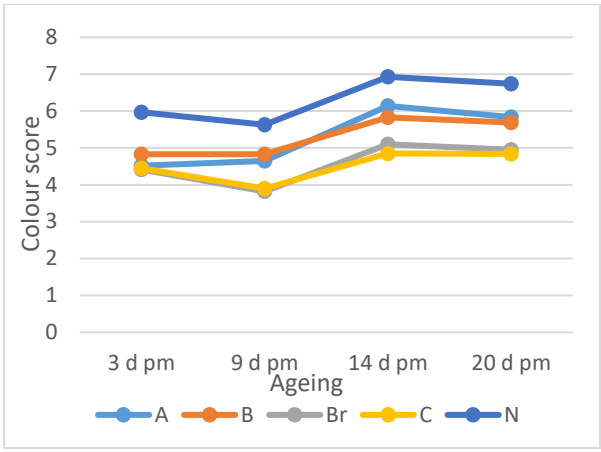
4.4.1 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form, Tables 3.2 and 3.3, and visual reference guides, Figures 3.6-3.9).

The effect of breed and ageing/packaging combination on visual colour, marbling, fibre separation, surface texture and structural integrity is summarised in Table 4.17 and Figure 4.5 (A-E). There was a significant interaction between means of breed and ageing/packaging for meat colour (P=0.009), marbling (P=0.014), fibre separation (P=0.001), surface texture (P=0.012), and structural integrity (P=0.006). The day 3 and 9 were aged in display cabinet and the panel could not see differences between steaks that were aged for 3 and 9 days for the Angus, Bonsmara and Nguni. Differences were observed for the Brahman and Charolais, steaks from these breeds became lighter with ageing. As compared to vacuum packaged steaks, it was observed that the steaks aged in display cabinet were lighter than those aged in vacuum bags, which were darker. There was not much differences between steaks aged for 14 and 20 days. Marbling seems to be more prominent with ageing for all the breeds, with the Angus having more marbling than the other breeds. The fibre separation ratings showed that fibre separation increases with ageing for all the breeds. There were not much significant differences between the breeds in surface texture of the steaks. Results for structural integrity showed that the steaks became more compressible as ageing progresses for all the breeds.

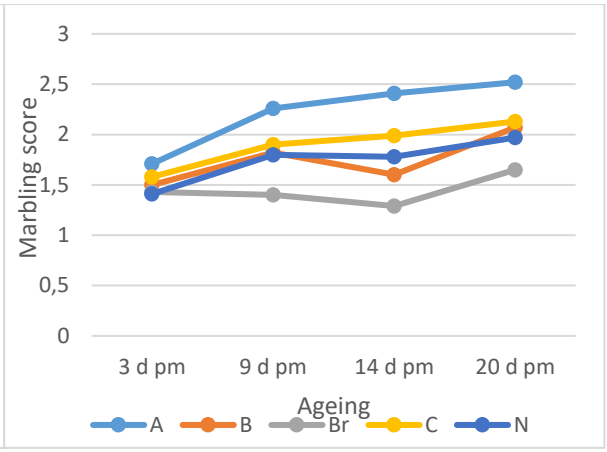
Table 4.17: Effect of breed X ageing interaction on sensory panel analyses of *m. longissimus dorsi* (LD)

	Beef Breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Colour[2]						0.084	0.009
3 d *post mortem*	4.52^{fgh}	4.83^{defg}	4.41^{h}	4.45^{gh}	5.97^{cb}		
9 d *post mortem*	4.65^{efgh}	4.83^{defg}	3.82^{i}	3.90^{i}	5.63^{c}		
14 d *post mortem*	6.14^{b}	5.83^{cb}	5.10^{d}	4.85^{efd}	6.93^{a}		
20 d *post mortem*	5.84^{cb}	5.69^{c}	4.95^{de}	4.84^{defg}	6.74^{a}		
Marbling[2]						1.117	0.014
3 d *post mortem*	1.71^{ghij}	1.50^{klm}	1.43^{lm}	1.58^{jkl}	1.41^{lm}		
9 d *post mortem*	2.26^{bc}	1.82^{fgh}	1.40^{lm}	1.90^{efg}	1.80^{fghi}		
14 d *post mortem*	2.41^{ab}	1.60^{ijkl}	1.29^{m}	1.99^{def}	1.78^{fghij}		
20 d *post mortem*	2.52^{a}	2.07^{cde}	1.65^{hijk}	2.13^{cd}	1.97^{def}		
Fibre separation[2]						1.101	0.001
3 d *post mortem*	1.86^{g}	1.91^{fg}	1.60^{h}	1.91^{fg}	2.11^{def}		
9 d *post mortem*	2.08^{def}	2.08^{def}	1.99^{efg}	2.17^{de}	2.22^{de}		
14 d *post mortem*	2.74^{a}	2.47b^{c}	2.16^{de}	2.74^{a}	2.47^{bc}		
20 d *post mortem*	2.61^{abc}	2.20^{d}	2.20^{d}	2.67^{ab}	2.44^{c}		
Surface texture[2]						0.983	0.012
3 d *post mortem*	2.42^{defg}	2.34^{efgh}	2.03^{j}	2.36^{defgh}	2.70^{a}		
9 d *post mortem*	2.53^{abcd}	2.34^{efgh}	2.22^{hi}	2.34^{efgh}	2.65^{abc}		
14 d *post mortem*	2.66^{abc}	2.35^{defgh}	2.14^{ij}	2.62^{abc}	2.67^{ab}		
20 d *post mortem*	2.48^{cde}	2.30^{fghi}	2.28^{ghi}	2.51^{bcde}	2.48^{cdef}		
Structural integrity[2]						0.011	0.006
3 d *post mortem*	1.58^{k}	1.88^{hi}	1.62^{jk}	1.87^{hi}	1.82^{ij}		
9 d *post mortem*	1.96^{ghi}	1.97^{ghi}	1.97^{ghi}	2.16^{efg}	2.07^{fgh}		
14 d *post mortem*	2.48^{bc}	2.41^{bcd}	2.25^{def}	2.53^{b}	2.31^{cde}		
20 d *post mortem*	2.79^{a}	2.56^{b}	2.37^{bcd}	2.86^{a}	2.42^{bcd}		

[1] Standard error of means
[2] Visual/sensory measurements
^{a,b,c,d,e,f,g,h,I,j,k} Means in the same row with a different superscript letter differ (P<0.05)



A



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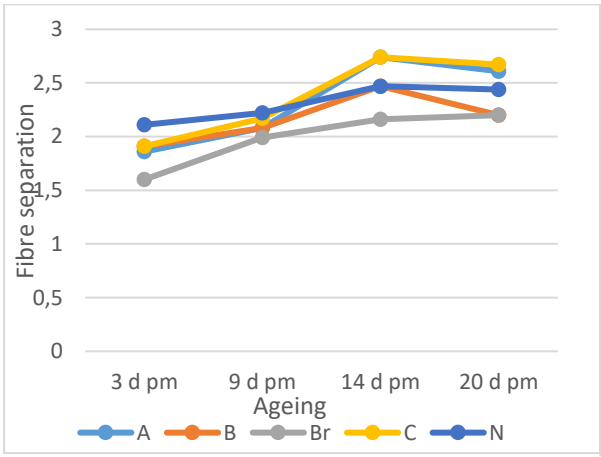

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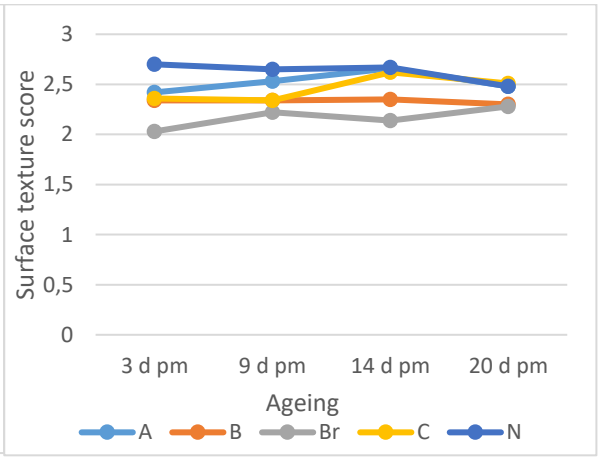

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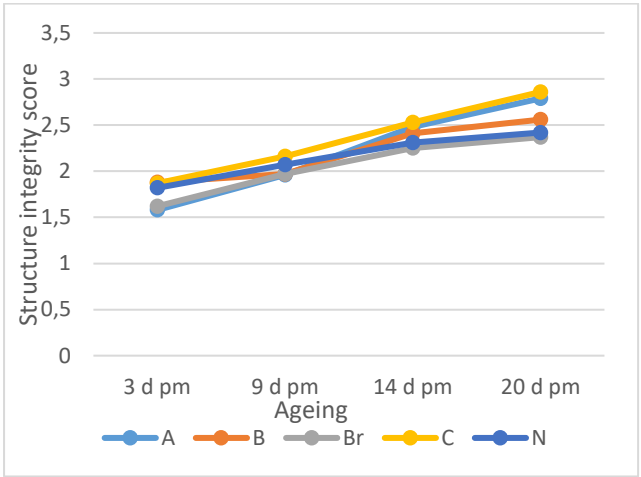

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Figure 4.4: Effect of breed X ageing interaction on colour (A), marbling (B), fibre separation (C), Surface texture and (D) and structural integrity of *m. longissimus dorsi* (LD).

4.4.2 Protein denaturation and lipid oxidation

Table 4.18: Effect of breed X ageing interaction on protein denaturation and lipid oxidation of *m. longissimus dorsi* (LD).

	Beef breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Drip-loss						0.707	0.513
3 d *post mortem*	0.09^{f}	0.40^{f}	0.27^{f}	0.29^{f}	0.18^{f}		
9 d *post mortem*	0.30^{f}	0.36^{f}	0.42^{f}	0.43^{f}	0.37^{f}		
14 d *post mortem*	1.16^{e}	1.56^{bcde}	1.88^{abc}	1.49^{cde}	1.36^{de}		
20 d *post mortem*	1.49^{cde}	1.66^{bcd}	1.95^{ab}	2.14^{a}	1.51^{bcde}		
WHC						0.00161	0.566
3 d *post mortem*	0.35^{efg}	0.37^{abc}	0.35^{cdef}	0.34^{fgh}	0.37^{ab}		
9 d *post mortem*	0.35^{efgh}	0.35^{efgh}	0.35^{def}	0.34^{fgh}	0.38^{a}		
14 d *post mortem*	0.34^{fghi}	0.37^{abcd}	0.33^{ghi}	0.32^{i}	0.36^{bcde}		
20 d *post mortem*	0.34^{fgh}	0.38^{a}	0.35^{efgh}	0.33^{hi}	0.37^{abc}		
TPS (mg/g)						1.379	0.0001
3 d *post mortem*	109.80^{h}	119.97^{g}	117.82^{g}	118.40^{g}	121.99^{g}		
9 d *post mortem*	137.35^{f}	145.27^{e}	149.61^{cde}	147.54^{de}	146.41^{de}		
14 d *post mortem*	168.50^{ab}	167.49^{b}	169.85^{ab}	156.02^{c}	174.79^{a}		
20 d *post mortem*	164.11^{b}	165.56^{b}	153.25^{cd}	153.25^{cd}	168.53^{ab}		
MPS (mg/g)						1.735	<0.0001
3 d *post mortem*	56.81^{l}	64.57^{k}	64.83^{k}	66.15^{k}	69.14^{jk}		
9 d *post mortem*	73.94^{ij}	76.88^{hi}	87.50^{g}	83.42^{gh}	79.17^{hi}		
14 d *post mortem*	107.28^{abc}	104.37^{bcd}	110.56^{ab}	95.18^{ef}	113.62^{a}		
20 d *post mortem*	100.06^{cde}	99.60^{de}	103.19^{cd}	88.34^{fg}	100.61^{cde}		
SPS (mg/g)						1.126	0.382
3 d *post mortem*	52.99^{i}	55.40^{i}	52.99^{i}	52.25i	52.85^{i}		
9 d *post mortem*	63.42^{defg}	68.39^{a}	62.11^{efgh}	64.13^{cdef}	67.23^{abc}		
14 d *post mortem*	61.22^{fgh}	63.12^{defg}	59.29^{h}	60.84^{gh}	61.18^{fgh}		
20 d *post mortem*	64.05^{cdef}	65.96^{abcd}	62.67^{efg}	64.91^{bcde}	67.92^{ab}		
TBARS (mg/kg)						0.00106	0.390
3 d *post mortem*	0.04^{c}	0.05^{c}	0.06^{c}	0.06^{c}	0.04^{c}		
9 d *post mortem*	0.18^{b}	0.29^{a}	0.23^{ab}	0.19^{b}	0.17^{b}		
14 d *post mortem*	0.04^{c}	0.04^{c}	0.04^{c}	0.03^{c}	0.04^{c}		
20 d *post mortem*	0.023^{c}	0.03^{c}	0.04^{c}	0.03^{c}	0.03^{c}		
Thiols (µM/mg)						2.138	0.992
3 d *post mortem*	48.70^{abcd}	50.32^{a}	50.72^{a}	49.37^{abc}	49.71^{ab}		
9 d *post mortem*	47.48^{bcde}	47.48^{bcde}	48.49^{abcde}	49.11^{abcd}	48.28^{abcde}		

14 d *post mortem*	46.94^{cde}	46.65^{de}	48.60^{abcde}	47.62^{bcde}	46.89^{cde}		
20 d *post mortem*	46.34^{e}	46.52^{e}	47.50^{bcde}	46.92^{cde}	46.62^{de}		
MSH (µg)						4.098	0.069
3 d *post mortem*	80.77^{efgh}	74.62^{ghij}	70.340^{ij}	73.160^{hij}	69.510^{j}		
9 d *post mortem*	85.40^{ef}	81.27^{efgh}	84.211^{ef}	82.295^{efg}	75.060^{ghij}		
14 d *post mortem*	78.626^{fghi}	86.23^{ef}	85.860^{ef}	112.620^{b}	87.460^{e}		
20 d *post mortem*	106.59^{cb}	98.24^{d}	128.147^{a}	129.474^{a}	99.768^{cd}		

[1] Standard error of means

[a,b,c,d,e,f,g,h,I,j] Means in the same row with a different superscript letter differ (P<0.05)

WHC- Water holding capacity

TPS- Total protein solubility

MPS- Myofibril protein hydrophobicity

SPS- Sarcoplasmic protein solubility

TBARS- Thiobarbituric acid reactive substances

MSH- Myofibril surface hydrophobicity

Table 4.18 shows the effect of breed and ageing/packaging combination on protein, lipid denaturation and water holding capacity. There were differences between means for the interactions of breed and ageing period for total protein solubility (P=0.0001), and myofibrillar protein solubility (P<0.0001). Solubility of total protein and myofibrillar proteins increased with ageing for all the breeds. There were no significant interactions (P>0.05) between the means for breed and ageing period for water holding capacity, sarcoplasmic protein solubility, TBARS, free thiol groups and myofibril surface hydrophobicity. This basically means that although significant differences were observed between the ageing periods, the pattern of significance was similar between the different breeds. For the drip loss, there was an increase in drip loss with ageing, with the day three *post mortem* having lower drip loss than the day nine *post mortem*. The vacuum packaged samples had higher drip loss than the steaks aged in display cabinet, with the day 20 *post mortem* higher than the day 14 *post mortem*. Sarcoplasmic protein solubility increased with ageing, with the day 9 *post mortem* higher than the day 3 *post mortem*, and the day 20 *post mortem* was not significantly different to the day 14 *post mortem*. TBARS were higher for the day 9, and days 3, 14 and 20 were not significantly different. The amount of free thiol groups increased with ageing. The concentration of hydrophobic myofibrillar proteins also increased with ageing.

4.4.3 Colour related parameters

Table 4.19: Effect of breed and ageing combination on colour related parameters of *m. longissimus dorsi* (LD)

	Beef breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
DeoxyMb %						0.882	0.468
3 d *post mortem*	93.12^{a}	95.06^{a}	91.5^{a}	93.51^{a}	93.51^{a}		
9 d *post mortem*	72.54^{ef}	75.67^{g}	77.67^{fg}	70.88^{fg}	71.13^{fg}		
14 d *post mortem*	100.8^{bcd}	99.3^{cd}	101.1^{bcd}	97.3^{de}	101.9^{bcd}		
20 d *post mortem*	103.0^{bc}	104.7^{bc}	103.5^{bc}	102.1^{bcd}	105.7^{b}		
OxyMb %						0.491	0.463
3 d *post mortem*	6.81^{c}	8.85^{ab}	9.09^{a}	9.35	9.63^{a}		
9 d *post mortem*	0.96^{h}	2.04^{gh}	3.96^{g}	0.72^{h}	2.42^{gh}		
14 d *post mortem*	5.57^{de}	5.85^{de}	6.49^{c}	8.77^{ab}	6.33^{c}		
20 d *post mortem*	7.33^{bc}	7.04^{bc}	7.76^{bc}	5.26^{de}	5.1^{def}		
MetMb %						0.260	0.361
3 d *post mortem*	2.15^{f}	1.306^{ef}	1.71^{f}	1.779^{f}	2.25^{f}		
9 d *post mortem*	12.75^{b}	16.275^{b}	20.25^{a}	14.6^{35b}	15.239^{b}		
14 d *post mortem*	2.45^{cde}	3.30^{cd}	4.037^{c}	2.548^{cde}	0.80^{cdef}		
20 d *post mortem*	0.34^{def}	1.25^{ef}	1.01^{ef}	0.30^{def}	0.247^{def}		
MRA (nM reduced/min/g)						1.46E-17	0.003
3 d *post mortem*	8.51E-8^{ghijk}	8.34E-8^{jk}	8.31E-8^{jk}	8.66E-8^{defgh}	8.40E-8^{ijk}		
9 d *post mortem*	8.76E-8^{bcdef}	8.84E-8^{bcde}	8.83E-8^{bcde}	8.95E-8^{abc}	8.97E-8^{ab}		
14 d *post mortem*	8.61E-8^{efghi}	8.46E-8^{hijk}	8.46E-8^{hijk}	8.87E-8^{abcd}	9.08E-8^{a}		
20 d *post mortem*	8.53E-8^{fghij}	8.29E-8^{k}	8.69E-8^{defg}	8.70E-8^{defg}	8.72E-^{cdefg}		
Meat colour characteristics							
L*						1.676	0.319
3 d *post mortem*	40.4^{de}	39.1^{f}	41.5^{c}	42.6^{ab}	37.6^{h}		
9 d *post mortem*	37.8^{h}	37.8^{gh}	40.3^{de}	41.3^{cd}	35.4^{i}		
14 d *post mortem*	38.9^{fg}	39.6^{ef}	41.7^{bc}	43.2^{a}	37.1^{h}		

20 d *post mortem*	39.6^{ef}	39.2^{f}	42.1^{bc}	43.3^{a}	37.5^{h}		
a*						1.417	0.582
3 d *post mortem*	12.2^{cd}	11.9^{d}	11.5^{d}	11.5^{d}	12^{d}		
9 d *post mortem*	9.3^{e}	8.1^{f}	8^{f}	7.8^{f}	8.2^{f}		
14 d *post mortem*	13.7^{ab}	14^{a}	13.9^{a}	13.1^{ab}	13.9^{a}		
20 d *post mortem*	13.9^{a}	13.4^{ab}	13.6^{ab}	12.9^{bc}	13.9^{ab}		
b*						1.091	0.440
3 d *post mortem*	9.7^{a}	9^{bc}	9.7^{a}	9.6^{ab}	8.9^{bcd}		
9 d *post mortem*	8.9^{bcd}	8.5^{cde}	9.4^{ab}	9.2^{ab}	7.9^{efg}		
14 d *post mortem*	7.2^{h}	7.6^{fgh}	8.1^{ef}	7.9^{efg}	7.1^{h}		
20 d *post mortem*	7.5^{fgh}	7.3^{gh}	8.3^{de}	8^{efg}	7.5^{fgh}		
Chroma						0.517	0.359
3 d *post mortem*	15.6^{abc}	14.9^{c}	15^{bc}	14.9^{c}	15^{c}		
9 d *post mortem*	13^{d}	11.8^{ef}	12.4^{de}	12.1^{def}	11.4^{f}		
14 d *post mortem*	15.4^{abc}	15.9^{ab}	16.2^{a}	15.3^{abc}	15.6^{abc}		
20 d *post mortem*	15.8^{abc}	15.3^{abc}	16^{ab}	15.2^{abc}	15.7^{abc}		
Hue angle						0.264	0.726
3 d *post mortem*	38.4^{c}	37.2^{c}	40.3^{b}	39.9^{b}	36.7^{c}		
9 d *post mortem*	43.7^{b}	46.1^{b}	49.6^{a}	49.7^{a}	44.1^{b}		
14 d *post mortem*	27.7^{g}	28.5^{g}	30.3^{f}	31.1^{f}	27.1^{g}		
20 d *post mortem*	28.4^{e}	28.6^{de}	31.3^{cd}	31.7^{f}	28.9^{cde}		

[1] Standard error of means, d *post mortem*=days *post mortem*

^{a,b,c,d,e,f,g,h,I,j,k} Means in the same row with a different superscript letter differ (P<0.05)

DeoxyMb- Deoxymyoglobin
OxyMb- Oxymyoglobin
MetMb-Metmyoglobin
MRA- Metmyoglobin reductase activity

The effect of breed and ageing combination on meat colour related parameters is shown in Table 4.19. There were significant interactions between means for breeds and ageing/packaging for metmyoglobin reductase activity (P= 0.003). There were no significant interactions between the means for deoxymyoglobin, oxymyoglobin, metmyoglobin, *L**, *a**, *b**, Chroma and Hue angle (P> 0.05) between breeds and ageing periods. Deoxymyoglobin increased with ageing for all the breeds, oxymyoglobin decreased with ageing between the days 3 and 9, and increased with ageing between the days 14 and 20 *post mortem*. The concentration

of metmyoglobin was higher for day 9 *post mortem* for all the breeds. Metmyoglobin reductase activity increased with ageing for all the breeds. The *L** values showed that the meat became lighter between day 3 and 9 *post mortem*; and with vacuum packaging, the meat became darker between days 14 and 20 *post mortem*. The *a**, *b**, Chroma and hue angle values decreased with ageing between days 3 and 9, and there were no significant differences between days 14 and 20.

4.4.4 Muscle energy metabolism

Table 4.20: Effect of breed and breed X ageing on muscle energy metabolism

	Cattle breed						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM**	***P*-Value**
Lactate (µM/g)							
1 hr *post mortem*	31.41	29.91	34.45	31.66	32.19	0.713	0.320
20 hr *post mortem*	72.75^{a}	74.06^{a}	72.08^{a}	65.36^{b}	69.76^{ab}	1.932	0.008
Glucose (µM/g)							
1 hr *post mortem*	1.35^{b}	1.50^{ab}	1.41^{b}	1.42^{b}	1.72^{a}	0.380	0.030
20 hr *post mortem*	3.23	3.26	3.37	2.95	3.35	0.530	0.389
Glycogen (µM/g)							
1 hr *post mortem*	30.55	33.66	34.37	30.68	28.48	1.203	0.476
20 hr *post mortem*	9.50	11.68	9.13	8.01	7.85	0.343	0.352
Glucose-6-Phosphate (µM/g)							
1 hr *post mortem*	4.00^{a}	4.22^{a}	4.24^{a}	3.14^{b}	3.62^{ab}	1.231	0.028
20 hr *post mortem*	7.21	7.62	8.36	6.81	6.51	0.489	0.176
ATP (µM/g)							
1 hr *post mortem*	5.85	5.62	5.71	5.83	5.61	0.597	0.788
20 hr *post mortem*	2.53	2.43	2.31	2.62	2.22	0.401	0.274
Creatine Phosphate (µM/g)							
1 hr *post mortem*	3.23	3.14	2.93	3.14	2.87	1.377	0.854
20 hr *post mortem*	1.80	1.64	1.61	1.84	1.56	0.127	0.064

[1] Standard error of means, d *post mortem*=days *post mortem*
[a,b] Means in the same row with a different superscript letter differ ($P<0.05$)

Table 4.20 summarises the effect of breed and time *post mortem* in hours on muscle energy metabolism. There were differences between breed and time *post mortem* means for lactate concentration at 20 hours ($P=0.008$), glucose concentration at one hour ($P=$ 0.030), glucose-6-phosphate concentration at one hour ($P=0.028$), as shown in Table 4.20. The Angus, Bonsmara, Brahman and Nguni had higher lactate concentrations, while the Charolais had the lowest lactate concentration. The concentration of glucose at one hour was higher for the Nguni

and lower for the other breeds. Glucose-6-Phosphate concentration was higher for the Angus, Bonsmara, Brahman and Nguni and lower for the Charolais. There were no significant differences between the breeds for lactate one hour, glucose 20 hours, Glucose-6-phosphate 20 hours, ATP one hour and 20 hours, and creatine phosphate one hour and 20 hours.

4.4.5 Tenderness related measurements

Table 4.21: Effect of breed and ageing interaction on tenderness related of *m. longissimus dorsi* (LD)

	Cattle breed						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM**	***P*-Value**
WBSF (N)						0.445	<0.0001
3 d *post mortem*	7.11^{a}	6.52^{b}	6.59^{b}	5.84^{c}	7.11^{a}		
9 d *post mortem*	5.25^{e}	4.70^{f}	5.54^{d}	4.76^{f}	5.60^{cd}		
14 d *post mortem*	4.23^{gh}	3.94^{i}	4.66^{f}	3.97^{hi}	4.35^{g}		
20 d *post mortem*	3.63^{j}	3.35^{kl}	3.82^{ij}	3.24^{l}	3.62^{jk}		
MFL (µm)						0.175	0.0001
3 d *post mortem*	39.58^{a}	28.84^{c}	38.98^{a}	32.35^{b}	33.81^{b}		
9 d *post mortem*	26.17^{de}	21.41^{ij}	26.84^{cd}	24.23^{efgh}	25.37^{def}		
14 d *post mortem*	23.15^{fghi}	20.56^{jk}	25.02^{defg}	22.53^{hij}	24.20^{efgh}		
20 d *post mortem*	21.95^{hij}	18.73^{k}	22.54^{hij}	20.60^{jk}	22.58^{ghij}		
VIA muscle fibre structure measurements							
Fibre detachment (%White to red area)						1.151	0.018
3 d *post mortem*	21.34^{bcde}	16.92^{gh}	19.42^{defg}	17.00^{gh}	16.04^{h}		
9 d *post mortem*	19.826^{cdef}	18.63^{fg}	18.20^{fgh}	18.82^{efg}	18.12^{fgh}		
14 d *post mortem*	21.28^{bcde}	21.80^{abcd}	22.22^{abc}	22.81^{ab}	23.45^{ab}		
20 d *post mortem*	23.94^{a}	22.98^{ab}	21.86^{abcd}	23.249^{ab}	24.01^{a}		
Fibre breaks score (1-5)						1.235	0.302
3 d *post mortem*	3.14^{abcd}	2.39^{defg}	2.59^{bcdefg}	2.47^{cdefg}	2.00^{g}		
9 d *post mortem*	2.72^{abcdefg}	2.30^{fg}	2.58^{bcdefg}	2.33^{efg}	2.00^{g}		
14 d *post mortem*	2.60^{abcdefg}	2.91^{abcdef}	2.80^{abcdef}	2.80^{abcdef}	3.18^{abc}		
20 d *post mortem*	3.25^{ab}	3.22^{abc}	2.88^{abcdef}	3.10^{abcde}	3.38^{a}		
% Separation score						1.420	0.921
3 d *post mortem*	20.00^{abcdefg}	13.21^{g}	20.00^{abcdefg}	15.30^{defg}	16.72^{cdefg}		
9 d *post mortem*	14.01^{fg}	15.00^{efg}	16.58^{cdefg}	17.92^{bcdefg}	15.76^{defg}		
14 d *post mortem*	25.16^{abcde}	27.33^{abc}	24.86^{abcdef}	23.38^{abcdef}	29.44^{a}		
20 d *post mortem*	28.06^{ab}	22.50^{abcdefg}	22.50^{abcdefg}	26.39^{abcd}	24.41^{abcdef}		
Fat score 1-5						0.050	0.943

3 d *post mortem*	2.58[a]	2.38[a]	1.78[a]	2.60[a]	2.67[a]		
9 d *post mortem*	2.70[a]	2.31[a]	1.77[a]	2.70[a]	2.36[a]		
14 d *post mortem*	2.57[a]	2.10[a]	2.75[a]	2.56[a]	2.82[a]		
20 d *post mortem*	2.7[a]	1.88[a]	2.08[a]	2.87[a]	2.91[a]		
Analyst tenderness score (1-5)						0.946	0.002
3 d *post mortem*	3.70[abcd]	2.65[gh]	3.30[cdef]	2.80[fgh]	2.55[h]		
9 d *post mortem*	3.30[cdef]	3.10[efgh]	3.15[defg]	3.35[cdef]	2.65[gh]		
14 d *post mortem*	3.45[bcde]	3.80[abc]	3.85[abc]	3.80[abc]	4.00[ab]		
20 d *post mortem*	4.00[ab]	4.05[a]	3.65[abcde]	4.20[a]	4.15[a]		

[1] Standard error of means

[a,b,c,d,e,f,g,h] Means in the same row with a different superscript letter differ (P<0.05).

Table 4.21 shows the effect of breed and ageing on tenderness related measurements. There were significant interactions between the breed and ageing/packaging means for Warner Bratzler shear force (P<0.0001), myofibril fragment length (P=0.0001), fibre detachment (P=0.018), and analyst tenderness score (P=0.002). Shear force decreased with ageing for all the breeds. At day 3 *post mortem*, the Angus and Nguni breeds produced the least tender meat but at day 20, the tenderness was not different to the Bonsmara and Brahman. The Charolais produced more tender meat at all ageing periods than all other breeds. The Angus and Brahman had the longest myofibril fragments, but at day 20, the MFLs were not significantly different to the Nguni and Charolais. Bonsmara produced the shortest myofibril fragments at all ageing periods. Fibre detachment increased with ageing for all the breeds. Bonsmara and Brahman had the highest detachment of fibre score at day 3, but at day 20 *post mortem*, the detachment score was not different to the other breeds. Analyst tenderness score increased with ageing for all breeds. At day 3 *post mortem*, Angus breed had the lowest tenderness score, but at day 20, tenderness score was not significantly different for all breeds. There were no significant interactions (P>0.05) between breed and ageing period means for fibre breaks score, percentage fibre separation score, and fat area score. There were no significant differences in fibre breaks score between the ageing periods for the Angus, Brahman and Charolais. Significant differences were only observed as ageing progresses for Bonsmara and Nguni. Fat area score was not significantly different for all breeds in all ageing periods. Tenderness score increased with ageing for Bonsmara, Charolais and Nguni, with no significant differences observed in all ageing periods for the Angus and Brahman.

4.5 Correlation coefficients

Correlation coefficients between visual measurements (colour, marbling, fibre separation, texture and structural integrity) and all tested meat quality attributes are shown in Tables 4.22-4.25. The $P<0.05$ indicates significant correlations between variables and $P>0.05$ indicates insignificant correlations between variables. Only significant correlations will be mentioned ($P<0.05$), and are bolded on the Tables. Correlations above $r= 0.3$ are acceptable and regarded as good, correlations above $r=0.7$ are regarded as very good.

Table 4.22 shows correlation coefficients between visual attributes (colour, marbling, fibre separation, texture, and structural integrity ratings; and drip-loss, water holding capacity, sarcoplasmic protein solubility, total protein solubility, myofibrillar protein solubility, and myofibril hydrophobicity.

The results shows that visual colour had very low but significant correlations with the drip loss ($r=0.131$, $P=0.009$), water holding capacity ($r=0.202$, $P<0.0001$), sarcoplasmic protein solubility ($r=0.243$, $P<0.0001$), and TBARS ($r=0.119$, $P=0.018$). Marbling also had very low but significant relationship with drip loss ($r=0.161$, $P=0.001$), sarcoplasmic protein solubility ($r=0.205$, $P<0.0001$), total protein solubility ($r=0.240$, $P<0.0001$), myofibrillar protein solubility ($r=0.191$, $P=0.0001$), myofibril surface hydrophobicity ($r=0.197$, $P=0.0001$), and thiols ($r=-0.146$, $P=0.004$). The fibre separation showed a very low but significant relationship with the drip loss ($r=0.210$, $P<0.0001$), TBARS ($r=-0.136$, $P=0.007$) and Thiols ($r=-0.143$, $P=0.005$). There were good and significant correlations between the fibre separation and sarcoplasmic protein solubility ($r=0.303$, $P<0.0001$), myofibrillar protein solubility ($r=0.391$, $P<0.0001$), and myofibril surface hydrophobicity ($r=0.332$, $P<0.0001$). Texture had good significant correlation with total protein solubility, and very low and significant relationship with water holding capacity ($r=0.164$, $P=0.0010$) and sarcoplasmic protein solubility ($r=0.150$, $P=0.003$). On the other hand, structural integrity showed good and significant relationship with drip loss ($r=0.345$, $P<0.0001$), sarcoplasmic protein solubility ($r=0.302$, $P<0.0001$), total protein solubility ($r=0.515$, $P<0.0001$), and myofibril protein solubility ($r=0.462$, $P =<0.0001$). Low but significant correlations were observed between the structural integrity and the water holding capacity ($r=0.221$, $P<0.0001$), TBARS ($r=-0.153$, $P=0.002$), and Thiols ($r=-0.213$, $P<0.0001$).

Table 4.22: Correlation matrix showing correlation coefficients between visual attributes and protein of *m. longissimus dorsi* (LD).

	Colour[2]	Marbling[2]	Fibre separation[2]	Texture[2]	Structural Integrity[2]
Drip-loss	**0.131**	**0.161**	**0.210**	-0.106	**0.350**
	0.009	**0.001**	**<0.0001**	0.035	**<0.0001**
WHC	**0.202**	0.048	-0.027	**0.164**	**0.221**
	<0.0001	0.343	0.589	**0.001**	**<0.0001**
SPS (mg/g)	**0.243**	**0.205**	**0.303**	**0.150**	**0.302**
	<0.0001	**<0.0001**	**<0.0001**	**0.003**	**<0.0001**
TPS (mg/g)	**0.383**	**0.240**	0.085	**0.515**	**0.515**
	<0.0001	**<0.0001**	0.090	**<0.0001**	**<0.0001**
MPS (mg/g)	**0.336**	**0.191**	**0.391**	0.039	**0.462**
	<0.0001	**0.0001**	**<0.0001**	0.438	**<0.0001**
MSH (µg)	0.014	**0.197**	**0.332**	0.058	**0.436**
	0.777	**0.0001**	**<0.0001**	0.257	**<0.0001**
TBARS (mg/kg)	**0.119**	0.031	**-0.136**	-0.022	**-0.153**
	0.018	0.55	**0.007**	0.660	**0.002**
Thiols (µM/mg)	-0.058	**-0.146**	**-0.143**	-0.055	**-0.213**
	0.252	**0.006**	**0.005**	0.278	**<0.0001**

WHC- Water holding capacity
TPS- Total protein solubility
MPS- Myofibril protein hydrophobicity
SPS- Sarcoplasmic protein solubility
TBARS- Thiobarbituric acid reactive substances
MSH- Myofibril surface hydrophobicity

Table 4.23 shows correlation coefficients between visual attributes (colour, marbling, fibre separation, texture and structural integrity ratings) and colour coordinates (*L**, *a**, *b**), Chroma, Hue angle, deoxymyoglobin, oxymyoglobin, metmyoglobin, and metmyoglobin reductase activity.

Good negative and significant correlations were found between visual colour and *L** (*r*=-0.589, *P*<0.0001) and *b** (*r*=-0.541, *P*<0.0001) values as shown in Table 4.23. Good positive and significant correlations were found between the visual colour and hue angle (*r*=0.642, *P*<0.0001). Very low but significant correlations were found between the visual colour and *a** (*r*=0.293, *P*<0.0001) values, deoxymyoglobin (*r*=-0.133, *P*=0.008), oxymyoglobin (*r*=0.217, *P*<0.0001) and metmyoglobin (*r*=-0.212, *P*<0.0001).

Table 4.23: Correlation matrix showing correlation coefficients between visual attributes and colour related parameters of *m. longissimus dorsi* (LD).

	Colour[2]	Marbling[2]	Fibre separation[2]	Texture[2]	Structural integrity[2]
L*	**-0.588**	-0.039	0.027	-0.290	0.190
	<0.0001	0.437	0.591	<0.0001	0.0001
a*	0.293	0.122	0.260	-0.024	**0.337**
	<0.0001	0.015	<0.0001	0.635	**<0.0001**
b*	**-0.541**	-0.111	-0.225	-0.191	-0.213
	<0.0001	0.027	<0.0001	0.0001	<0.0001
Chroma	0.052	0.073	0.152	-0.094	0.231
	0.302	0.148	0.002	0.059	<0.0001
Hue angle	**0.642**	0.169	**0.324**	0.124	**0.382**
	<0.0001	0.001	**<0.0001**	0.014	**<0.0001**
DeoxyMb %	-0.133	-0.299	**-0.340**	-0.077	**-0.447**
	0.008	<0.0001	**<0.0001**	0.129	**<0.0001**
OxyMb %	0.217	**0.353**	**0.443**	0.097	**0.541**
	<0.0001	**<0.0001**	**<0.0001**	0.055	**<0.0001**
MetMb %	-0.212	**-0.031**	-0.018	-0.003	-0.095
	<0.0001	**0.537**	0.721	0.947	0.060
MRA (nM reduced/min/g)	0.003	0.001	0.117	0.050	0.085
	0.949	0.983	0.020	0.320	0.091

Mb- Myoglobin
MRA- Metmyoglobin reductase activity
DeoxyMb- deoxymyoglobin
OxyMb-oxymyoglobin
MetMb- metmyoglobin

Table 4.24 shows correlation coefficients between visual attributes (colour, marbling, fibre separation, texture and structural integrity ratings) and muscle fibre typing. There were very low correlations, which were not significant between the visual attributes (colour, marbling, fibre separation, texture and structural integrity ratings) and red fibre areas (Type1), intermediate fibre areas (Type IIA), and white fibre areas (Type IIB). There were also very low correlations, which were not significant, between the visual attributes and the percentage red fibres, percentage intermediate fibres and percentage white fibres.

Table 4.24: Correlation matrix showing correlation coefficients between visual attributes and muscle fibre typing of *m. longissimus dorsi* (LD).

	Colour[2]	Marbling[2]	Fibre separation[2]	Texture[2]	Structural integrity[2]
Fibre areas (µm²):					
Red Type 1	0.154	0.053	0.047	0.105	-0.083
	0.285	0.713	0.743	0.467	0.565
Intermediate type IIA	0.047	0.020	0.134	0.131	-0.101
	0.744	0.889	0.355	0.364	0.483
White type IIB	0.126	-0.183	0.052	0.102	-0.131
	0.384	0.202	0.720	0.481	0.364
% Fibre type:					
Red	-0.073	-0.173	-0.110	-0.086	-0.043
	0.615	0.230	0.443	0.554	0.766
Intermediate	0.357	0.167	0.195	0.296	-0.183
	0.011	0.245	0.175	0.037	0.205
White	-0.204	0.046	-0.039	-0.143	0.186
	0.156	0.750	0.789	0.323	0.196

Table 4.25: Correlation matrix showing correlation coefficients between visual attributes and tenderness related measurements of *m. longissimus dorsi* (LD).

	Colour[2]	Marbling[2]	Fibre separation[2]	Texture[2]	Structural integrity[2]
WBSF (N)	**-0.258**	**-0.357**	**-0.423**	-0.024	**-0.604**
	<0.0001	**<0.0001**	**<0.0001**	0.633	**<0.0001**
MFL (µm)	**-0.181**	**-0.190**	**-0.412**	-0.027	**-0.516**
	0.0003	**0.0001**	**<0.0001**	0.590	**<0.0001**
Sarcomere length	0.011	-0.047	**0.355**	0.171	**0.201**
	0.914	0.640	**0.0003**	0.089	**0.045**
Fibre detachment	0.131	**0.108**	**0.223**	0.068	**0.280**
(%White to red area)	0.009	**0.032**	**<0.0001**	0.177	**<0.0001**
Fibre breaks score (1-5)	0.046	0.049	0.049	0.028	**0.141**
	0.361	0.332	0.332	0.580	**0.005**
% fibre separation score	**0.111**	0.040	**0.116**	0.066	**0.116**
	0.041	0.465	**0.032**	0.223	**0.032**
Fat area score (1-5)	0.027	**0.140**	0.092	0.065	0.131
	0.682	**0.035**	0.166	0.331	0.048
Analyst tenderness score	0.072	0.084	**0.169**	0.029	**0.262**
(1-5)	0.151	0.095	**0.001**	0.558	**<0.0001**

WBSF-Warner Bratzler shear force
SL- Sarcomere length
MFL- Myofibrillar fragment length
IMF- Intramuscular fat

Correlation coefficients between the visual attributes and tenderness related measurements are shown in Table 4.25. Low but significant correlations were found between the visual colour and Warner Bratzler shear force (r=-0.258, P<0.0001), myofibril fragment length (r=-0.181, P=0.0003) and percentage fibre separation (r=0.111, P=0.041). Good negative and significant correlations were found between the marbling and shear force (r=-0.357, P<0.0001). Very low but significant correlations were found between marbling and myofibril fragment length (r=-0.190, P=0.0001), fibre detachment (r=0.108, P=0.032), and fat area score (r=0.140, P=0.035). Fibre separation showed good negative and significant correlations with shear force (r=-0.423, P<0.0001), myofibril fragment length (r=-0.412, P<0.0001) and good positive and significant correlations with sarcomere length (r=0.355, P=0.0003). The structural integrity also showed good negative and significant correlations with the shear force (r=-0.604, P<0.0001) and myofibril fragment length (r=-0.516, P<0.0001). Significant but low correlations were observed between the structural integrity and the sarcomere length (r=0.201, P=0.045), fibre detachment (r=0.278, P<0.0001), % fibre separation score (r=0.116, P=0.032), and analyst tenderness score (r=0.262, P <0.0001).

CHAPTER 5
RESULTS OF PHASE 2

5.1 Effect of breed on carcass characteristics, visual sensory panel evaluation of meat characteristics, meat colour measurements/evaluations, muscle energy metabolism, protein denaturation, lipid oxidation and meat tenderness.

Table 5.1: ANOVA table to summarise *P*-values for Phase 2 results

TREATMENTS				
	Breed	**Ageing**	**Treatment**	**Breed X ageing**
Carcass characteristics	**p109**		**P124**	
Live animal (kg)	<0.0001			
Warm carcass mass(kg)	<0.0001		0.681	
Cold carcass mass	<0.0001		0.667	
Dressing %	<0.0001			
Eye Muscle Area (mm^2)	0.001		0.508	
Ultimate pH (pHu)	<0.0001			
Visual appraisals	**p111**	**p119**	**p124**	**p130**
Colour[2]	<0.0001	<0.0001	0.659	<0.0001
Marbling[2]	<0.0001	<0.0001	0.826	<0.0001
Fiber separation[2]	0.003	<0.0001	0.264	0.001
Surface texture[2]	0.0001	<0.0001	0.867	0.001
Structural integrity[2]	<0.0001	<0.0001	0.133	0.036
Protein and lipid denaturation	**p111**	**p119**	**p125**	**p131**
Drip-loss	<0.0001	<0.0001	0.173	0.002
Water holding capacity	0.012	<0.0001	0.490	0.296
Total protein solubility (mg/g)	<0.0001	<0.0001	0.392	0.005
Myofibrill protein solubility (mg/g)	<0.0001	<0.0001	0.458	<0.0001
Sarcoplasmic protein solubility (mg/g)	0.0001	<0.0001	0.010	<0.0001
Myofibrill surface hydrophobicity (µg)	<0.0001	<0.0001	0.596	0.005
TBARS (mg/kg)	0.817	<0.0001	0.658	0.602
Thiol (µM/mg)	0.503	0.0002	0.145	0.2861
Instrumental colour	**p113**	**p120**	**p126**	**p137**
Deoxymyoglobin %	0.012	<0.0001	0.221	<0.0001
OxyMyoglobin %	0.0002	0.0001	0.311	0.048
Metmyoglobin %	0.001	<0.0001	0.848	0.024
Metmyoglobin reductase activity	0.503	<0.0001	0.024	0.584
*L**	<0.0001	0.001	0.859	0.002
*a**	<0.0001	<0.0001	0.312	0.0004
*b**	<0.0001	<0.0001	0.543	<0.0001
Chroma	<0.0001	<0.0001	0.371	0.0004

Hue angle	<0.0001	<0.0001	0.984	<0.0001
Muscle energy	**p114**		**p127**	**p139**
Lactate (µM/g)	< 0.0001		<0.0001	0.0043
Glucose (µM/g)	<0.0001		<0.0001	<0.0001
Glycogen (µM/g)	<0.0001		0.069	<0.0001
G-6-P (µM/g)	<0.0001		0.405	<0.0001
ATP (µM/g)	0.001		0.003	0.0285
Creatine Phosphate (µM/g)	0.0004		0.269	0.0285
Fibre typing	**p115**			
Fibre areas (μm^2)				
Red Type 1	0.025			
Intermediate type IIA	0.011			
White type IIB	0.0002			
% Fibre type				
Red	0.954			
Intermediate	0.350			
White	0.523			
Tenderness related measurements	**p116**	**p123**	**p129**	**p142**
Warner Bratzler shear force (N)	<0.0001	0.0003	0.084	0.137
Sarcomere length (µm)	<0.0001		0.116	
Myofibril fragment length (µm)	0.492	<0.0001	0.947	0.306
Total collagen (mg/g)	0.196			
Insoluble collagen (mg/g)	0.308			
Collagen solubility (%)	0.007			
Intra muscular fat (%)	0.219			
VIA muscle fibre structure measurements	**p116**	**p123**	**p129**	**p142**
Fibre detachment (%White to red area)	0.054	<0.0001	0.675	0.137
Fibre breaks score (1-5)	0.037	<0.0001	0.811	0.084
% Fibre separation score	0.546	0.002	0.393	0.033
Fat area score (1-5)	0.126	0.044	0.004	0.228
Fibre diameter (µm)	<0.0001	0.014	0.284	0.974
Average analyst tenderness score (1-5)	0.060	0.358	0.06	0.226

Table 5.1 summarises the *P*-values for all the analysis for Phase 1 results. Values with $P<0.05$ indicates significant differences between variables as highlighted in green and $P>0.05$ indicates non-significant differences between variables.

5.1.1 Carcass characteristics

All carcasses assessed in this study were of A-age Class, fatness Class 2 and confirmation Code 3. Results of carcass characteristics are summarised in Table 5.2. Breed had an effect on carcass characteristics like live animal weight ($P<0.0001$), warm carcass mass ($P<0.0001$), cold carcass mass ($P<0.0001$), dressing % ($P<0.0001$) and eye muscle area ($P=0.001$). Angus and Charolais breeds had the highest live weight, warm carcass mass and cold carcass mass. Nguni presented the lowest live weight, warm carcass mass, cold carcass mass, and eye muscle

area. Brahman and Charolais had the highest dressing percentage. This table also highlights differences between breeds for ultimate pH (pHu), there were differences between breeds (P<0.0001). Charolais and Nguni had higher pHu than Angus, Bonsmara and Brahman, which had lower pHu that were not significantly different. Figure 5.1 shows the effect of breed on carcass pH and temperature at 1, 3, 6 and 20 hours' *post mortem*. Results show that Charolais and Nguni had slower pH/temperature drop at 1, 3, 6 and 20 hours compared to other breeds that had pH/temperature drops, which were not significantly different.

Table 5.2: Effect of beef breed on carcass characteristics of *m. longissimus dorsi* (LD).

	Beef Breed						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Live animal (kg)	453.3^{a}	403.7^{b}	412.3^{b}	436.6^{a}	314.0^{c}	8.40	<0.0001
Warm carcass mass (kg)	247.9^{a}	229.7^{b}	227.5^{b}	247.5^{a}	172.9^{c}	5.53	<0.0001
Cold carcass mass (kg)	242.6^{a}	224.5^{b}	222.2^{b}	241.^{a}	169.0^{c}	5.43	<0.0001
Dressing %	54.1^{b}	54.5^{b}	56.3^{a}	56.0^{a}	54.4^{b}	0.40	<0.0001
Eye Muscle Area (mm^2)	6063^{ab}	5584^{bc}	5835^{b}	6521^{a}	5117^{c}	206.95	0.001
pHu	5.46^{b}	5.46^{b}	5.38^{b}	5.70^{a}	5.78^{a}	0.78	<0.0001

[1] Standard error of means

^{a,b,c} Means in the same row with a different superscript letter differ (P<0.05)

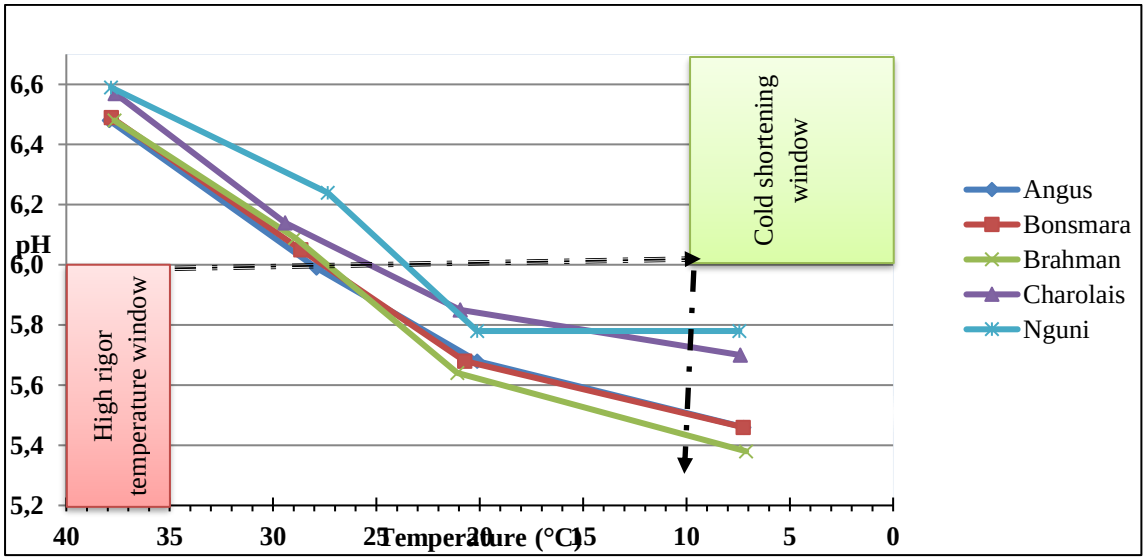


Figure 5. 1: Temperature and pH decline profiles at 1, 3, 6 and 20 hours *post mortem* of five beef breeds (Angus, Bonsmara, Brahman, Charolais and Nguni) of *m. longissimus dorsi* (LD). Cold shortening and high rigor temperature windows according to Pearson and Young (1989) and as discussed in the review of Thompson, (2002).

5.1.2 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form, Table 3.2, and visual reference guides, Figures 3.6-3.9).

Visual evaluations of meat colour were conducted using a 10-member trained panel, by using posters as references. The panel evaluated differences in visual colour between the five different cattle breeds. Results of the effect of breed on meat visual surface structural properties are summarised in Table 5.3. Breed had a significant effect on means of the evaluated sensory attributes, visual colour ($P<0.0001$), marbling ($P<0.0001$), fibre separation ($P=0.003$), surface texture ($P=0.0001$), and structural integrity ($P<0.0001$).

The sensory panel rated steaks from Nguni breed as the darkest, and the Brahman steaks as the lightest. Steaks from the Nguni breed were rated as light cherry red to cherry red; steaks from the Angus, Bonsmara and Charolais were rated as pink to light cherry red and steaks from Brahman were rated as light pink to pink according to Meat Colour Reference Guide, ARC-API, Meat Science, 2013 Addendum 3.

The Angus, Charolais and Nguni breed steaks had more marbling (visible intramuscular fat), rated as slight to small, and the Brahman and Bonsmara steaks had less marbling, rated as practically devoid of marbling to slight, according to the Marbling Reference Guide, ARC-API, Meat Science, 2013 Addendum 3.

The sensory panel also observed that steaks from Nguni, Bonsmara and Brahman breeds had less fibre separation, rated as slight to moderate. Steaks from Angus and Charolais had more fibre separation, rated as slight to moderate separation, although these breeds had more marbling, the rating was similar, according to the Reference Guide, ARC-API, Meat Science, 2013 Addendum 3.

The surface texture was rated as smooth to slightly coarse for steaks from all the breeds, according to Visual Surface Texture Reference Guide, ARC-API, Meat Science, 2013 Addendum 3. The Angus and the Charolais steaks had slightly coarse visual texture (fibre bundles well distinguished), the Bonsmara, Brahman and the Nguni steaks had smooth visual surface texture (can distinguish fibre bundles).

Steaks from the Angus and Charolais breeds were more compressible, where Nguni steaks were the least compressible, as evaluated using the structural integrity guidelines (Addendum 3).

Table 5.3: Effect of breed on visual sensory measurements as judged by the sensory panel on *m. longissimus dorsi* (LD).

	Beef Cattle Breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Colour[2]	5.3[b]	4.9[b]	4.2[c]	5.1[b]	6.3[a]	0.184	<0.0001
Marbling[2]	2.1[ab]	1.9[bc]	1.8[c]	2.2[a]	2.3[a]	0.082	<0.0001
Fibre separation[2]	2.4[a]	2.2[bc]	2.2[bc]	2.3[ab]	2.1[c]	0.597	0.003
Surface texture[2]	2.6[a]	2.4[bc]	2.3[c]	2.5[ab]	2.3[c]	0.052	0.0001
Structural integrity[2]	2.6[a]	2.3[b]	2.3[b]	2.5[ab]	2.1[c]	0.062	<0.0001

[1] Standard error of means
[2] Visual/sensory measurements
[a,b,c] Means in the same row with a different superscript letter differ (P<0.05)
[2] Average of panel ratings as define under methods

5.1.3 Protein and lipid denaturation.

Table 5.4: Effect of breed on protein denaturation and lipid oxidation of *m longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Drip-loss	0.951[b]	0.999[b]	1.57[a]	0.785[b]	0.621[c]	0.884	<0.0001
WHC	0.41[ab]	0.40[b]	0.40[b]	0.44[a]	0.42[ab]	0.092	0.012
TPS (mg/g)	176.7[c]	183.6[b]	186.4[ab]	186.7[ab]	188.4[a]	14.11	<0.0001
MPS (mg/g)	105.1[b]	108.1[b]	114.5[a]	116.4[a]	116.8[a]	15.35	<0.0001
SPS (mg/g)	71.57[bc]	75.68[a]	71.95[bc]	70.297[c]	72.72[b]	6.95	0.0001
MSH (µg)	79.32[c]	78.20[c]	86.00[ab]	89.23[a]	81.48[bc]	14.67	<0.0001
TBARS (mg/kg)	0.023	0.024	0.027	0.023	0.023	0.008	0.817
Thiols (µM/mg)	74.54	74.65	74.97	73.59	74.13	4.45	0.503

[1] Standard error of means
[a,b,c] Means in the same row with a different superscript letter differ (P<0.05)
WHC- Water holding capacity
TPS- Total protein solubility
MPS- Myofibril protein hydrophobicity
SPS- Sarcoplasmic protein solubility
TBARS- Thiobarbituric acid reactive substances
MSH- Myofibril surface hydrophobicity

The results show that breed had a significant effect on drip-loss (P<0.0001), water holding capacity (P=0.012), total protein solubility (P<0.0001), myofibrillar protein solubility (P<0.0001), sarcoplasmic protein solubility (P=0.0001) and myofibrillar surface hydrophobicity (P<0.0001), as shown in Table 5.4. Brahman breed had higher drip-loss, followed by Angus, Bonsmara and Charolais. Nguni breed had the lowest drip-loss. Brahman, Charolais and Nguni had higher total protein solubility and myofibrillar protein solubility. Angus and Bonsmara had lower total and myofibrillar protein solubility. Bonsmara had higher

sarcoplasmic protein solubility and Angus, Bonsmara and Charolais had lower sarcoplasmic protein solubility. Bonsmara and Charolais had higher myofibril hydrophobicity, Angus and Bonsmara had lower myofibril hydrophobicity.

There were no differences between breeds for thiobarbituric acid reactive substances (P=0.352) and Thiols (P=0.503).

Colour related parameters, which include myoglobin derivatives (deoxymyoglobin, oxymyoglobin and metmyoglobin), metmyoglobin reductase activity and meat colour characteristics (L^*, a^*, b^*, Chroma and Hue angle) were evaluated using both extraction methods and Minolta meter (CIE colour measurements). There were differences between breeds for deoxymyoglobin (P=0.012), oxymyoglobin (P=0.002), metmyoglobin (P=0.001) as shown in Table 5.5. There were higher deoxymyoglobin and oxymyoglobin content and lower metmyoglobin content in Angus and Bonsmara. Brahman, Charolais and Nguni had lower deoxymyoglobin content and oxymyoglobin content and higher metmyoglobin content. The breeds did not show any differences in the amount of metmyoglobin reductase activity (P>0.05).

Differences were observed between breeds for L^*, a^*, b^*, Chroma and Hue angle (P<0.0001), as shown in Table 5.5. Steaks from Nguni were the darkest, followed by Angus, Bonsmara and Charolais, and steaks from Brahman were lightest (as seen by L^* values). Steaks from Angus and Brahman were redder, and Charolais and Nguni steaks were less red (as seen by a^* values). The red colour was more intense in Angus and Brahman and less intense in Nguni and Charolais (referred to as Chroma). Hue angle, which refers to discolouration, shows that steaks from Nguni breed discoloured quicker, followed by those from Angus, Bonsmara and Charolais.

5.1.4 Minolta measured colour and related parameters

Table 5.5: Effect of breed on meat colour related parameters of *m. longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
DeoxyMb %	90.60^{ab}	90.78^{a}	88.53^{c}	88.53^{c}	88.94^{bc}	5.414	0.012
OxyMb %	10.11^{a}	10.10^{ab}	8.95^{bc}	8.18^{c}	7.95^{c}	3.664	0.0002
MetMb %	1.79^{b}	1.61^{b}	4.60^{a}	5.67^{a}	5.27^{a}	6.947	0.001
MRA (nM reduced/min/g)	7.36 x 10^{-8}	7.18 x 10^{-8}	7.27 x10^{-8}	7.18 x10^{-8}	7.05 x 10^{-8}	1.29 x 10^{-16}	0.503
Meat colour characteristics (CIE)							
L*	40.4^{b}	41.4^{b}	44^{a}	41.1^{b}	37.1^{c}	5.944	<0.0001
a*	14.2^{a}	12.6^{b}	13.2^{ab}	11^{c}	11^{c}	3.494	<0.0001
b*	8.7^{b}	8.2^{b}	9.9^{a}	6.7^{c}	6.1^{c}	3.537	<0.0001
Chroma	16.7^{a}	15.1^{b}	16.6^{a}	13^{c}	12.6^{c}	4.602	<0.0001
Hue angle	31.5^{b}	32.9^{b}	36.8^{a}	31.4^{b}	29^{c}	0.651	<0.0001

[1] Standard error of means
[a,b,c] Means in the same row with a different superscript letter differ (P<0.05).
MRA- Metmyoglobin reductase activity
DeoxyMb -deoxymyoglobin
OxyMb -oxymyoglobin
MetMb- metmyoglobin

5.1.5 Muscle energy metabolism

Muscle energy metabolism is an important meat quality factor. The conversion of muscle to meat is a process of energy metabolism with glycolysis after slaughter (Binke, 2004). The concentrations of lactate, glycogen, creatine phosphate, ATP and glucose-6-phosphate were determined according to the method of Dalrymple and Hamm (1973). Results shows that there were differences between breeds for lactate (P<0.001), glucose (P<0.0001), glycogen (P<0.0001), glucose-6-phosphate (P<0.0001), ATP (P=0.001) and creatine phosphate (P=0.0004), as shown in Table 5.6. Lactate and glycogen concentrations were higher for Brahman, which was similar to Angus and Bonsmara. Nguni had the lowest lactate and glycogen concentrations, which was similar to Charolais. For glucose concentration, Charolais and Nguni had the lowest concentrations and Angus, Bonsmara and Brahman had the highest concentrations. Glucose-6-phosphate was highest for Brahman, followed by Angus and Bonsmara and Charolais and Nguni had the lowest concentration. ATP concentration was higher for Angus, Brahman and Charolais, and lower for Brahman and Nguni. Creatine phosphate concentration was highest for Charolais and lower for the other breeds.

Table 5.6: Effect of breed on lactate, glucose, glycogen, glucose-6-phosphate (G-6-P), adenosine triphosphate (ATP) and creatine phosphate (CP) of *m. longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Lactate (µM/g)	42.30^{ab}	43.27^{ab}	46.05^{a}	39.43^{bc}	36.44^{c}	3.668	< 0.0001
Glucose (µM/g)	2.53^{a}	2.19^{a}	2.42^{a}	1.60^{b}	1.30^{b}	1.496	<0.0001
Glycogen (µM/g)	31.38^{a}	22.78^{b}	35.61^{a}	16.89^{bc}	12.73^{c}	22.217	<0.0001
G-6-P (µM/g)	4.38^{b}	3.91^{b}	5.39^{a}	2.30^{c}	2.21^{c}	2.741	<0.0001
ATP (µM/g)	5.10^{a}	5.02^{a}	4.54^{b}	5.17^{a}	4.48^{b}	1.254	0.001
CP (µM/g)	7.07^{b}	7.04^{b}	6.28^{c}	7.68^{a}	6.81^{bc}	1.888	0.0004

[1] Standard error of means
[a,b,c] Means in the same row with a different superscript letter differ (P<0.05)
G-6-P- Glucose-6-phosphate
ATP- Adenosine triphosphate
CP- Creatine phosphate

5.1.6 Muscle fibre typing

The effect of breed on fibre typing is illustrated in Table 5.7. No significant differences were observed between breeds for percentage red fibre, percentage intermediate fibre, and percentage white fibre. Differences were observed for red fibre areas (Type1) (P=0.025), intermediate fibre areas (Type IIA) (P=0.011), and white fibre areas (Type IIB) (P=0.0002). Charolais had the smallest red fibre (Type1) areas that was not significantly different to that of bonsmara and Nguni. Angus and Brahman had the largest red fibre areas (Type I) and was not significantly different from Bonsmara and Nguni. Bonsmara had the largest intermediate fibre areas (Type IIA) which was similar to that of Angus and Brahman. Charolais had the smallest intermediate fibres (Type IIA) areas, which were similar to that of Nguni. White fibre areas (Type IIB) were larger in Bonsmara, and smaller in Charolais, which was similar to that of Nguni and Angus.

Table 5.7: Effect of breed on muscle fibre typing of *m. longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Fibre areas (µm²):							
Red Type 1	2713[a]	2385[ab]	2698[a]	2016[b]	2422[ab]	96.7	0.025
Intermediate Type IIA	3711[ab]	3862[a]	3539[ab]	2902[c]	3189[bc]	39.1	0.011
White Type IIB	5529[bc]	6986[a]	6106[b]	4885[c]	5592[bc]	53.6	0.0002
% Fibre type:							
Red	35.53	34.66	35.47	35.78	35.77	2.541	0.954
Intermediate	29.20	29.90	28.85	27.12	30.34	0.558	0.350
White	35.26	35.44	35.69	37.10	33.89	1.908	0.523

[1] Standard error of means

[a,b,c] Means in the same row with different superscript letter differ ($P<0.05$).

5.1.7 Tenderness related measurements and muscle fibre related VIA measurements of *m. longissimus dorsi* (LD).

Results that represent mechanisms related to tenderness are summarised in Table 5.8. There were differences between breeds for Warner Bratzler shear force ($P<0.0001$), sarcomere length ($P<0.0001$), and percentage collagen solubility ($P=0.007$). Nguni and Angus were found to be more tender than Bonsmara, but similar to Brahman and Charolais, these breeds had lower shear force values. Bonsmara, Angus and Brahman had longer sarcomere lengths and Nguni had the shortest sarcomere length. Percentage collagen solubility was the highest for Charolais, and significantly different from all other breeds, which did not differ from one another. There were no significant differences between breeds for myofibril fragment length (MFL), total collagen, insoluble collagen and intramuscular fat (IMF).

Table 5.8 also summarises the effect of breed on VIA muscle fibre structure measurements. Fibre breaks score ($P=0.037$) and fibre diameter ($P<0.0001$) showed differences between breeds. Angus, Bonsmara and Charolais had higher fibre breaks score, while Nguni had the lowest fibre break score, which was similar to that of Bonsmara. Brahman and Nguni had the highest fibre diameter compared to other breeds, which had lower but similar fibre diameters. There were no significant differences between breeds for percentage white area measured (Fibre detachment), percentage fibre separation score, and fat area score.

Table 5.8: Effect of breed on tenderness related measurements of *m. longissimus dorsi* (LD).

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
WBSF (N)	39.83[b]	46.40[a]	40.91[ab]	43.46[ab]	37.87[b]	2.117	<0.0001
SL (µm)	1.89[ab]	1.93[a]	1.91[ab]	1.87[b]	1.81[c]	0.082	<0.0001
MFL (µm)	25.67	25.26	27.00	27.47	26.31	8.660	0.492
Total collagen (mg/g)	1.67	1.55	1.47	1.65	1.46	0.24	0.196
Insoluble collagen (mg/g)	1.43	1.34	1.27	1.38	1.26	0.21	0.308
Collagen solubility (%)	13.9[b]	14.1[b]	14.0[b]	16.7[a]	13.8[b]	1.9	0.007
% IMF	1.21	1.05	1.32	1.37	1.34	0.341	0.219
VIA muscle fibre structure measurements							
Fibre detachment (%White to red area)	19.29	20.82	18.67	21.43	19.44	0.543	0.054
Fibre breaks score (1-5)	2.12[a]	2.33[a]	1.90[ab]	2.17[a]	1.58[b]	1.588	0.037
% Fibre separation score	33.6	38.2	36.9	42.5	37.8	1.4	0.546
Fat area score (1-5)	0.73	0.69	0.57	0.67	1.01	1.128	0.126
Fibre diameter (µm)	50.00[b]	50.00[b]	60.00[a]	50.00[b]	60.00[a]	0.009	<0.0001
Analyst tenderness score (1-5)	3.11[bc]	3.66[a]	3.00[c]	3.45[ab]	2.93[c]	1.374	0.060

[1] Standard error of means
[a,b,c] Means in the same row with a different superscript letter differ ($P<0.05$.)
WBSF-Warner Bratzler shear force
SL- Sarcomere length
MFL- Myofibrillar fragment length
IMF- Intramuscular fat
VIA – video image analyses

5.2 Effect of ageing on visual meat characteristics, meat colour measurements/evaluations, muscle energy metabolism, protein denaturation, lipid oxidation and meat tenderness.

5.2.1 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form, Table 3.2 and 3.3, and visual reference guides, Figures 3.6-3.9)

The effect of ageing and packaging on the visual analysis of meat surface is summarised in Table 5.9. The results show that there were differences between ageing period/packaging and meat colour, marbling, fibre separation, surface texture, and structural integrity ($P<0.0001$). Meat colour was rated as light cherry red to cherry red for steaks that were aged for three days (propylene cling-wrap packaging) in the display cabinet. Steaks that were aged for 9, 14 and 20 (vacuum packaged) were rated as cherry red to dark red, and there were no significant differences between these ageing periods. Marbling, which can be seen as flecks or thin strips of fat evenly distributed in a cut of meat and can also be referred to as intramuscular fat, seemed to become more noticeable and seemed to increase with ageing, where the day 3 aged steaks were rated as practically devoid of or only showing slight marbling. There were no significant differences between day 9 and 14 vacuum pack aged steaks; the steaks were rated as having slight marbling. The day 20 vacuum pack aged steaks were rated as having slight marbling, but were significantly different to other ageing periods.

Fibre separation was rated as having no separation (fibres fit tightly together) to slight separation (can just see separation between fibres) for day 3 aged steaks. Days 9, 14 and 20 aged steaks were rated as having slight separation (separation between fibres just visible), and there were no significant differences between these two ageing periods.

Surface texture for day 3 aged steaks were rated as smooth (can just distinguish between fibres) and days 9, 14 and 20 aged steaks were rated as smooth (but can distinguish fibres) to slightly coarse (fibres well distinguished), and there were no significant differences between the days 9, 14 and 20 ageing periods. The structure of the meat became more compressible or softer with ageing. At day 3 *post mortem*, the structural integrity was rated as stiff or hard. Days 9, 14 and 20 were rated as compressible to soft, but there were significant differences between means for ageing periods, with the structure becoming softer with ageing.

Table 5.9: Effect of ageing on visual meat characteristics of *m. longissimus dorsi* (LD)

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM**	***P*-Value**
Colour[2]	4.51[b]	5.40[a]	5.41[a]	5.46[a]	0.067	<0.0001
Marbling[2]	1.64[c]	2.04[b]	2.17[b]	2.33[a]	0.050	<0.0001
Fibre separation [2]	1.74[b]	2.40[a]	2.37[a]	2.35[a]	0.037	<0.0001
Surface texture[2]	2.25[b]	2.49[a]	2.50[a]	2.44[a]	0.034	<0.0001
Structural integrity[2]	1.72[d]	2.18[c]	2.60[b]	2.93[a]	0.041	<0.0001

[1] Standard error of means
[2] Visual/sensory measurements
[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05).

5.2.2 Protein denaturation and lipid oxidation

Table 5.10: Effect of ageing on protein denaturation and lipid oxidation of *m. longissimus dorsi* (LD).

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM[1]**	***P*-Value**
Drip-loss	0.01[b]	1.37[a]	1.37[a]	1.42[a]	0.397	<0.0001
WHC	0.43[ab]	0.42[b]	0.37[c]	0.44[a]	0.092	<0.0001
TPS (mg/g)	101.16[b]	212.36[a]	213.28[a]	210.90[a]	14.109	<0.0001
MPS (mg/g)	49.63[c]	139.01[a]	136.26[a]	123.10[b]	15.354	<0.0001
SPS (mg/g)	51.56[d]	73.35[c]	77.03[b]	87.75[a]	6.682	<0.0001
MSH (µg)	65.58[d]	76.04[c]	87.17[b]	102.23[a]	11.960	<0.0001
TBARS(mg/kg)	0.022[b]	0.021[b]	0.026[a]	0.028[a]	0.009	<0.0001
Thiols (µM/mg)	73.82[b]	73.52[b]	75.30[a]	74.97[a]	3.359	0.0002

[1] Standard error of means, d *post mortem*=days *post mortem*,
[a,b,c,d] Means in the same row with a different superscript letter differ (P<0.05).
WHC- Water holding capacity
TPS- Total protein solubility
MPS- Myofibril protein hydrophobicity
SPS- Sarcoplasmic protein solubility
TBARS- Thiobarbituric acid reactive substances
MSH- Myofibril surface hydrophobicity

The effect of ageing on protein and lipid denaturation and water binding is summarised in Table 5.10. There were differences between ageing periods for drip-loss (P<0.0001), water holding capacity (P<0.0001), total protein solubility (P<0.0001), myofibrillar protein solubility (P<0.0001), sarcoplasmic protein solubility (P<0.0001), TBARS (P<0.0001), Thiols (P=0.0002), and surface myofibril hydrophobicity (P<0.0001). Drip-loss and total protein solubility were lower for day 3 aged samples, and no significant differences could be found between days 9, 14 and 20. Water holding capacity was higher for day 9, which was similar to

day 3. Day 14 had the lowest water holding capacity. Myofibril protein solubility was higher for days 9 and 14. Day 20 had the lowest myofibril protein solubility. There was an increase in sarcoplasmic protein solubility and myofibril surface hydrophobicity with ageing.

5.2.3 Minolta measured colour and related parameters

The effect of ageing on colour related parameters is summarised in Table 5.11. There were differences between ageing periods for deoxymyoglobin (P<0.0001), oxymyoglobin (P=0.0001), metmyoglobin (P<0.0001), metmyoglobin reducing activity (P<0.0001), L^* (P=0.001), a^* (P<0.0001), b^* (P<0.0001), Chroma (P<0.0001) and Hue (P<0.0001). High levels of deoxymyoglobin were detected for day 9 *post mortem*; day 20 had lower levels of deoxymyoglobin, followed by the days 3 and 14. The levels of oxymyoglobin were higher in day 9, followed by day 3, after which days 14 and 20 had lower levels. The levels of metmyoglobin were higher for days 14 and 20 and there were no significant differences between them, day 9 had the lowest metmyoglobin content followed by day 3. The metmyoglobin reducing activity was higher for day 3 *post mortem* and showed a linear increase with ageing for days 9, 14 and 20. Day 3 *post mortem* steaks were lighter (higher L^* values) and there were no differences between days 9, 14 and 20. Day 3 *post mortem* steaks were less red (lower a^* values) and there was a linear decrease in redness of the steaks that were aged for days 9, 14 and 20. The b^* values were higher for day 3 *post mortem* and there was no significant differences between days 9, 14 and 20. Chroma was higher for days 9 and 14, and there was no significant differences between them and lower for days 3 and 20 and there was no significant differences between them. Hue angle was higher for days 9 and 14 and lower for day 3.

Table 5.11: Effect of ageing on colour related parameters of *m. longissimus dorsi* (LD).

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM[1]**	***P*-Value**
DeoxyMb%	87.4^{b}	88.98^{a}	86.42^{bc}	85.99^{c}	3.999	<0.0001
OxyMb%	9.18^{b}	9.96^{a}	8.45^{c}	8.70^{bc}	2.495	0.0001
MetMb %	3.42^{b}	1.47^{c}	4.99^{a}	5.18^{a}	4.876	<0.0001
MRA (nM reduced/min/g)	$5.41x+10^{-8d}$	8.88×10^{-8a}	7.53×10^{-8ab}	7.03×10^{-8b}	1.02×10^{-8}	<0.0001
Meat colour characteristics (CIE)						
L*	41.3^{a}	40.6^{b}	40.6^{b}	40.8^{b}	1.669	0.001
a*	11.1^{d}	13.4^{a}	12.9^{b}	12.3^{c}	1.696	<0.0001
b*	9.2^{a}	7.6^{b}	7.6^{b}	7.5^{b}	0.726	<0.0001
Chroma	14.5^{b}	15.4^{a}	15.1^{a}	14.4^{b}	1.956	<0.0001
Hue angle	39.5^{a}	29.6^{c}	30.5^{ab}	31.3^{b}	0.038	<0.00001

[1] Standard error of means, d *post mortem*-days *post mortem*
[a,b,c] Means in the same row with a different superscript letter differ (P<0.05).
DeoxyMb- Deoxymyoglobin
OxyMb- Oxymyoglobin
MetMb-Metmyoglobin
MRA- Metmyoglobin reductase activity

5.2.4 Tenderness related measurements of *m. longissimus dorsi* (LD).

Figure 5.2 and Table 5.12 indicates the effect of ageing on myofibrillar fragment length (MFL) and Warner Bratzler shear force (WBSF). There were differences between ageing periods for both myofibrillar fragment length (P< 0.0001) and Warner Bratzler shear force (P=0.003). There was a linear decrease in the length of myofibril fragments. The day 3 had longer myofibril fragment length, followed by day 9, and then day 14 and the day 20 had the shortest myofibril fragment length. The results also show that tenderness of meat improved with ageing. The day 3 *post mortem* steaks had higher shear values, followed by the day 9 *post mortem* and then day 14 *post mortem*. The day 20 *post mortem* steaks had the lowest shear force values and were thus more tender.

Table 5.12 also shows the effect of ageing on VIA muscle fibre structure measurements. There were differences between ageing periods for the percentage white to red area (P<0.0001), breaks score (P=0.001), percentage fibre separation score (P=0.002), fat area score (P=0.043), and fibre diameter (P=0.014). There were no significant differences between ageing periods for the average analyst tenderness score (P>0.05). The percentage white to red area increased with ageing, the fibre breaks score was higher for day 20 *post mortem* that was similar to day 14 *post mortem* and day 9 *post mortem* had the lowest breaks score. The percentage fibre

separation and fat area score were lower for the day 3 *post mortem* and similar for the days 9, 14 and 20. The fibre diameter was lower for days 14 and 20 and higher for days 3 and 9.

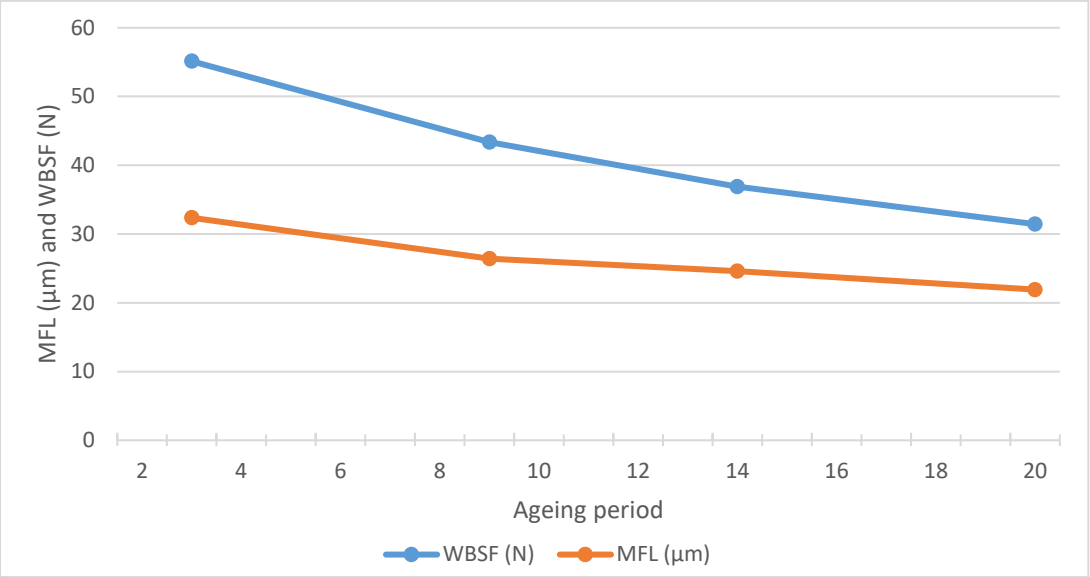


Figure 5.2: Effect of ageing on myofibril fragment length (MFL) and Warner Bratzler shear force (WBSF) of *m. longissimus dorsi* (LD).

Table 5.12: Effect of ageing on tenderness related measurements of *m. longissimus dorsi* (LD).

	Ageing					
	3 d *post mortem*	**9 d *post mortem***	**14 d *post mortem***	**20 d *post mortem***	**SEM[1]**	***P*-Value**
WBSF (N)	55.13[a]	43.35[b]	36.89[c]	31.44[d]	1.376	0.0003
MFL (µm)	32.36[a]	26.42[b]	24.60[c]	21.92[d]	3.041	<0.0001
VIA muscle fibre structure measurements						
% Fibre detachment (% White to red area)	16.11[d]	18.43[c]	21.18[b]	23.72[a]	4.397	<0.0001
% Fibre separation score	29.63[b]	38.20[a]	40.12[a]	42.50[a]	25.185	0.002
Fat area score (1-5)	0.51[b]	0.67[ab]	0.77[ab]	0.97[a]	1.150	0.044
Fibre diameter (µm)	56.74[a]	55.40[ab]	54.10[bc]	53.00[c]	0.008	0.014
Analyst tenderness score (1-5)	3.39	2.92	3.47	3.85	3.677	0.358

[1] Standard error of means, d *post mortem*-days *post mortem*
[a,b,c,d] Means in the same row with a different superscript letter differ ($P<0.05$).
WBSF- Warner Bratzler shear force
MFL – Myofibril fragment length

5.3 Effect of *post mortem* treatment (electrical stimulation and delayed chilling) on carcass characteristics, meat visual characteristics and related physiological characteristics, colour measurements/evaluations, muscle energy metabolism, protein denaturation, lipid oxidation and meat tenderness.

The effect of electrical stimulation (ES) and non-electrical stimulation (NS) on temperature and pH decline during a 20 hours *post mortem* is illustrated in Figure 5.3 for of the study. Carcasses that were not electrically stimulated experienced higher pH values as compared to those that were electrically stimulated. The pH decline for NS carcasses was faster than the pH decline for ES carcasses. At 20 hours *post mortem*, there were no significant differences between the ultimate pH for both treatments (5.52 and 5.57 respectively). Electrically stimulated carcasses initially (at one hour) had very high temperatures. However, from three hours onwards, the temperature decline was similar to carcasses that were not stimulated.

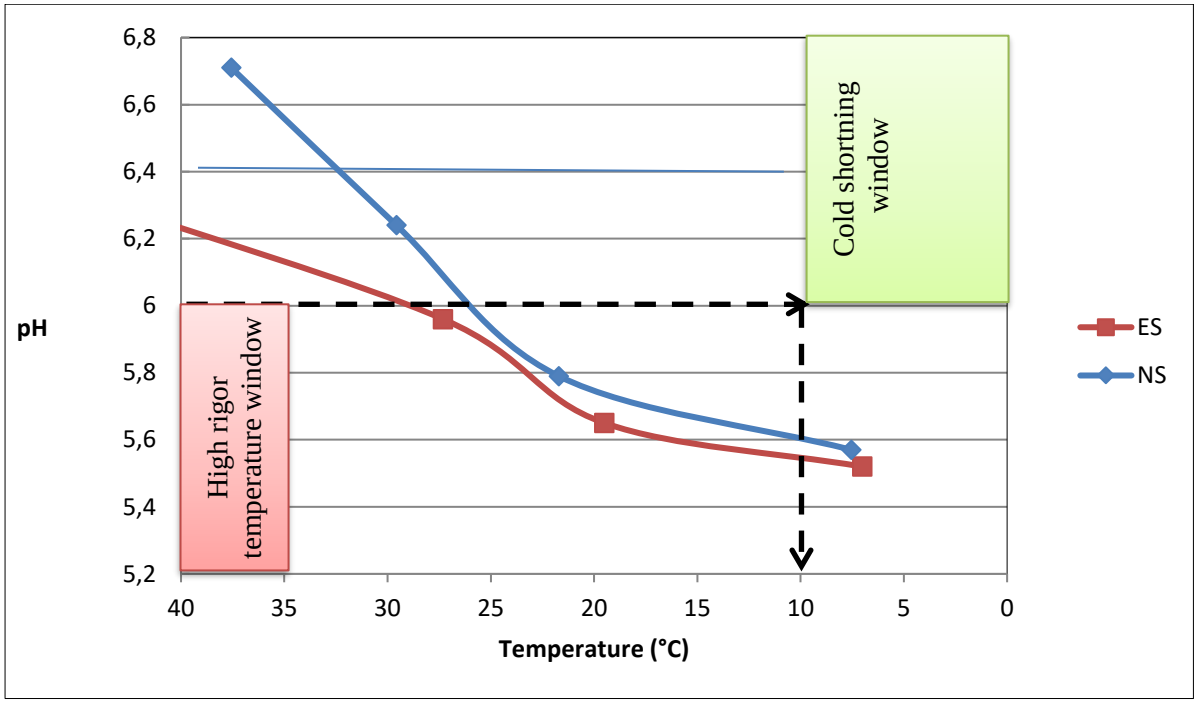


Figure 5.3: Effect of *post mortem* treatment (ES and NS) on temperature and pH decline profiles at 1, 3, 6 and 20 hours *post mortem* of *m. longissimus dorsi* (LD). Cold shortening and high rigor temperature windows according to Pearson and Young (1989) and as discussed in the review of Thompson, (2002)

Table 5.13: Effect of treatment (ES and NS) on carcass characteristics of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
Warm carcass mass (kg)	112.74	104.53	10.85	0.681
Cold carcass mass (kg)	110.21	112.02	10.48	0.669
Eye Muscle Area (mm^2)	6118	5584	65.20	0.508

[1] Standard error of means

Table 5.13 shows the effect of electrical stimulation on carcass characteristics. There were no significant differences in carcass characteristics between electrically stimulated and non-stimulated carcasses. These included warm carcass mass, cold carcass mass, and eye muscle area.

5.3.2 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form (Tables 3.2 and 3.3) and visual reference guides, Figures 3.6-3.9).

Table 5. 14: Effect of treatment (ES and NS) on the visual meat characteristics of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
Colour[2]	5.16	5.21	0.116	0.659
Marbling[2]	2.04	2.05	0.052	0.826
Fibre separation[2]	2.18	2.24	0.039	0.264
Surface texture[2]	2.41	2.42	0.033	0.867
Structural integrity[2]	2.30	2.39	0.042	0.133

[1] Standard error of means

[2] Visual/sensory measurements

The effect of treatment on visual meat characteristics is summarised in Table 5.14. There were no significant differences between electrically stimulated steaks and steaks that were not electrically stimulated for visual analysis measurements which included the meat colour, marbling, fibre separation, surface texture and structural integrity.

5.3.3 Protein denaturation and lipid oxidation

Table 5.15 summarises the effect of treatment (ES and NS) on protein and lipid denaturation and water binding. There were no differences (P>0.05) between electrically stimulation and non-electrica1 stimulation for steaks that were analysed for drip loss, water holding capacity, total protein solubility, myofibrillar protein solubility, TBARS, free thiol groups, myofibril hydrophobicity (P>0.05). Sarcoplasmic protein solubility showed differences between treatments (P=0.01) with electrically stimulated steaks having lower sarcoplasmic protein solubility and non-stimulated steaks having higher protein solubility.

Table 5.15: Effect of treatment (ES and NS) on protein and lipid denaturation and water binding of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
Drip-loss	0.94	1.06	0.884	0.173
WHC	0.41	0.42	0.092	0.490
TPS (mg/g)	183.7	185.0	14.109	0.392
MPS (mg/g)	112.7	111.6	15.354	0.458
SPS (mg/g)	71.55[b]	73.39[a]	6.947	0.010
MSH (µg)	82.34	83.12	14.669	0.596
TBARS(mg/kg)	0.023	0.023	0.008	0.658
Thiol (µM/mg)	74.07	74.73	4.454	0.145

[1] Standard error of means
[a,b] Means in the same row with a different superscript letter differ (P<0.05).
WHC- Water holding capacity
TPS- Total protein solubility
MPS- Myofibril protein hydrophobicity
SPS- Sarcoplasmic protein solubility
TBARS- Thiobarbituric acid reactive substances
MSH- Myofibril surface hydrophobicity

5.3.4 Colour related parameters

The effect of treatment (ES and NS) on colour related parameters is summarised in Table 5.16. There were no differences (P>0.05) between treatments for deoxymyoglobin content, oxymyoglobin content, metmyoglobin content, L^*, a^*, b^*, Chroma and Hue (P>0.05). Treatments for metmyoglobin reductase activity in steaks from electrically stimulated carcasses differed (P=0.024), showing to have higher MRA than in non-stimulated steaks.

Table 5.16: Effect of treatment (ES and NS) on colour related parameters of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
DeoxyMb %	86.75	87.42	5.414	0.221
OxyMb %	9.6	8.89	3.664	0.311
MetMb %	3.84	3.69	7.679	0.848
MRA(nM reduced/min/g)	7.34×10^{8a}	7.08×10^{8b}	1.136×10^{8}	0.024
Meat colour characteristics (CIE)				
L*	40.8	40.8	9.945	0.859
a*	12.3	12.6	3.496	0.312
b*	7.9	8.1	3.537	0.543
Chroma	14.7	15.1	4.603	0.371
Hue angle	32.7	32.6	0.655	0.984

[1] Standard error of means
DeoxyMb- Deoxymyoglobin
OxyMb- Oxymyoglobin
MetMb-Metmyoglobin
MRA- Metmyoglobin reductase activity

5.3.5 Muscle energy metabolism

Results of the effect of treatment (ES and NS) on lactate, glucose, glycogen, glucose-6-phosphate, ATP and creatine phosphate are highlighted in Table 5.17. There were differences in the treatments for the lactate ($P<0.0001$), glucose ($P<0.0001$), and ATP concentrations Lactate and glucose concentrations were higher for electrically stimulated carcasses, and lower for carcasses that were not stimulated, but stepwise delayed chilled. The concentration of ATP was higher for stepwise delayed chilled carcasses and lower for electrically stimulated carcasses. There were no differences ($P>0.05$) between treatments for glycogen and creatine phosphate concentrations.

Table 5.17: Effect of treatment (ES and NS) on lactate, glucose, glycogen, glucose-6-phosphate, ATP and creatine phosphate of *m. longissimus dorsi* (LD).

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
Lactate (µM/g)	45.45[a]	37.81[b]	13.454	<0.0001
Glucose (µM/g)	2.49[a]	1.55[b]	1.496	<0.0001
Glycogen (µM/g)	22.21	26.29	22.218	0.069
Glucose-6- phosphate (µM/g)	3.81	3.59	2.741	0.405
ATP (µM/g)	4.66[b]	5.04[a]	1.254	0.003
Creatine phosphate (µM/g)	7.05	6.84	1.888	0.269

[1] Standard error of means
[a,b] Means in the same row with a different superscript letter differ (P<0.05).

5.3.6 Tenderness related parameters

Table 5.18 shows the effect of treatment on tenderness related measurements which are Warner Bratzler shear force, myofibril fragment length, and muscle fibre related VIA measurements (percentage white to red area, fibre breaks score (1-5), percentage fibre separation score, fat area score (1-5), fibre diameter, and overall tenderness score). There were no differences (P>0.05) between treatments for Warner Bratzler Shear force, sarcomere length and myofibril fragment length.

Table 5.18: Effect of treatment on tenderness related measurements of *m. longissimus dorsi* (LD)

	Treatment			
	ES	**NS**	**SEM[1]**	***P*-Value**
WBSF	39.83	43.46	2.117	0.084
SL (µm)	1.90	1.87	0.082	0.116
MFL (µm)	26.36	26.31	8.660	0.947
VIA muscle fibre structure measurements				
Fibre detachment (%White to red area)	20.00	19.74	6.543	0.675
Fibre breaks score (1-5)	2.03	2.00	1.588	0.811
% Fibre separation score	38.98	36.30	31.452	0.393
Fat area score (1-5)	0.90[a]	0.56[b]	1.128	0.004
Fibre diameter	50.00	60.00	0.009	0.284
Analyst tenderness score (1-5)	3.69	3.13	3.713	0.06

[1] Standard error of means
[a,b] Means in the same row with a different superscript letter differ (P<0.05).
WBSF-Warner Bratzler shear force
SL- Sarcomere length
MFL- Myofibrillar fragment length

There were no differences between ES and NS for percentage white to red area**,** fibre breaks score, percentage fibre separation score, fibre diameter and tenderness score 1-5 as shown in Table 5.18. Differences were only found between treatments for fat area score (*P*=0.004) with ES having higher fat area score that NS.

5.4 Effect of breed and ageing interaction on visual sensory panel evaluations of meat characteristics, meat colour measurements/evaluations, muscle energy metabolism, protein denaturation, and lipid oxidation and meat tenderness.

5.4.1 Visual sensory panel evaluations of meat characteristics (with reference to sensory evaluation form, Tables 3.2 and 3.3, and visual reference guides, Figures 3.6-3.9).

The effect of breed and ageing/packaging combination on visual colour, marbling, fibre separation, surface texture and structural integrity is summarised in Table 5.19 and Figure 5.4 (A-E). There was a significant interaction between means of ageing and packaging for meat colour (*P*<0.0001), marbling (*P*<0.0001), fibre separation (*P*=0.001), surface texture (*P*=0.001) and structural integrity (*P*=0.036). The panel observed colour differences between the two different packaging types, where steaks that were aged in display cabinet were lighter than vacuum packaged aged steaks for all breeds. Overall, there were no significant differences between days 9, 14 and 20 vacuum packaged steaks for Angus, Bonsmara, Charolais, and Nguni. Bonsmara breed increased in redness until day 14 *post mortem*, and decreased at day 20.

The panel observed differences in fibre separation between ageing periods/between the two packaging methods. There were differences between the day 3 aged steaks and the days 9, 14 and 20 *post mortem* vacuum package aged steaks for all breeds. There were no significant differences between the steaks that were aged at 9, 14 and 20 days vacuum packaged aged steaks. The fibre separation for day 3 *post mortem* steaks was rated as no separation (fibres fit tightly together) to slight separation (can just see separation between fibre bundles) and the day 9, 14 and 20 *post mortem* were rated as slight separation (can just see separation between fibre bundles).

There were differences that were observed between ageing periods for all breeds for the visual surface texture. For Angus and Charolais, the day 3 *post mortem* steaks had smooth (can distinguish fibre bundles) surface visual texture and the days 9, 14 and 20 aged steaks had smooth to slightly coarse (fibre bundles well distinguished) visual texture. Bonsmara, Brahman

and Nguni did not show any differences in surface visual texture between ageing periods. The panel also observed that structure became more compressible/soft with ageing for all breeds.

Table 5.19: Effect of breed X ageing interaction on sensory panel analyses of *m. longissimus dorsi* (LD).

	Beef Breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Colour[2]						0.150	<0.0001
3 d *post mortem*	4.40^{fgh}	4.04^{hi}	3.93^{i}	4.49^{fg}	5.75^{b}		
9 d *post mortem*	5.67^{bc}	5.22^{de}	4.55^{f}	5.04^{e}	6.41^{a}		
14 d *post mortem*	5.48^{bcd}	5.16^{de}	4.44^{fgh}	5.37^{bcde}	6.58^{a}		
20 d *post mortem*	5.75^{b}	5.31^{cde}	4.08^{ghi}	5.52^{bcd}	6.57^{a}		
Marbling[2]						0.112	<0.0001
3 d *post mortem*	1.68^{ghi}	1.50^{i}	1.48^{i}	1.86^{fgh}	1.67^{hi}		
9 d *post mortem*	2.19^{bcde}	1.82^{fgh}	1.63^{hi}	2.21^{bcde}	2.29^{abcd}		
14 d *post mortem*	2.21^{bcde}	1.98^{defg}	1.92^{efgh}	2.19^{bcde}	2.53^{a}		
20 d *post mortem*	2.22^{bcde}	2.35^{abc}	2.13^{cdef}	2.47^{ab}	2.49^{ab}		
Fibre separation[2]						0.083	0.001
3 d *post mortem*	1.78^{g}	1.73^{g}	1.82^{g}	1.73^{g}	1.65^{g}		
9 d *post mortem*	2.68^{a}	2.43^{bcde}	2.15^{f}	2.47^{abcd}	2.24^{def}		
14 d *post mortem*	2.48^{abc}	2.48^{abc}	2.26^{cdef}	2.48^{abc}	2.16^{f}		
20 d *post mortem*	2.62^{ab}	2.22^{ef}	2.42^{bcde}	2.40^{bcde}	2.12^{f}		
Surface texture[2]						0.003	0.001
3 d *post mortem*	2.35^{def}	2.29^{def}	2.17^{f}	2.16^{f}	2.25^{ef}		
9 d *post mortem*	2.81^{a}	2.49^{bcd}	2.24^{f}	2.58^{bc}	2.32d^{ef}		
14 d *post mortem*	2.62^{abc}	2.46^{bcde}	2.29^{def}	2.64^{abc}	2.47^{bcde}		
20 d *post mortem*	2.60^{abc}	2.34^{def}	2.45^{cde}	2.66^{ab}	2.16^{f}		
Structural integrity[2]						0.092	0.036
3 d *post mortem*	1.83^{hi}	1.79^{i}	1.85^{hi}	1.68^{i}	1.41^{j}		
9 d *post mortem*	2.54^{cde}	2.22^{fg}	2.05^{gh}	2.37^{ef}	1.73^{i}		
14 d *post mortem*	2.75^{bc}	2.57^{bcde}	2.58^{bcde}	2.69^{b}	2.42^{def}		
20 d *post mortem*	3.26^{a}	2.82^{b}	2.82^{b}	3.17^{a}	2.63^{bcd}		

[1] Standard error of means
[2] Visual/sensory measurements
[a,b,c,d,e,f,g,h,i] Means in the same row with a different superscript letter differ (P<0.05)

The structural integrity of steaks from all breeds increased with ageing and it was rated as compressible to soft for all steaks. The structural integrity for Nguni was less compressible

as compared to other breeds for days 3 and 9, but at days 14 and 20 *post mortem*, the structural integrity was similar as that of Bonsmara and Brahman. At day 20 *post mortem*, the structural integrity for Angus and Charolais had softer structure, as rated by the panel.

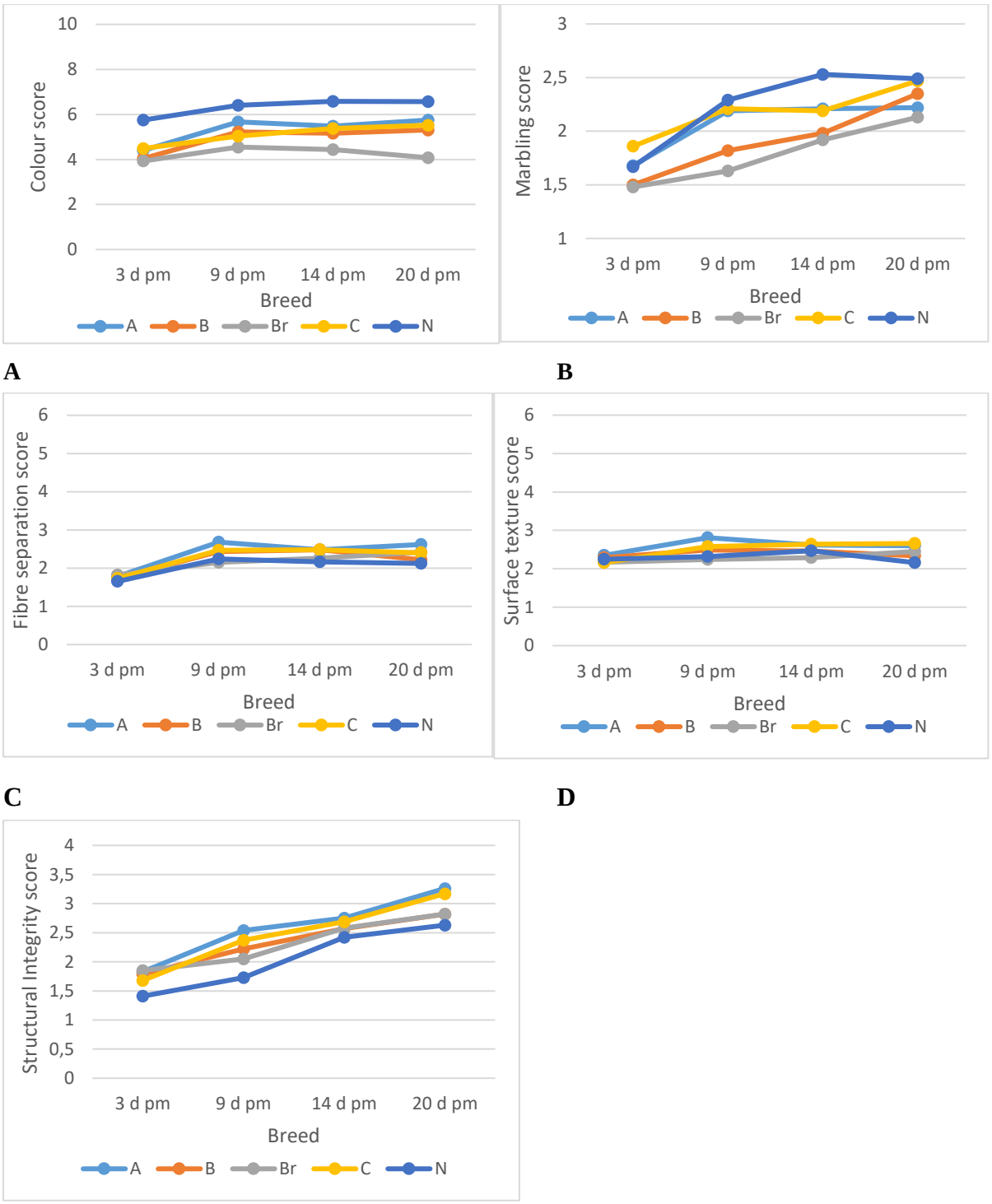


Figure 5.4: Effect of breed X ageing interaction on colour (A), marbling (B), fibre separation (C), Surface texture and (D) and structural integrity of *m. longissimus dorsi* (LD).

5.4.2 Protein denaturation and lipid oxidation

Table 5.20 shows the effect of breed and ageing combination protein denaturation, lipid denaturation, drip-loss and water holding capacity. There were significant interaction between means for breed and ageing period for drip-loss (P=0.002), total protein solubility (P=0.005), myofibrillar protein solubility (P<0.0001), sarcoplasmic protein solubility (P<0.0001), surface myofibril hydrophobicity (P=0.005). There were no significant interactions (P>0.05) between the means for breed and ageing period for water holding capacity, TBARS and free thiol groups.

Drip-loss increased with ageing for all breeds and was very low for day 3 *post mortem*. Bonsmara breed showed the highest drip-loss, especially at day 9 *post mortem*. Nguni and Charolais had drip-loss that increased with ageing, with the highest drip-loss observed at day 20 *post mortem* with no significant differences between days 9 and 14 *post mortem*. There were no significant differences between days 14 and 20 *post mortem* for Angus, Brahman, and Bonsmara.

Total protein solubility increased with ageing for all breeds, but there were no significant differences between days 9, 14 and 20 *post mortem* for all breeds. The only ageing period that had lower total protein solubility was the day 3 *post mortem*. Angus showed lower total protein solubility as compared to other breeds for days 3, 9 and 14 *post mortem.*

Myofibrillar protein solubility increased with ageing for all breeds. At day 3 *post mortem*, all breeds had similar myofibrillar protein solubility. There were no significant differences between ageing periods for Angus, Brahman and Bonsmara. The day 20 *post mortem* had lower myofibrillar protein solubility for these breeds. For Charolais and Nguni, there were no significant differences between days 9, 14 and 20 *post mortem*.

Sarcoplasmic protein solubility increased with ageing in all breeds, the day 20 *post mortem* had higher sarcoplasmic protein solubility for all breeds. Bonsmara and Bonsmara had the highest sarcoplasmic protein solubility, followed by Nguni and Angus and Charolais had the lowest sarcoplasmic protein solubility. Surface myofibril hydrophobicity increased with ageing in all breeds. The day 3 *post mortem* had lower surface myofibril protein solubility for all breeds, and the day 20 *post mortem* had the highest myofibril surface hydrophobicity.

Table 5.20: Effect of breed X ageing interaction on protein denaturation and lipid oxidation of *m. longissimus dorsi* (LD).

	Beef breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Drip-loss						0.397	0.002
3 d *post mortem*	0.01^{i}	0.17^{i}	0.01^{h}	0.01^{i}	0.01^{j}		
9 d *post mortem*	1.12^{defg}	1.46^{c}	2.13^{a}	1.07^{efg}	0.97^{fg}		
14 d *post mortem*	1.46^{c}	1.35d^{c}	1.98^{ab}	1.03^{fg}	0.93^{g}		
20 d *post mortem*	1.32^{cde}	1.38^{c}	1.83^{b}	1.32^{dc}	1.21^{cdef}		
WHC						0.059	0.296
3 d *post mortem*	0.43	0.42	0.40	0.45	0.43		
9 d *post mortem*	0.41	0.40	0.42	0.43	0.41		
14 d *post mortem*	0.35	0.37	0.35	0.39	0.40		
20 d *post mortem*	0.44	0.43	0.43	0.48	0.42		
TPS (mg/g)						11.414	0.005
3 d *post mortem*	100.83^{gh}	102.80^{gh}	97.06^{h}	104.39^{g}	101.43^{gh}		
9 d *post mortem*	202.34^{f}	211.48^{bcde}	218.58^{ab}	212.58^{abcd}	216.22^{abcd}		
14 d *post mortem*	205.30^{ef}	210.52^{cde}	216.89^{abcd}	216.36^{abcd}	217.28^{abc}		
20 d *post mortem*	198.33^{f}	209.88^{de}	214.15^{abcd}	213.55^{abcd}	218.70^{a}		
MPS (mg/g)						13.144	<0.0001
3 d *post mortem*	49.02^{i}	49.44^{i}	46.41^{i}	53.72^{i}	50.32^{i}		
9 d *post mortem*	128.03^{fg}	135.37^{def}	148.13^{a}	138.07^{bcd}	144.46^{abc}		
14 d *post mortem*	126.58^{g}	131.40^{efg}	145.51^{ab}	140.82^{abcd}	136.50^{cde}		
20 d *post mortem*	116.90^{h}	115.74^{h}	118.21^{h}	133.09^{defg}	132.79^{defg}		
SPS (mg/g)						6.682	<0.0001
3 d *post mortem*	51.80^{h}	53.36^{h}	50.64^{h}	50.67^{h}	51.34^{h}		
9 d *post mortem*	74.30^{efg}	76.11^{de}	70.44^{g}	74.51^{efg}	71.77^{fg}		
14 d *post mortem*	78.72^{cd}	79.12^{cd}	71.37^{fg}	75.54^{def}	80.78^{c}		
20 d *post mortem*	81.43^{c}	94.13^{a}	95.35^{a}	80.46^{c}	85.92^{b}		
MSH (µg)						11.960	0.005
3 d *post mortem*	63.06^{k}	62.786^{k}	66.72^{ijk}	65.83^{jk}	69.89^{hijk}		
9 d *post mortem*	78.40^{efg}	72.45^{ghi}	78.56^{efg}	74.61^{ghi}	76.39^{fgh}		
14 d *post mortem*	80.25^{efg}	83.50^{ef}	84.44^{e}	102.93^{b}	85.95^{de}		
20 d *post mortem*	98.61^{bc}	93.57^{cd}	112.25^{a}	114.74^{a}	93.19^{cd}		
TBARS (mg/kg)						0.003	0.602
3 d *post mortem*	0.021	0.023	0.022	0.023	0.020		

9 d *post mortem*	0.021	0.019	0.021	0.021	0.019		
14 d *post mortem*	0.025	0.025	0.025	0.024	0.024		
20 d *post mortem*	0.026	0.024	0.025	0.025	0.026		
Thiols (µM/mg)						2.541	0.286
3 d *post mortem*	73.39	74.03	73.46	73.56	74.65		
9 d *post mortem*	73.54	73.30	74.19	72.70	73.72		
14 d *post mortem*	75.28	75.15	76.33	74.48	75.07		
20 d *post mortem*	75.92	76.10	75.89	73.63	73.08		

[1] Standard error of means

[a,b,c,d,e,f,g,h,i] Means in the same row with a different superscript letter differ (P<0.05).

WHC- Water holding capacity

TPS- Total protein solubility

MPS- Myofibril protein hydrophobicity

SPS- Sarcoplasmic protein solubility

TBARS- Thiobarbituric acid reactive substances

MSH- Myofibril surface hydrophobicity

5.4.3 Colour related parameters

The effect of breed and ageing combination on meat colour related parameters is shown in Table 5.21. There were significant interactions between means for breeds and ageing periods for deoxymyoglobin (P<0.0001), oxymyoglobin (P=0.048), metmyoglobin (P=0.024), L^* (P= 0.002), a^* (P= 0.0004), b^* (P< 0.0001), Chroma (P= 0.0004) and Hue angle (P< 0.0001). No differences (P>0 0.05) were observed for metmyoglobin reducing activity between breeds and ageing periods. The concentration of deoxymyoglobin was higher for day 3 *post mortem* for all breeds, but there were no significant differences between days 9, 14 and 20 *post mortem* for all breeds except for that of Nguni, which had higher deoxymyoglobin concentration at day 9 *post mortem*. Overall, day 9 *post mortem* had higher oxymyoglobin concentration, which was significantly different to days 14 and 20 *post mortem* for Bonsmara and Nguni, but similar to the day 3 *post mortem*. Charolais breed showed the lowest oxymyoglobin concentration at day 3 *post mortem*, but at days 9, 14 and 20, the oxymyoglobin concentration was similar to that of other breeds.

Nguni breed showed higher L^* values at all ageing periods, followed by Charolais and Angus. Brahman had the lightest steaks for all ageing periods. The a^* value was lower for the day 3 *post mortem* for all breeds. Charolais and Nguni had the lowest a^* values at all ageing periods and there were no significant differences between these breeds. Angus and Brahman

had the highest *a** values for day 9 *post mortem*, and there were no significant differences between these breeds for day 9 *post mortem*. Angus had the highest *a** values at days 9 and 14 *post mortem*.

The *b** value decreased with ageing, the day 3 *post mortem* had the highest values for all breeds. Nguni had the lowest *b** values, followed by Charolais and then Angus, Bonsmara and Brahman, which had the highest *b** values. There were no significant differences between ageing periods for Bonsmara breed. Nguni breed had the lowest *b** values for all ageing periods. There were no significant differences between ageing periods for days 9, 14 and 20 *post mortem* for Angus, Bonsmara, Charolais, and Nguni.

For Chroma, there were no significant differences between ageing periods for Charolais and Nguni. Chroma values for these two breeds were the lowest. Chroma increased with ageing for Angus, Bonsmara and Brahman. Hue angle increased with ageing for all breeds. Hue angle for the day 3 *post mortem* was significantly different from days 9, 14 and 20 that were similar. Nguni had the highest Hue angle, followed by Angus and Charolais, and then Bonsmara and Brahman, which had the lowest Hue angle.

Table 5.21: Effect of breed and ageing combination on colour related parameters of *m. longissimus dorsi* (LD)

	Beef cattle breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
DeoxyMb %						3.999	<0.0001
3 d *post mortem*	76.26^{b}	75.89^{bc}	72.90^{defg}	72.95^{defg}	72.05^{efgh}		
9 d *post mortem*	75.39^{bcd}	75.01^{bcd}	73.92^{bcdef}	73.51^{cdef}	80.10^{a}		
14 d *post mortem*	74.30^{bcde}	74.45^{bcde}	72.47^{efgh}	73.26^{cdef}	70.74^{gh}		
20 d *post mortem*	73.79^{bcdef}	75.09^{bcd}	72.17^{efgh}	71.72^{fgh}	70.19^{h}		
OxyMb %						2.495	0.048
3 d *post mortem*	9.76^{abcd}	10.69^{ab}	9.41^{bcdef}	7.18^{gh}	8.63^{cdefg}		
9 d *post mortem*	11.03^{a}	9.85^{abc}	10.21^{b}	9.43^{bcdef}	9.21^{bcdef}		
14 d *post mortem*	10.47^{ab}	9.50^{abcde}	7.89^{fgh}	7.98^{efg}	6.42^{h}		
20 d *post mortem*	9.205^{bcdef}	10.35^{ab}	8.29^{defg}	8.13^{efg}	7.53^{gh}		
MetMb %						4.876	0.024
3 d *post mortem*	1.14^{gh}	0.46^{gh}	3.48^{defg}	6.56^{abc}	5.73^{bcd}		
9 d *post mortem*	0.46^{gh}	1.92^{fg}	2.53^{efg}	4.54^{cdef}	0.01^{h}		
14 d *post mortem*	2.12^{fg}	2.64^{efg}	6.10^{abcd}	5.17^{cde}	8.87^{a}		
20 d *post mortem*	3.44^{defg}	1.43^{g}	6.26^{abcd}	5.17^{cde}	8.36^{ab}		
MRA (nM reduced/min/g)						1.04 x 0^{16}	0.584
3 d *post mortem*	5.57 x 10^{8}	5.57 x 10^{8}	5.42 x 10^{8}	5.33 x 10^{8}	5.13 x 10^{8}		
9 d *post mortem*	8.84 x 10^{8}	9.03 x 10^{8}	8.87 x 10^{8}	8.96 x 10^{8}	8.68 x 10^{8}		
14 d *post mortem*	7.70 x 10^{8}	7.26 x 10^{8}	7.45 x 10^{8}	7.46 x 10^{8}	7.78 x 10^{8}		
20 d *post mortem*	7.33 x 10^{8}	6.85 x 10^{8}	7.31 x 10^{8}	6.98 x 10^{8}	6.61 x 10^{8}		
Meat colour characteristics							
L*						1.670	0.002
3 d *post mortem*	41.2^{de}	42.2^{c}	43.4^{b}	41.9^{cd}	37.5^{h}		
9 d *post mortem*	40^{g}	41.2^{de}	43.9^{ab}	40.4^{feg}	37.1^{h}		
14 d *post mortem*	40.1^{fg}	40.8^{ef}	43.9^{b}	41.1^{de}	36.9^{h}		
20 d *post mortem*	40.5^{feg}	41.2^{de}	44.7^{a}	40.9^{fe}	36.7^{h}		
a*						1.696	0.0004
3 d *post mortem*	12.3^{fe}	11.4^{gh}	11.6^{fg}	10.3^{ij}	10.1^{j}		
9 d *post mortem*	15.1^{a}	13.6^{bc}	15.1^{a}	11.1^{igh}	11.5^{fgh}		
14 d *post mortem*	15^{a}	13.2^{dc}	13.6^{bc}	11.5^{gh}	11.3^{gh}		
20 d *post mortem*	14.3^{ab}	12.3^{fe}	12.6^{de}	11.1^{igh}	10.8^{ijh}		
b*						0.726	<0.0001

3 d *post mortem*	10[a]	9.8[a]	10.1[a]	8.5[b]	7.4[e]		
9 d *post mortem*	8.2[bc]	7.9[ced]	9.8[a]	5.9[gfh]	5.8[gfh]		
14 d *post mortem*	8.4[b]	7.7[ed]	9.8[a]	6.3[f]	5.7[gh]		
20 d *post mortem*	8.2[cbd]	7.4[e]	9.8[a]	6.2[gf]	5.4[h]		
Chroma						1.956	0.0004
3 d *post mortem*	15.8[def]	15[fg]	15.4[ef]	13.4[h]	12.5[ih]		
9 d *post mortem*	17.2[ab]	15.7[def]	18[a]	12.6[hi]	12.9[hi]		
14 d *post mortem*	17.2[ab]	15.3[efg]	16.8[bc]	13.1h	12.7[hi]		
20 d *post mortem*	16.5[bcd]	14.4[g]	16[cde]	12.7[hi]	12.1[i]		
Hue angle						0.038	<0.0001
3 d *post mortem*	40[bc]	40.5[a]	41[a]	39.4[ab]	36.3[cd]		
9 d *post mortem*	28.5[gh]	30[ef]	33[def]	27.9[hi]	26.6[jk]		
14 d *post mortem*	29.4[g]	30.3[ef]	35.8[cde]	28.7[hi]	26.6[jkl]		
20 d *post mortem*	29.6[f]	31[def]	37.9[c]	29[gh]	26.5[kl]		

[1] Standard error of means, d *post mortem*=days *post mortem*

[a,b,c,d,e,f,g,h,i] Means in the same row with a different superscript letter differ (P<0.05)

DeoxyMb- Deoxymyoglobin
OxyMb- Oxymyoglobin
MetMb- Metmyoglobin
MRA- Metmyoglobin reductase activity

5.4.4 Muscle energy metabolism

Table 5.22 and Figure 5.5 summarises the effect of breed and time *post mortem* in hours on muscle energy metabolism. There were differences between breed and time *post mortem* means for lactate concentration in the muscle at 3, 6 and 20 hours *post mortem* (P=0.004). All breeds had similar lactate concentrations at one hour *post mortem*. Nguni breed had lower lactate concentration at 3, 6 and 20 hours *post mortem* but similar to Charolais. Lactate concentration increased with ageing. There were differences in muscle glucose, glycogen and glucose-6-phosphate concentrations between breeds at 1, 3, 6 and 20 hours *post mortem* (P<0.0001), as shown in Figure 5.5 B, C, D and Table 5.22. Charolais and Nguni had the lowest and similar glucose, glycogen and glucose-6-phospate concentrations for 1, 3, 6 and 20 hours. The glucose concentrations for Angus, Bonsmara and Brahman were the highest and similar for all the hours and glycogen concentrations were higher for Bonsmara and lowest for Angus and Bonsmara at 1, 3 and 6 hours. Glucose-6-phosphate was highest for Bonsmara at 3 and 20 hours and similar to the Angus and Bonsmara at 1 and 6 hours.

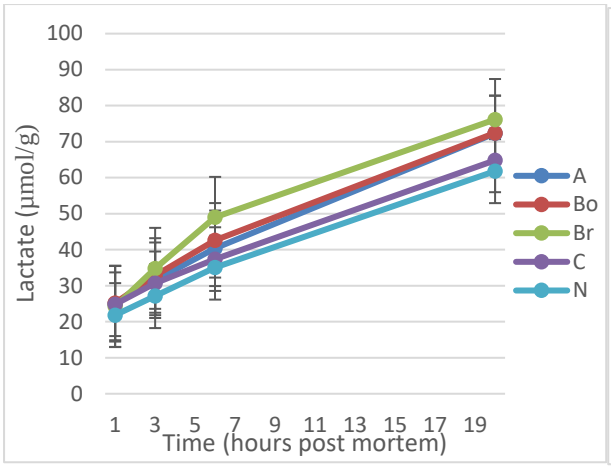
There were no significant differences between breeds for ATP and creatine phosphate concentrations at 1, 3, 6 and 20 hours *post mortem*.

Table 5.22: Effect of breed and ageing interaction on muscle energy (lactate, glucose, glycogen, glucose-6-phosphate, ATP and creatine phosphate).

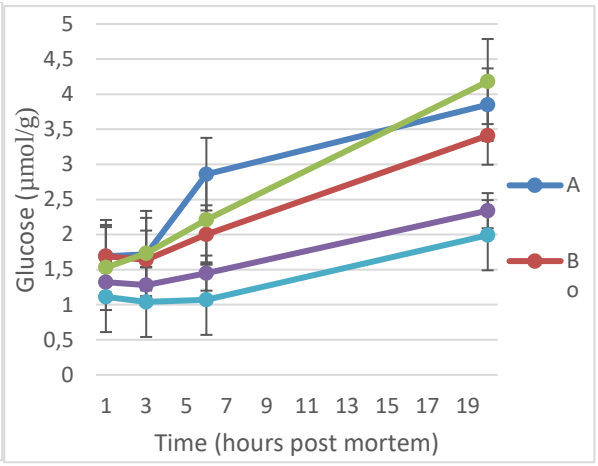
	Cattle breed						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
Lactate (µM/g)						2.723	0.004
1 h *post mortem*	24.98^{ij}	25.21^{ij}	24.25^{ij}	24.86^{ij}	21.84^{j}		
3 h *post mortem*	31.52^{g}	32.85^{fg}	34.82^{fg}	30.66^{gh}	27.11^{hi}		
6 h *post mortem*	40.39^{de}	42.60^{d}	48.98^{c}	37.39^{ef}	35.02^{fg}		
20 h *post mortem*	72.30^{a}	72.42^{a}	76.16^{a}	64.81^{b}	61.81^{b}		
Glucose (µM/g)						0.844	<0.0001
1 h *post mortem*	1.69^{fg}	1.69^{efg}	1.53^{fgh}	1.32^{gh}	1.11^{h}		
3 h *post mortem*	1.72^{efg}	1.64^{fg}	1.73^{efg}	1.28^{gh}	1.04^{h}		
6 h *post mortem*	2.86^{c}	2.00^{def}	2.21^{de}	1.45^{gh}	1.07^{h}		
20 h *post mortem*	3.85^{ab}	3.41^{b}	4.18^{a}	2.34^{cd}	1.99^{def}		
Glycogen(µM/g)						6.728	<0.0001
1 h *post mortem*	41.83^{bc}	31.40^{d}	49.02^{a}	24.54^{efg}	20.79^{gh}		
3 h *post mortem*	40.13^{c}	26.23^{ef}	45.16^{ab}	22.19^{fg}	14.38^{ijk}		
6 h *post mortem*	28.37^{de}	22.00^{g}	31.38^{d}	14.46^{ijk}	10.32^{kl}		
20 h *post mortem*	15.17^{ij}	11.49^{jk}	16.87^{hi}	6.38^{lm}	5.41^{m}		
Glucose-6-Phosphate (µM/g)						1.275	<0.0001
1 h *post mortem*	4.27^{de}	3.97^{def}	4.56^{d}	2.19^{ijk}	2.83^{ghi}		
3 h *post mortem*	2.62^{hi}	2.59^{hij}	3.59^{efg}	1.83^{jk}	1.62^{k}		
6 h *post mortem*	3.10^{gh}	3.28^{fgh}	3.98^{def}	1.81^{jk}	1.40^{k}		
20 h *post mortem*	7.52^{b}	5.79^{c}	9.44^{a}	3.36^{fgh}	3.00^{gh}		
ATP (µM/g)						1.275	0.029
1 h *post mortem*	6.51^{a}	6.40^{a}	6.18^{ab}	6.51^{a}	5.97^{b}		
3 h *post mortem*	5.93^{bc}	5.91^{bc}	5.54^{cd}	5.91^{bc}	5.26^{de}		
6 h *post mortem*	5.04^{ef}	4.82^{f}	4.13^{g}	4.94^{ef}	4.06^{g}		
20 h *post mortem*	2.93^{i}	2.95^{hi}	2.32^{j}	3.33^{h}	2.62^{ij}		
Creatine Phosphate (µM/g)						1.046	0.029
1 h *post mortem*	7.79^{cb}	7.94^{b}	7.12^{de}	8.71^{a}	7.34^{bcde}		
3 h *post mortem*	7.52^{bcd}	6.99^{def}	6.77^{efg}	7.78^{bc}	6.88^{defg}		
6 h *post mortem*	7.17^{cde}	6.79^{efg}	6.35^{fgh}	7.24^{cde}	6.69^{efg}		
20 h *post mortem*	5.79^{h}	6.43^{fgh}	4.87^{i}	6.98^{def}	6.31^{gh}		

[1] Standard error of means, d *post mortem*=days *post mortem*

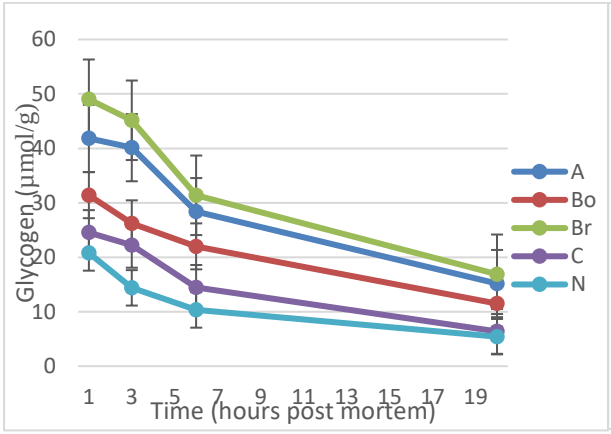
^{a,b,c,d,e,f,g,h,I,j,k} Means in the same row with a different superscript letter differ (P<0.05).



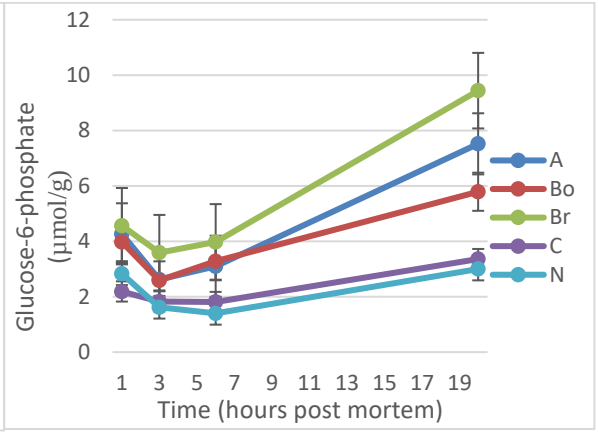
A



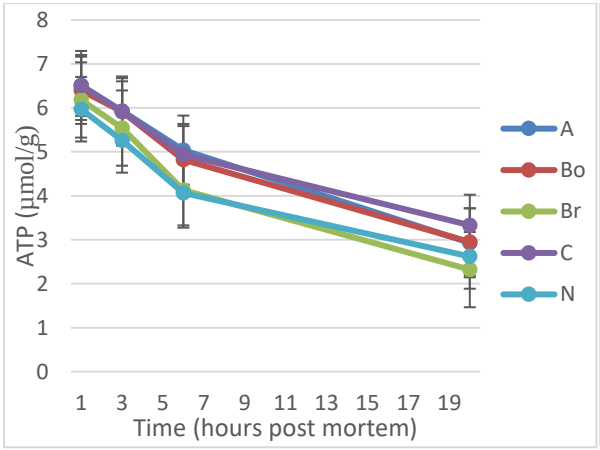
B



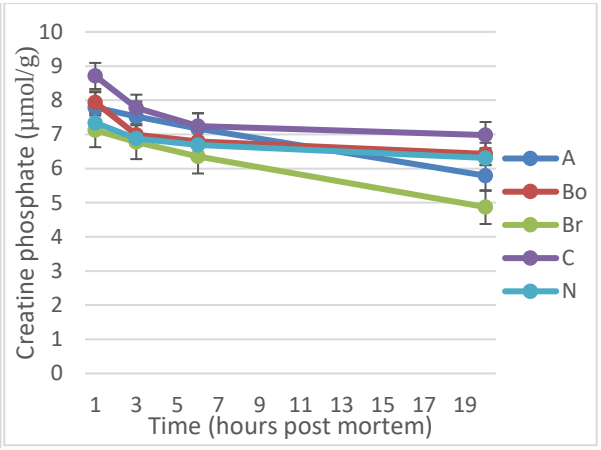
C



D



E



F

Figure 5.5 E-F: Effect of breed and time *post mortem* in hours on lactate (A), glucose (B), glycogen (C), glucose-6-phosphate (D), ATP (E) and creatine phosphate (F) of *m. longissimus dorsi*.

5.4.5 Tenderness related measurements

Table 5.23: Effect of breed and ageing interaction on tenderness related measurements of *m longissimus dorsi* (LD)

	Beef breeds						
	Angus	**Bonsmara**	**Brahman**	**Charolais**	**Nguni**	**SEM[1]**	***P*-Value**
WBSF (N)						4.397	0.137
3 d *post mortem*	54.74^{b}	59.35^{a}	53.27^{b}	55.43^{b}	52.19^{b}		
9 d *post mortem*	41.59^{de}	46.76^{c}	42.28^{d}	47.97^{c}	38.75^{ef}		
14 d *post mortem*	33.26^{g}	42.81^{d}	37.08^{f}	38.36^{ef}	32.96^{gh}		
20 d *post mortem*	29.82^{hi}	36.77^{f}	30.90^{gh}	32.37^{gh}	27.37^{i}		
MFL (µm)						3.041	0.306
3 d *post mortem*	32.55^{a}	30.05^{b}	33.23^{a}	33.84^{a}	32.18^{a}		
9 d *post mortem*	25.86^{cde}	25.59^{def}	27.59^{c}	26.93^{cd}	26.00^{cde}		
14 d *post mortem*	23.62^{gh}	23.85^{fgh}	25.48^{defg}	25.42^{defg}	24.62^{efg}		
20 d *post mortem*	20.66^{i}	21.55^{i}	21.47^{i}	23.67^{gh}	22.43^{hi}		
Fibre detachment (%White to red area)						4.397	0.137
3 d *post mortem*	15.03^{hi}	19.26^{def}	14.56^{i}	17.06^{fghi}	14.93^{i}		
9 d *post mortem*	16.43^{ghi}	18.90^{defg}	17.71^{efgh}	20.52^{cd}	18.86^{defg}		
14 d *post mortem*	20.85^{cd}	22.34^{bc}	20.25^{cde}	22.37^{bc}	20.31^{cde}		
20 d *post mortem*	24.83^{ab}	22.78^{bc}	22.14^{bc}	25.53^{a}	23.67^{ab}		
Fibre breaks score (1-5)						1.164	0.084
3 d *post mortem*	1.69^{efg}	2.61^{abc}	1.91^{cdef}	1.97^{bcdef}	1.10^{g}		
9 d *post mortem*	1.64^{efg}	1.63^{efg}	1.94^{bcdef}	2.10^{bcdef}	1.40^{fg}		
14 d *post mortem*	2.30^{abcde}	2.64^{ab}	1.57^{efg}	2.10^{bcdef}	1.88^{def}		
20 d *post mortem*	2.86^{a}	2.44^{abcd}	2.16^{abcde}	2.51^{abcd}	1.95^{bcdef}		
% Fibre separation score						1.140	0.033
3 d *post mortem*	28.88^{efg}	30.63^{defg}	22.99^{g}	32.36^{cdefg}	33.13^{cdefg}		
9 d *post mortem*	32.00^{cdefg}	47.25^{abc}	36.36^{bcdefg}	36.68^{bcdefg}	38.75^{abcdef}		
14 d *post mortem*	41.88^{abcde}	39.13^{abcdef}	45.00^{abcd}	49.82^{ab}	25.25^{fg}		
20 d *post mortem*	31.50^{defg}	35.63^{bcdefg}	43.07^{abcde}	49.05^{ab}	53.88^{a}		
Fat area score (1-5)						1.149	0.228
3 d *post mortem*	0.40^{e}	0.55^{bcde}	0.50^{de}	0.44^{de}	0.60^{bcde}		
9 d *post mortem*	0.70^{abcde}	0.50^{de}	0.36^{e}	0.94^{abcde}	0.90^{abcde}		
14 d *post mortem*	0.40^{e}	1.15^{bacd}	0.45^{de}	0.58^{bcde}	1.25^{abc}		
20 d *post mortem*	1.40^{a}	0.55^{cde}	0.91^{abcde}	0.67^{bcde}	1.30^{ab}		
Fibre diameter (µm)						0.008	0.974
3 d *post mortem*	57.0^{abc}	55.5^{abcde}	57.8^{ab}	52.5^{bcdef}	60.0^{a}		
9 d *post mortem*	55.0^{abcde}	54.5^{bcde}	57.3^{abc}	52.2^{cdef}	57.5^{ab}		
14 d *post mortem*	51.5^{def}	54.5^{bcde}	55.9^{abcd}	50.6^{ef}	57.5^{ab}		

20 d *post mortem*	51.5^{ef}	54.5^{bcde}	54.5^{bcde}	47.8^{f}	57.0^{abc}		
Analyst tenderness score (1-5)						0.858	0.226
3 d *post mortem*	2.40^{gh}	3.50^{bcd}	2.32^{gh}	2.82^{efg}	2.20^{h}		
9 d *post mortem*	2.50^{gh}	3.25^{cde}	2.82^{efg}	3.39^{bcd}	2.70^{fgh}		
14 d *post mortem*	3.50^{bcd}	4.05^{a}	3.27^{cde}	3.50^{bcd}	3.05^{def}		
20 d *post mortem*	4.05^{a}	3.85^{ab}	3.59^{abc}	4.0556^{a}	3.75^{abc}		

[1] Standard error of means
[a,b,c,d,e,f,g,h] Means in the same row with a different superscript letter differ (P<0.05).

Table 5.23 shows the effect of breed and ageing on tenderness related measurements. There were no significant interactions (P>0.05) between breed and ageing period means for Warner Bratzler shear force, myofibril fragment length, percentage white to red area, fibre break scores 1-5, percentage fibre separation score, fat area score, fibre diameter, analyst tenderness score. Differences were found between breed and ageing period for percentage fibre separation score (P=0.033). There was an increase in the percentage fibre separation score for Nguni and Charolais with ageing period, Angus, Bonsmara and Brahman had an increase in fibre separation score, with ageing then suddenly a decrease at day 20 *post mortem*.

5.5 Correlation coefficients

Table 5.24: Correlation matrix showing correlation coefficients between visual attributes carcass characteristics.

	Colour[2]	**Marbling[2]**	**Fibre separation[2]**	**Texture[2]**	**Structural Integrity[2]**
Live weight	**-0.435**	-0.103	**0.320**	0.269	**0.387**
	0.002	0.483	**0.025**	0.061	**0.006**
Total warm carcass	**-0.508**	-0.150	0.281	0.198	**0.350**
Mass	**0.0002**	0.305	0.051	0.173	**0.014**
Total cold carcass	**-0.507**	-0.151	0.284	0.199	**0.351**
mass	**0.0002**	0.301	0.048	0.170	**0.014**

Correlation coefficients between visual measurements (colour, marbling, fibre separation, texture and structural integrity) and all tested meat quality attributes are shown in Tables 5.24- 5.29. The P<0.05 indicates significant correlations between variables and P>0.05 indicates insignificant correlations between variables. Only significant correlations will be mentioned (P<0.05) and bolded on the Tables. Correlations which are above r= 0.3 are acceptable and good, correlations above r=0.7 are regarded as very good.

Table 5.24 shows correlation coefficients between visual attributes (colour, marbling, fibre separation, texture and structural integrity ratings) and drip-loss, water holding capacity, sarcoplasmic protein solubility, total protein solubility, myofibrillar protein solubility, and myofibril hydrophobicity.

Very low but significant correlations were found between visual colour and water holding capacity (r=0.254, P<0.0001) and myofibril protein solubility (r=0.254, P<0.0001); while good and significant correlations were found between visual colour and sarcoplasmic protein solubility (r=0.304, P <0.0001) and total protein solubility (r=0.309, P <0.0001). Marbling showed very low, but significant correlations with drip loss (r=0.170, P=0.001), water holding capacity (r=0.125, P=0.019), and myofibril protein solubility (r=0.296, P<0.0001). Good positive and significant correlations were found between marbling and sarcoplasmic protein solubility (r=0.365, P<0.0001), total protein solubility (r=0.351, P<0.0001), and myofibril surface hydrophobicity (r=0.353, P<0.0001).

Fibre separation showed good positive and significant correlations with drip loss, sarcoplasmic protein solubility, total protein solubility, and myofibrillar protein solubility. Low but significant correlations were found between fibre separation and water holding capacity, and myofibril protein solubility. Texture only had significant but very low correlations with myofibril surface hydrophobicity (r=0.116, P<0.034). Structural integrity showed good and significant correlations with drip loss (r=0.408, P<0.0001), sarcoplasmic protein solubility (r=0.554, P<0.0001), total protein solubility (r=0.514, P<0.0001), myofibrillar protein solubility (r=0.428, P<0.0001), and myofibril surface hydrophobicity (r=0.461, P<0.0001). There were very low but significant correlations with TBARS (r=0.145, P=0.005) and Thiols (r=0.106, P=0.037) (Table 5.25).

Table 5.25: Correlation matrix showing correlation coefficients between visual attributes and protein denaturation and lipid oxidation of *m. longissimus dorsi* (LD).

	Colour[2]	Marbling[2]	Fibre separation[2]	Texture[2]	Structural Integrity[2]
Drip-loss	-0.093	0.170	**0.377**	0.083	**0.408**
	0.066	0.001	**<0.0001**	0.128	**<0.0001**
WHC	0.254	0.125	-0.129	0.034	-0.021
	<0.0001	0.019	0.018	0.372	0.856
SPS (mg/g)	**0.304**	**0.365**	**0.401**	0.189	**0.554**
	<0.0001	**<0.0001**	**<0.0001**	0.0003	**<0.0001**
TPS (mg/g)	**0.309**	**0.351**	**0.485**	0.207	**0.514**
	<0.0001	**<0.0001**	**<0.0001**	<0.0001	**<0.0001**
MPS (mg/g)	**0.265**	**0.296**	**0.452**	0.181	**0.428**
	<0.0001	**<0.0001**	**<0.0001**	0.001	**<0.0001**
MSH (µg)	0.043	**0.353**	**0.229**	**0.116**	**0.461**
	0.439	**<0.0001**	**<0.0001**	**0.034**	**<0.0001**
TBARS (mg/kg)	0.012	0.100	0.086	0.143	**0.145**
	0.809	0.050	0.093	0.006	**0.005**
Thiols (µM/mg)	-0.072	-0.012	0.051	0.029	**0.106**
	0.157	0.812	0.321	0.564	**0.037**

WHC- Water holding capacity
TPS- Total protein solubility
MPS- Myofibril protein hydrophobicity
SPS- Sarcoplasmic protein solubility
TBARS- Thiobarbituric acid reactive substances
MSH- Myofibril surface hydrophobicity

Table 5.26: Correlation matrix showing correlation coefficients between visual attributes and colour related parameters of *m. longissimus dorsi* (LD).

	Colour[2]	Marbling[2]	Fibre separation[2]	Texture[2]	Structural integrity[2]
L*	**-0.809**	-0.194	0.067	-0.061	0.080
	<0.0001	0.0001	0.196	0.224	0.125
a*	**-0.185**	0.005	0.232	0.079	0.157
	0.0002	0.848	<0.0001	0.166	0.003
b*	**-0.698**	-0.289	-0.095	-0.142	-0.106
	<0.0001	<0.0001	0.049	0.004	0.027
Chroma	**-0.428**	-0.123	0.115	-0.008	0.063
	<0.0001	0.019	0.032	0.754	0.2849
Hue angle	**0.797**	**0.360**	0.250	0.219	0.267
	<0.0001	**<0.0001**	<0.0001	<0.0001	<0.0001
DeoxyMb %	-0.045	-0.114	-0.003	-0.048	-0.164
	0.378	0.032	0.777	0.233	0.0004
OxyMb %	-0.203	-0.163	0.035	0.017	-0.098
	<0.0001	0.002	0.619	0.909	0.027
MetMb %	0.134	0.148	-0.023	0.008	0.137
	0.008	0.005	0.774	0.674	0.002
MRA (nM reduced/min/g)	0.184	0.186	**0.354**	0.196	0.188
	0.0003	0.0002	**<0.0001**	0.0002	0.0003

Mb- Myoglobin
MRA- Metmyoglobin reductase activity
DeoxyMb- deoxymyoglobin
OxyMb- oxymyoglobin
MetMb- metmyoglobin

Table 5.26 shows correlation coefficients between visual attributes (colour, marbling, fibre separation, texture and structural integrity ratings) and Minolta measured colour and related parameters. Visual colour showed very good negative and significant correlations with *L** (r=- 0.809, P<0.0001), *b** (r=-0.698, P<0.0001), and very high significant negative correlations with hue angle (r=0.797, P<0.0001). Good and significant negative correlations were also found between the visual colour and chroma (r=-0.428, P<0.0001). Very low but significant correlations were found between visual colour and *a** (-0.185, P=0.0002) oxymyoglobin (r=-0.203, P<0.0001), metmyoglobin (r=0.134, P=0.008) and metmyogobin reductase activity (r=0.184, P=0.0003). Marbing showed good correlation with hue angle (r=0.360, P<0.0001), and very low but significant correlations with *L** (r=-0.194, P=0.0001) *b** (r=-0.289, P<0.0001), Chroma (r=- 0.123, P=0.019), deoxymyoglobin (r=-0.114, P=0.032), oxymyoglobin (r=-0.163, P=0.002), metmyoglobin (r=0.148, P=0.005) and metmyoglobin reductase activity (r=0.186, P=0.0002). Fibre separation showed good

correlations with metmyoglobin reductase activity (r=0.354, P<0.0001) and very low but significant correlations with a^* (r=0.232, P<0.0001), b^* (r=-0.095, P=0.049), Chroma (r=0.115, P=0.032) and Hue angle (r=0.250, P<0.0001). Structural integrity showed very low but significant correlations with a^* (r=0.157, P=0.003), b^* (r=-0.106, P=0.027), Hue angle (r=0.267, P<0.0001), deoxymyoglobin (r=-0.164, P=0.0004), oxymyoglobin (r=-0.098, P=0.027), metmyoglobin (r=0.137, P=0.002), metmyoglobin reductase activity (r=0.188, P=0.0003).

Table 5.27: Correlation matrix showing correlation coefficients between visual attributes and muscle fibre typing of *m. longissimus dorsi* (LD).

	Colour[2]	**Marbling[2]**	**Fibre separation[2]**	**Texture[2]**	**Structural integrity[2]**
Fibre areas (µm²):					
Red Type 1	-0.031	-0.056	-0.151	-0.094	-0.098
	0.833	0.701	0.299	0.834	0.486
Intermediate type IIA	-0.129	-0.132	-0.112	-0.075	-0.095
	0.375	0.364	0.429	0.628	0.517
White type IIB	-0.181	-0.266	-0.266	-0.263	-0.093
	0.197	0.065	0.071	0.069	0.512
% Fibre type:					
Red	0.206	0.112	0.067	0.009	-0.019
	0.156	0.407	0.645	0.950	0.899
Intermediate	0.134	-0.090	0.048	0.110	0.058
	0.358	0.476	0.745	0.371	0.528
White	**-0.300**	-0.011	-0.102	-0.126	-0.067
	0.036	0.939	0.485	0.387	0.647

Table 5.28 shows correlation coefficients between visual attributes (colour, marbling, fibre separation, texture and structural integrity ratings) and tenderness related measurements. Visual colour showed good significant correlations with sarcomere length (r=-0.461, P<0.0001) and very low significant correlations with WBSF (r=-0.243, P<0.0001), fibre breaks score (r=-0.172, P=0.001) and fat area score (r=0.142, P=0.005). Marbling showed good and significant correlations with WBSF (r=-0.312, P<0.0001), and intramuscular fat (r=0.478, P=0.001), and low but significant correlations with MFL (-0.236, P<0.0001), Fibre detachment (0.207, P<0.0001), fat area score (r=0.117, P=0.021), and fibre diameter (r=- 0.108, P=0.028).

Table 5.28: Correlation matrix showing correlation coefficients between visual attributes and tenderness related measurements of *m. longissimus dorsi* (LD).

	Colour[2]	**Marbling[2]**	**Fibre separation[2]**	**Texture[2]**	**Structural integrity[2]**
WBSF (N)	-0.243	**-0.312**	**-0.401**	-0.151	**-0.506**
	<0.0001	**<0.0001**	**<0.0001**	0.003	**<0.0001**
MFL (µm)	-0.069	-0.236	**-0.360**	-0.171	**-0.484**
	0.173	<0.0001	**<0.0001**	0.0013	**<0.0001**
Sarcomere length	**-0.461**	-0.179	0.012	-0.163	-0.056
	<0.0001	0.078	0.906	0.109	0.586
IMF	0.015	**0.478**	0.081	0.101	-0.081
	0.916	**0.0005**	0.578	0.481	0.579
VIA muscle fibre structural measurements					
Fibre detachment (%White to red area)	-0.013	0.207	0.252	0.108	**0.381**
	0.810	<0.0001	<0.0001	0.043	**<0.0001**
Fibre breaks score (1-5)	-0.172	0.064	0.108	0.073	0.150
	0.0007	0.206	0.035	0.154	0.003
% fibre separation score	-0.018	0.061	0.123	-0.008	0.078
	0.726	0.212	0.019	0.870	0.127
Fat area score (1-5)	0.142	0.117	0.028	0.046	0.069
	0.005	0.021	0.577	0.392	0.175
Fibre diameter	-0.010	-0.108	-0.186	-0.182	-0.135
	0.851	0.028	0.0002	0.0003	0.009
Analyst tenderness score (1-5)	-0.072	0.020	0.012	0.010	0.090
	0.158	0.693	0.812	0.831	0.077

WBSF-Warner Bratzler shear force
SL- Sarcomere length
MFL- Myofibrillar fragment length
IMF- Intramuscular fat

Table 5.29: Correlation matrix showing correlation coefficients between visual attributes and pH and temperature and muscle energy status of *m. longissimus dorsi* (LD).

	Colour[2]	Marbling[2]	Fibre separation[2]	Texture[2]	Structural integrity[2]
pH	0.099	-0.278	**-0.548**	-0.241	**-0.582**
	0.167	<0.0001	**<0.0001**	0.0007	**<0.0001**
Temperature	**-0.361**	**-0.510**	**-0.627**	-0.268	**-0.800**
	<0.0001	**<0.0001**	**<0.0001**	0.0001	**<0.0001**
pH & Temperature	**0.409**	**0.504**	**0.555**	0.213	**0.781**
	<0.0001	**<0.0001**	**<0.0001**	0.0027	**<0.0001**
Lactate	0.104	**0.403**	**0.619**	0.266	**0.674**
	0.149	**<0.0001**	**<0.0001**	0.0002	**<0.0001**
Glucose	-0.183	0.258	**0.521**	0.245	**0.533**
	0.010	0.0003	**<0.0001**	0.001	**<0.0001**
Glycogen	**-0.548**	-0.250	-0.298	-0.170	**-0.342**
	<0.0001	0.0004	<0.0001	0.0169	**<0.0001**
Glucose-6-Phosphate	-0.298	0.137	**0.468**	0.179	**0.477**
	<0.0001	0.056	**<0.0001**	0.012	**<0.0001**
ATP	-0.229	**-0.417**	**-0.549**	-0.229	**-0.654**
	0.001	**<0.0001**	**<0.0001**	0.001	**<0.0001**
Creatine Phosphate	0.212	-0.139	**-0.356**	-0.194	-0.299
	0.003	0.051	**<0.0001**	0.007	<0.0001

Table 5.29 shows correlation coefficients between visual attributes (colour, Marbling, fibre separation, texture and structural integrity ratings) and pH, temperature and muscle energy status. Visual colour showed negative significant and good correlations with temperature (r=-0.361, P<0.0001) pH and temperature combination (r=0.409, P<0.0001) and glycogen (r=-0.548, P<0.0001). Low but significant correlations were observed with glucose (r=-0.183, P=0.0102), glucose-6-phosphate (r=-0.298, P<0.0001), ATP (r=-0.229, P=0.001) and creatine phosphate (r=0.212, P=0.003). Marbling showed good negative and significant correlations with temperature, and ATP and positive correlations with pH and temperature and lactate. Low but significant correlations were observed with pH (r=-0.278, P<0.0001), glucose (r=0.258, P=0.0003), glycogen (r=-0.249, P=0.0004) glucose-6-phosphate (r=0.137, P=0.056), and ATP (r=-0.417, P<0.0001).

Fibre separation showed good negative and significant correlations with pH (-0.548, P<0.0001), temperature (r=-0.627, P<0.0001), ATP (r=-0.549, P<0.0001) and creatine phosphate (r=-0.356, P<0.0001). While good positive and significant correlations were observed with pH and temperature (r=0.555, P<0.0001), lactate (r=0.619, P<0.0001), glucose

(r=0.521, P<0.0001), and glucose-6-phosphate (r=0.468, P<0.0001). Texture showed low but significant correlations with pH (r=-0.241, P=0.001), temperature (r=-0.268, P=0.0001), pH and temperature (r=0.213, P=0.003), lactate (r=0.266, P=0.0002), glucose (r=0.245, P=0.001), glycogen (r=-0.170, P=0.017), glucose-6-phosphate (r=0.179, P=0.012), ATP (r=-0.229, P=0.012), and creatine phosphate (r=-0.194, P=0.007). Structural integrity showed very good negative correlations with pH and very good positive correlations with pH and temperature. There were also good negative correlations with pH (r=-0.582, P<0.0001), ATP (r=-0.654, P<0.0001) and creatine phosphate (r=-0.300, P<0.0001), and positive correlations with lactate (r=0.674, P<0.0001), glucose (r=0.533, P<0.0001), and glucose-6-phosphate (r=0.477, P<0.0001).

CHAPTER 6
DISCUSSION

6.1 Aim of study and experimental description

This research project was carried out in two phases, with Phase 1 being an exploratory phase and implement the findings from the first phase into second phase 2, to allow for a more in-depth analysis in the second phase, in order to learn and implement knowledge from the first phase to improve second phase. This chapter presents a discussion of the results obtained in Phase 1 (pilot study) and Phase 2 in order to evaluate the merits of predicting meat tenderness through evaluating phenotypic meat colour characteristics and certain structural properties assessed on the surface of meat by visually and mechanically methods i.e. beef colour, surface morphology (texture), shelf life, tenderness, and juiciness.

Although similar beef cattle breeds were included in Phase 1 and Phase 2 of the study, results cannot be readily compared due to inherent differences between the phases such as genetic origin (different producers), seasonal variations (described in Chapter 3), differences in feeding management, and environmental conditions such as temperature and humidity.

Some Phase 2 analyses procedures were changed minimally from that of Phase 1, because the first phase was for exploratory purposes, whereas the second phase was for in-depth analysis. Differences between the two phases will be highlighted and explained where necessary. It should also be stressed that the aim of this study was not to compare Phase 1 and Phase 2, but to learn from Phase 1, to improve Phase 2. It should also be noted that some differences observed between breeds could be caused by other factors such as seasonal variations in feed quality, environmental differences (temperature and humidity), differences in early life management, as well as differences in management in the feedlot (stress, transportation, etc.).

The main aim of this study was to evaluate the possibility of predicting meat tenderness by evaluating meat colour characteristics and certain structural properties on the surface of meat objectively and instrumentally. Visual measurements included the visual colour; marbling (flecks of fat on the surface of the steak); fibre separation (degree of separation of fibres on the surface of the meat); texture (the degree of meat fineness or coarseness); and structure integrity (measures how intact the structure is with a slight finger press). The current study evaluated phenotypic differences in meat colour, tenderness and juiciness. Phenotypic differences were compared between five different beef cattle breeds (Angus, Bonsmara, Brahman, Charolais and Nguni), with an application of two treatments, electrical stimulation (ES) and step-wise chilling

(NS). Four different types of ageing period (days 3, 9, 14 and 20) with day 3 aged in polysterine plates covered with polypropylene cling wrap in a display cabinet and days 9, 14 and 20 aged in vacuum bags during Phase 2. During Phase 1, day 9 steaks were aged in polysterine plates covered with polypropylene cling wrap in display cabinet, where it was noted that steaks do not preserve for this long using this packaging method. The steaks started changing colour to brownish, greenish tinge, and an unpleasant mouldy odour. For this reason, during Phase 2, the packaging of day 9 was changed.

It should be noted in this study, a correlation of $r<0.3$ was regarded as low, $r>0.3$-$r<0.5$ as good, and $r>0.5$ as very good.

6.2 Effect of live animal and carcass characteristics on meat quality

Measurable and observable characteristics of the carcass are used to characterise value and quality of beef (Coleman *et al*., 2016). Carcass weight has been reported to influence pH/temperature ratios Frylinck *et al*. (2013) (Tables 4.2 and 5.2). Nguni carcasses have lower carcass weights, and studies have reported that the smaller frame of this breed has made them more prone to cold shortening. In this study, cold shortening was avoided by applying either step-wise chilling or electrical stimulation. Cold shortening can be assessed through measurement of sarcomere length. Even though Nguni breed had shorter sarcomere lengths than other breeds (1.7 µm and 1.8 µm respectively for Phases 1 and 2), the SLs were still longer than what was reported in other studies, and indicated that cold shortening phenomenon was avoided (> 1.5 µm) (Table 4.7 and 5.8). Studies by Vincenzi dos Santos *et al*. (2013)reported improved texture, with increasing slaughter weight in beef animals and the increase in slaughter weight was also associated with more juiciness and flavour. Differences in tenderness between the breeds showed that slaughter weight of animals for this study did not have an effect on meat tenderness (Tables 4.7 and 5.8). As expected, warm and cold carcass weights followed the same pattern as live weight of the five breeds, these characteristics showed good negative correlations with visual colour (r=-0.435, P=0.002; r=-0.508, P=0.0002; r=-0.507, P=0.0002, respectively) as shown in Table 5.24. These results show that the larger the live, cold and warm carcass weights of the animals were, the lighter the meat colour of the steaks were perceived to be by the visual panel. This correlation could also be because of the fact that some breeds like the Nguni naturally produces darker meat and exhibited low live weight, warm and cold carcass weights. Nevertheless, Nguni breed was found to have the lowest oxymyoglobin concentration Table 5.5. Live animal weight also correlated with fibre separation (r=0.320**,** P=0.023**)** and structure integrity (r=0.387, P=0.006). This means that for this study, animals

with higher live weights produced meat that was perceived to be more tender by the visual panel. These visual analysis results for tenderness measurements were in agreement with studies by Vincenzi dos Santos *et al*. (2013). Warm and cold carcass masses were also related to structure integrity. Steaks of animals which had higher live weights, warm and cold carcass weights were related to a more compressible structure (Table 5.24) (r=0.387, P=0.006; r=0.350, P=0.014; r=0.351 P=0.014 respectively**)**. The dressing percentage of Charolais and Brahman was higher, and Nguni, Angus and Bonsmara had similar dressing percentages. From the results of this study, it was evident that eye muscle area was related to carcass weight. Eye muscle area was higher when carcass weight was higher and vice versa. According to McKiernan (2007), the eye muscle area is not very useful as an indicator of animal or carcass quality. Eye muscle area is highly associated with animals, because as an animal gets older and bigger, its eye muscle area also increases.

6.3 Factors affecting the process of meat tenderness

Three "factors/stages" *post mortem* determine meat tenderness, i.e. background toughness, the toughening phase (*pre rigor*) and the tenderisation phase (*post rigor*). The toughening and tenderisation phases take place during ageing *post mortem*, while background toughness occurs at the time of slaughter and does not change during the ageing process. Variation in the background toughness is due to the connective tissue component of muscle (Koohmaraie and Geesink, 2006). Collagen is an abundant connective tissue protein, and is a factor that contributes to variations in meat tenderness, texture and solubility. The level of soluble collagen has been reported to affect tenderness and total collagen content has been reported to have limited value in tenderness prediction (Starkey *et al*., 2016). Results of this study shows that total collagen and insoluble collagen did not have any effect on breed differences, whereas soluble collagen did show differences, but the differences did not show any pattern in relation to shear force measurements, or any of the tenderness related measurements (Table 4.7 and 5.8). Studies have reported that collagen content appear to be affected mainly by the rate of growth of the cattle than breed per se (Muier *et al*., 2000). According to Warner *et al*. (2010), total collagen content is of limited value when predicting tenderness, whereas the level of collagen solubility would be expected to affect tenderness for the *longissimus* muscle. In this study, total collagen has been found to play a significant role in visual meat texture (r=0.306; P=0.035) (Table 5.28). These findings were in disagreement with studies by Warner *et al*. (2010).

The toughening phase occurs as a result of sarcomere shortening during rigor development (Koohmaraie *et al*., 1996; Wheeler and Koohmaraie, 1994), and is known to have an effect on meat tenderness, although this relationship is still controversial (Smulders *et al*., 1990; Veiseith *et al*., 2004). For beef, this process typically occurs within the first 24 hours *post mortem* (Wheeler and Koohmaraie, 1999). In this study, two "ideal" *post mortem* treatments with electrical stimulation (ES) followed by chilling within 1 hour at 4°C, and no electrical stimulation with step-wise chilling (NS) (six hours at 10°C followed by chilling at 4°C) were applied, as already mentioned above. The length of sarcomeres for NS carcasses were longer than ES carcasses in Phase 1 and in Phase 2, but there were no significant differences (Tables 4.16 and 5.18). Nevertheless, the SLs for both phases were longer than 1.8 µm, which indicates that cold shortening was not a factor in both these phases. Longer SL is advantageous as it usually indicate towards a relaxed myosin-actomyosin interaction and therefore more tender meat. Several workers reported that muscles with longer sarcomeres were generally more tender than those with shorter sarcomeres (Frylinck and Heinze, 2003; Kerth *et al*., 1999; Yu and Lee, 1986; Herring *et al*., 1967). In the present study, there was no correlation between visual tenderness measurements and sarcomere lengths (Table 5.28). This observation may possibly be related to the fact that sarcomere length is strongly correlated to meat tenderness only when sarcomeres are shorter than 1.7 µm (Veiseth *et al*., 2004; Herring *et al*., 1967), but some authors reported 2 µm (Wheeler *et al*., 2002; Bouton *et al*., 1973). Since all sarcomeres measured in this study were longer than 1.8 µm, meat tenderness was independent of muscle shortening. According to Marsh and Leet (1966), as cited by Strydom *et al*. (2011), sarcomere shortening of <20% produced nearly insignificant effects on beef tenderness. If the resting sarcomere length of bovine muscle is taken as 2.1 µm, then percentage average shortening for all the breeds was <20%. Sarcomere length breed differences were observed but again, these showed no relation with WBSF for the same reason that the lengths were longer than 1.7 µm. Nguni breed, which appeared to be more tender than other breeds had the shortest sarcomeres. On the other hand, Bonsmara breed, which in this study produced the least tender meat, had the longest sarcomeres. Strydom *et al*. (2000) reported shorter SLs for Bonsmara breed and longer SLs for the Nguni. In Phase 1, SL did give the same relation to shear force as many studies have reported, (longer sarcomere = tender meat). These two studies gave conflicting results. This shows that there could be other contributing factors to this phenomenon and it is suspected that the day temperature differences at slaughter could have played a role.

The tenderisation phase occurs as a result of ageing, and it was suggested that this is due to enzymatic activity (Półtorak *et al*., 2017). It is now well recognised that *post mortem* proteolysis of myofibrillar and myofibrillar-related proteins is accountable for this process (Koohmaraie and Geesink, 2006). Information related to the denaturation pattern of myofibrillar proteins is important, because these proteins are associated with the tenderness and water holding capacity (WHC) of meat (Chelh *et al*., 2006). Results of this study showed that there was a good relationship between myofibrillar protein solubility with fibre separation and structure integrity (r=0.452, P<0.0001; r=0.428, P<0.0001). These supports reports by other studies that these proteins are implicated in meat tenderness (Nowak, 2011; Laville *et al*., 2009; Chelh *et al*., 2006). Protein solubility increased with ageing between days 3 and 9, days 9 and 14 were similar, and day 20 was lower (Table 5.10). Inconsistency of these results suggests that *post mortem* ageing of red meat influence protein extractability. Inconsistent results were also reported by Eady *et al*. (2014), where the authors found that protein concentration of myofibrillar proteins were similar on day 1 and day 8 *post mortem* and lower on day 5 *post mortem*. Ageing related changes in protein extractability were likely due to modifications in protein molecular size, conformation and inter- and intramolecular bonds that occur with *post mortem* ageing (Eady *et al*., 2014).

Myofibrillar surface hydrophobic (MSH) proteins concentration seems to be related to both the amount of marbling, and the structural integrity (r=0.353, P<0.0001; r=0.461, P<0.0001 respectively). This implies that a higher amount of marbling and higher structure compressibility are associated with higher protein hydrophobicity (Table 5.25). Breed differences in MSH shows that for some breeds, the concentration of the hydrophobic myofibrils was lower (Tables 4.4 and 5.4). The pattern between the breeds for MSH and WHC was similar for some breeds more than for others. This could be because there were not much difference between the breeds for WHC. Hydrophobicity of surface myofibrils increased with ageing, showing that as ageing progresses, more myofibre fragments were released. This means that during ageing, proteins denature and result in loss of solubility due to aggregation, which exposes the hydrophobic groups to the surface of the proteins. This has been shown to correspond with the WBSF, which increases with ageing, and the drip loss and WHC, which increase and decrease with ageing, respectively (Table 4.9 and 5.10).

Solubility of sarcoplasmic protein solubility (SP) was reported to be more associated with meat colour changes than with tenderness. These proteins are known to be susceptible to denaturation early *post mortem*, due to rapid glycolysis (Eady *et al*., 2014). Results of this study showed that solubility of these proteins was related to fibre separation and structure integrity

(r=0.401, P<0.0001; r=0.554, P<0.0001). This relationship could be because solubility of sarcoplasmic protein solubility follows the same pattern as these visual attributes. It was observed that solubility of these proteins increased with ageing (Table 4.9 and 5.10). However, there is no clear relationship, which was reported on the relationship between solubility of these proteins and meat tenderness. Bowker *et al*. (2008) observed a decrease in sarcoplasmic protein solubility with ageing. Boles *et al.* (1992) observed day to day fluctuations in solubility of sarcoplasmic proteins but, could not find a clear trend with ageing from zero to seven days *post mortem*. These authors also reported that exogenous proteases associated with meat ageing are not known to degrade sarcoplasmic proteins. Therefore, it is highly unlikely that the increase in concentration with ageing can be attributed to direct proteolysis, as observed in this study.

Meat drip loss has been reported as one of the leading causes of meat sale drop. Meat drip loss is of high importance to meat quality, because beef with high drip loss has been found to have an unattractive appearance, and can turn consumers away and lead to drop in meat sales (Jama *et al*., 2008). In this study, drip loss was found to be correlated to visual fibre separation (r=0.377, P<0.0001) and structure integrity (r=0.408, P<0.0001) (Table 5.25). This implies that the higher the drip loss, the more fibre separation and more compressible surface structure. The formation of drip is a function of the WHC of meat, more drip is formed when the WHC is low and less when it is high (Warris, 2010). However, WHC was found to have a very low correlation with visual meat tenderness measurements. Drip loss has been reported to be related to lower pH values, the higher drip loss observed in the Brahman breed could be related to the pH values observed in this breed because this breed showed the lowest pH values (Table 5.2 and Table 5.4). However, it must be stressed that the lower pH values do not imply that carcasses from these breeds produced PSE (pale, soft and exudative) meat. The PSE phenomenon is said to occur when the pH of meat is <6 at 45 minutes after slaughter (Adzitey and Nurul, 2011). Nguni breed, which had the highest pH values, were seen to have lower drip loss. The water holding capacity of the Charolais and Nguni breeds were higher than that of the Angus, Bonsmara and Brahman (Table 5.4). With ageing, drip loss seems to remain similar between days 9, 14 and 20. Vacuum packaging seems to result in more drip than meat aged in display cabinet, this could be because during vacuuming the steaks are pressed, resulting in release of more drip.

The drip-loss and WHC is usually a dilute solution of sarcoplasmic proteins (Adeyemi and Sazili, 2014). Solubility of these proteins were found to have good correlations with marbling (r=0.365, P<0.0001), fibre separation (r=0.401, P<0.0001), and structural integrity

(r=0.554, P<0.0001) (Table 5.25) as mentioned above. Sarcoplasmic protein solubility (SPS) was the lowest (similar to the Charolais) for Brahman, but this breed showed higher drip loss. There was actually no clear pattern between solubility of sarcoplasmic proteins and drip loss between breeds. The inconsistent pattern between drip loss and the sarcoplasmic protein solubility in this study could be due to the fact that no PSE phenomenon was encountered in the study, as can be supported by the normal pH drop rate, pH at 45 minutes and ultimate pH values of the breed carcasses (Tables 4.9 and 5.10). Sarcoplasmic solubility and the drip loss/WHC normally show a good pattern if there are different quality classes of meat (Joo *et al*., 1999), especially in such case where PSE phenomenon is encountered. This could be the reason why there were such inconsistencies between the drip loss and sarcoplasmic protein solubility. Although there were significant differences between breeds in drip loss, there was not that much differences between the drip loss measurements. Drip loss and SPS both increased with ageing, which shows that as ageing progresses, drip loss increases and the more drip the more sarcoplasmic proteins in the drip (Table 4.9 and 5.10).

Tenderness and WHC have also been reported to be affected by the fibre type composition. Type I muscle fibres have been reported to be associated with WHC and shear force measurements. Hwang *et al*. (2010) reported that increasing the proportion of slow-twitch Type I fibres percentage and area improves tenderness. Maltin *et al*. (1998) reported that an increase in the proportion of the Type I fibres is associated with tenderness and juiciness. These postulations were based on the fact that fast-twitch glycolytic fibres exhibit a more extensive and efficient sarcoplasmic reticulum, higher acto-myosin-ATPase activity, and higher levels of glycogen than slow-twitch oxidative fibres, which, conversely, contain a higher number of mitochondria, as well as more phospholipids and myoglobin. When assessing the correlations between these muscle fibres and visual tenderness measurements, it was observed that there was no correlation with marbling, fibre separation, texture, and structural integrity, as shown in Table 5.27 (r=-0.056, P=0.7004; r=-0.151, P=0.299; r=-0.094. P=0.834; r=-0.0976, P=0.48 respectively). According to the visual measurements, it is therefore not the case in this study that Type I fibres can explain the WBSF and WHC differences between the breeds. Significant differences between breeds were observed in Type I muscle fibres with the Angus, Bonsmara, Charolais, and Nguni containing more of these fibre type than the Brahman (Table 4.6 and 5.7). In agreement, O'Neill (2016) also reported that the Nguni contained more Type 1 muscle fibre than the Brahman did.

Meat tenderness is also related to structural (and biochemical) properties of skeletal muscle fibres, particularly those of myofibrils and intermediate filaments. Histological studies

reported that myofibrils break into smaller sections, known as myofibrillar fragments, during *post mortem* storage of muscle. This process is called myofibril fragmentation. It is considered a useful ageing indicator of meat (Veiseth *et al*., 2001). Strydom *et al*. (2005) reported that myofibril fragmentation of beef was significantly influenced by ageing in beef (2 days 34.2 µm, 14 days 24.7 µm). Results of this study showed that there were no significant differences between the studied breeds for Phase 2; Phase 1 did show significant differences between breeds, but the pattern was different to the WBSF. The fact that WBSF values for breeds were too close to each other could be a contributing factor (Table 4.7 and 5.8). Results of this study showed that myofibril fragment length (MFL) could be a very good indicator of meat tenderness as ageing progresses, because the fragment length became shorter with ageing from three days to 20 days. These results partially agree with results from Strydom *et al*. (2005). The authors found that MFL was a good indicator of the development of tenderness during prolonged ageing, but not for the early *post mortem* variation in tenderness. This could be because their steaks were aged for a shorter period of two days and steaks for this study were aged for three days. Shorter MFLs (or higher myofibrillar indices) are normally associated with a higher degree of proteolysis and supposedly a larger degree of ageing (tenderisation) (Olson, *et al*., 1976 as cited by Strydom *et al*. 2005). Correlation results between fibre separation visual measurements and structure integrity showed that shorter MFLs are associated with more fibre separation, and a compressible structure (r=-0.360, P<0.0001) (r=-0.484, P<0.0001) (Table 5.28). This indicates that visual meat tenderness corresponds with MFL, and is a good predictor of meat tenderness in this study.

6.4 Influence of electrical stimulation on meat tenderness and related factors

In this study, the process of electrical stimulation gave different effects between Phase 1 and Phase 2. Nevertheless, beneficial effects were experienced in both phases. Electrical stimulation in Phase 2 had similar an effect on tenderness measurements than non-stimulation and delayed chilling. During Phase 1, electrically stimulated carcasses resulted in more tender steaks than steaks that were not stimulated. The different levels of effect between Phase 1 and Phase 2 could be attributed to different climatic conditions because during Phase 2, heat waves were experienced during certain days of slaughter as will be discussed below. Electrical stimulation is reported to have beneficial effects in accelerating *post mortem* glycolysis, such that when muscle enters *rigor*, it is prevented from excessive cold shortening at rapid chilling conditions; the electrical current causes the muscle to contract, thereby increasing the rate of glycolysis accompanied by a rapid decline in pH (Figure 4.3 and 5.3) (Ho, 1997). According

to Pearson and Young (1989), cold shortening occurs when the muscle pH is greater than 6.0 at a temperature of less than 10°C with ATP still available. In this study, the cold shortening phenomena can definitely not be considered by applying two optimal *post mortem* treatments (placed directly into chillers after electrical stimulation or step-wise chilling with no stimulation). Delayed chilling should benefit tenderisation, especially when processing smaller carcasses such as the Nguni. These conditions were chosen to eliminate cold shortening, as can be seen by SL which are longer than 1.7 µm (Table 4.7 and 5.8). On the other hand, care must be taken that the pH does not drop too quickly while the carcass temperature is too high, as this causes heat shortening, high protein denaturation, and high drip loss. Although care was taken not to over-stimulate, it is apparent from the data in Table 4.14 that ES treated carcasses had a higher drip compared to NS treated carcasses for Phase 1 and Table 5.15 shows that for Phase 2, ES carcasses had lower drip loss, similar to NS carcasses.

Due to stimulation, muscle glycogen level was higher in ES and lower in NS, even though there were no significant differences. Noticeable differences can be seen in the level of lactate (Table 5.16). Muscle energy status immediately *post mortem* has been reported to affect both meat tenderness and colour (Frylinck *et al*., 2013; Scheffler *et al*., 2011; Monin and Sellier, 1985). The concentration of glycogen, had significant relationship with visual meat colour (r=-0.548, P<0.0001) and structure integrity (r=-0.342, P<0.0001) (Table 5.28). Higher lactate concentration and pH and temperature interaction in muscle resulted in more prominent marbling (r=0.403, P<.0001; r=0.504, P <.0001, respectively), more separation of fibres (r=0.619, P<.0001; r=0.555, P<.0001, respectively) and a more compressible structure (r=0.266, P=0.0002; r=0.781, P<.0001, respectively). Breeds that exhibited lower lactate levels (Table 5.6) also showed higher pH levels (Table 5.2) and lower glucose and glycogen concentrations. Other studies associate higher ultimate pH with more tender meat (Maltin *et al*., 2003) and darker meat colour. Consistently Nguni breed, which had the highest ultimate pH i.e. 5.78, gave rise to the most tender meat (Table 5.3 and 5.4) and darkest meat colour. This was because the reduction in glycolytic metabolites causes more rapid ATP depletion, earlier *rigor* and allowed prolonged activity of proteases (Scheffler *et al*., 2011). In support of this, Table 5.22 shows that ATP levels were depleted faster for Nguni breed than other breeds. However, Charolais and Angus breeds did not follow the same trends. Although Angus breed had an ultimate pH of 5.46, it had similarly tender meat as that of Nguni. Charolais, which had similar ultimate pH to that of Nguni (pH 5.7), had muscle glycogen, and lactate levels higher than Nguni, and tenderness and colour averages different from Nguni (Table 5.6). As an

indigenous breed, Nguni is adapted to sub-tropical climate and harsh conditions. This could explain its lower energy needs, lower glycogen and other energy components, and explain the natural tendency to produce darker meat (Frylinck *et al*., 2013). On the other hand, the higher ultimate pH, lower energy components and darker meat of Charolais is not normal for this breed, and could be caused by heat stress pre-slaughter caused by harsh conditions experienced during Phase 2. Both Phase 1 and Phase 2 slaughters were during spring (October-November) and the maximum South African temperatures this season is around 27ºC, but during Phase 2, there were several days where environmental temperatures reached a higher maximum of 31ºC (see http://www.pretoria.climatemps.com/october.php#table). During Phase 1 of this study, Charolais produced lighter pinkish meat, which was the lightest compared to the other breeds (Table 4.5).

6.5 Evaluation of meat tenderness using objective and instrumental analysis

Studies have shown that consumer re-purchase decisions depend on meat eating quality characteristics, mainly tenderness followed by juiciness and flavour (Shackelford *et al*., 2001). Attempts to analyse tenderness characteristics using different surface structural properties of meat showed great potential in this study for some structural properties (Table 4.3 and 5.3). Marbling level, fibre separation score, surface texture, and structural integrity are some of the visual characteristics believed to have potential in predicting meat tenderness. According to both Phase 1 and Phase 2 results, the visual panel was able to pick-up tenderness differences between breeds using fibre separation, texture and structure integrity sensory measurements. According to fibre separation measurements for Phase 2, Angus and Charolais breeds showed to have more fibre separation, meaning these breeds were perceived as more tender. Brahman and Nguni breeds had less fibre separation, and these breeds were perceived as less tender. Meat texture, which is known as the degree of meat fineness or coarseness, showed that Angus and Charolais had a coarse structure. Nguni had more of a smooth structure than other breeds, meaning that this breed was perceived as producing less tender meat, while Angus and Charolais were perceived as producing more tender meat. It should also be stressed that this is known as the most difficult visual measurement to distinguish. Fibre separation and surface texture both showed that Nguni was least tender and Angus and Charolais were more tender. Structure integrity which was analysed by a slight finger press showed that Angus and Charolais breeds had less compressible structure and Nguni a more compressible structure. This implies that using this technique, Nguni is the one which was perceived as more tender (Table 5.3). Phase 1 results showed that Angus, Charolais and Nguni were more tender and

Brahman was least tender using the fibre separation and texture visual measurements (Table 4.3). Fibre separation increased with ageing between the two packaging methods but ageing in vacuum packages did not show any differences between the days 9, 14 and 20. Similar results were observed with surface texture. Structure integrity decreased with ageing, meaning that with ageing the structure became less intact and hence the meat was more tender. These results were similar for both Phase 1 and Phase 2 (Table 4.8 and 5.9). The panel did not pick-up differences between steaks from electrically stimulated carcasses and those that were not stimulated, probably because there were not much differences as will be discussed in the following paragraphs. The effect of both treatments were similar on visual tenderness characteristics. The amount of marbling has been found to be associated with instrumental meat tenderness in some studies (Frylinck *et al*., 2015) and sensory tenderness (Cannata *et al*., 2010). The visual panel also noticed differences in visual marbling between breeds. Breeds with more marbling were found to be Charolais, Nguni and Angus. Brahman had less marbling. With ageing, the amount of marbling seems to increase (Table 5.8 and 4.8). This does not mean that the meat suddenly develops more marbling as ageing progresses. It seems that as ageing progresses, the fibres separate, exposing more marbling.

When assessing the relationship between Warner Bratzler shear force (WBSF) and visual tenderness measurements, it was revealed that certain structural visual measurements on the meat surface have potential to predict meat tenderness. Meat tenderness, as measured by WBSF showed significant negative relationship with marbling, fibre separation and structural integrity (r=-0.312, P<0.0001; r=-0.401, P<0.0001; r=-0.506, <0.0001, respectively) as shown in Table 5.27. This implies that tender meat is associated with higher marbling, less fibre separation and more compressible structure. Dinh (2008) also reported that marbling score practically has a great effect on meat tenderness and cooking quality. The author reported that marbling is more responsible for meat juiciness, because during cooking, most marbling is lost. A certain percentage remains and helps with lubricating the meat, contributes to mouth feeling, and is a more positive addition to whole flavour of meat. Visual texture showed very little relationship with the WBSF (r=-0.151, P=0.003). Because of the WBSF measurement values that were too close to one another, the panel could not distinguish differences between breeds. It should also be noted that this measurement is known as a very difficult visual measurement to distinguish. According to WBSF measurements, Nguni and Angus produced the most tender meat, while Bonsmara produced the least tender. Although WBSF had significant relationship with certain visual tenderness measurements, the pattern of which breed produced the most tender meat did

not correspond. This relationship could be based more on ageing than breed as tenderness using WBSF seems to increase with ageing (Table 5.11 and 4.10) and seems to correspond with visual marbling, fibre separation and structure integrity measurements. The reason for the less correspondence between the visual tenderness measurements and WBSF between the breeds could be that WBSF measurements are so near to each other (Table 5.8 and 4.7), and therefore the differences were not that apparent visually; with visual measurements, although differences between scores were picked up, the scores were still near to each other.

According to Davey and Dickson (1970), Rowe (1989), Will (1980), changes, which occur post mortem, include detachment of the endomysium, breaks in the sarcomeres (Gothard 1966, Davey & Gilbert., 1969, Olson, 1977), and fibre contraction (Davey & Olson., 1969) as cited by Taylor and Frylinck (2003). These structural changes have been associated with shear force measures (Ho, 1997; Taylor, 1995). According to Taylor and Frylinck (2003), fibre detachment and breaks across the diameter of the fibre are events, which relate to meat tenderness. For this study, fibre detachment (% white to red area), fibre breaks score, % fibre separation score, fat area score, fibre diameter and analyst tenderness scores were evaluated using a video image analyser. These measurements were expected to correlate well with visual tenderness measurements because they somewhat measure the same properties. In addition, studies by Taylor and Frylinck. (2003) reported that sensory tenderness and Warner Bratzler shear force measurements were significantly associated with fibre breaks at 7 and 21 days, but did not find a relationship with fibre detachment or contraction. This study showed that only fibre detachment showed a good relationship with structure integrity (r=0.381, P<0.0001) (Table 5.28). This shows that as the structure became less intact, the fibres became detached.

Fibre detachment score (% white to red area) which also measure the percentage of fat to meat was also expected to have a relationship with the marbling score. This was expected to be so for the fact that as the fibres separates/detaches more marbling is exposed and becomes visible on the surface, but the correlation was rather lower than expected (r=0.207, P<0.0001) (Table 5.28). Nevertheless, fibre detachment did not show differences between breeds, this was also the case with a study by (Taylor and Frylinck, 2003). Again, WBSF measurements, which were close to each other, could be the reason why there were no differences between the breeds. With ageing, these measurements showed to have the same pattern as the WBSF/ fibre separation/ structure integrity, increasing with ageing (Table 4.11 and 5.12).

Although there were significant correlations between the visual tenderness measurements and some VIA measurements, the correlations were very low (Table 5.28). Fibre breaks score,

fibre diameter and analyst tenderness scores showed differences between breeds (Table 5.8) but the pattern was neither similar to breed differences in WBSF nor visual fibre separation, texture or structure integrity. With ageing the fibre breaks score increased, indicating that as ageing progresses there is more fibre breaks. With ageing, the fibre detachment and fibre diameter increased, showing the same pattern as WBSF, and visual tenderness measurements. Fat area score also increased with ageing, similar in pattern to that of marbling as ageing progresses (Table 5.12). These results shows that there could be a possible link between objective tenderness measurements and instrumental tenderness measurements, using the WBSF and very little link using the VIA measurements. It is believed that in an experiment where treatments are chosen to give more significant differences, these technologies can correlate more on a higher level. It should also be noted that visual colour measurements results are means taken over a 10 member visual panel whereas the VIA measurements are based on measurements of a single experienced classifier (but with replications). Electrical stimulation for both Phase 2 and Phase 1 had the same effect as non-stimulation for the VIA measurements (Table 4.16and 5.18).

6.6 Objective and instrumental evaluation of meat colour

Consumers evaluate steaks using colour in making their purchase decisions and the colour associated with fresh beef steaks is usually cherry red (Carpenter *et al*., 2001). Consumers will tend to discriminate against meat of any colour deviating from this. The relationship between objective and instrumental colour measurements would give an indication of how accurately the naked eye can judge meat colour. The oxidation level of myoglobin would also offer an opportunity to understand the chemistry behind meat colour, shelf life and packaging. The fact that different breeds might produce darker meat than others could also influence consumer purchase decisions, and result in discrimination against breeds with darker meat colour. It is not known if these visual colour differences could have an effect on the structure or other quality parameters of meat. Hence, it was necessary to study phenotypic differences between these breeds to understand the impact on other quality characteristics.

In this study, visual analysis of colour was carried out using a trained 10-member visual panel. Visual colour analysis data showed that breed differences exist in terms of visual colour between the Angus, Bonsmara, Brahman, Charolais and Nguni (Table 4.3 and 5.3) for Phase 1 and Phase 2. Nguni produced visually darker meat than all other breeds, while Brahman, and Charolais produced the lightest meat during Phase 1. During Phase 2, Charolais steaks were darker and similar in colour to Angus and Bonsmara. Meat colour from Angus, Bonsmara,

Brahman and Nguni was comparable for the visual analysis for both Phase 1 and Phase 2 for all breeds, except for that of Charolais. Charolais produced the lightest meat during Phase 1, and meat from this breed was darker during Phase 2. According to these results, this breed seems to be more sensitive to environmental conditions, as were experienced during Phase 2. During Phase 2, heat waves were experienced during several slaughter days, as already mentioned above. European cattle breeds *(Bos taurus*), such as the Charolais (in the case of this study) have been reported to have low heat tolerance (O'Neill, 2016), due to the nature of these breeds, the excessive heat seems to have affected pH decline, and hence meat colour. Excessive heat caused stress in these animals prior to slaughter.

The visual panel was also able to pick-up differences between aged steaks for the different packaging types. Visual results showed that meat colour deteriorates faster for some packaging types than it does for others. For Phase 2 of the study, the panellist results showed that days 9, 14 and 20 were similar in colour and Day 3 *post mortem* was less red. According to these results, the colour of fresh vacuum packaged meat can be preserved up to at least 20 days *post mortem* (Table 5.8). Phase 1 results also showed that colour is preserved in vacuum packaged steaks as 14 days *post mortem* was similar to 20 days *post mortem*. Differences were observed only in steaks aged in display cabinets, where these steaks seems to become lighter as ageing progressed (Table 4.8). The combination of breed and ageing showed that during ageing, the breeds behaved differently (P <0.05) according to the visual analysis measurements (Table 5.19 and Figure 5.4). It is evident that vacuum packaging seems to preserve the meat colour for at least up to 20 days *post mortem* for the Angus, Bonsmara and Nguni breeds, based on conditions of this study. Meat colour from these breeds were similar for nine, 14 and 20 days *post mortem*. Whereas the Charolais and Brahman behaved differently, the colour of steaks from the two breeds seemed to deteriorate a little from nine to 20 days *post mortem*. The significant differences observed between three days *post mortem* and nine, 14 and 20 days *post mortem* could be due to the packaging used. Because the 3 days packaged meat had access to oxygen throughout ageing, this could explain the lighter meat that was perceived by the panel. The same observation was made for the Phase 1 of the study. Even though Phase 1 steaks were aged in polypropylene cling wrap up to nine days and vacuum packaged for 14 and 20 days, results still prove that the Brahman and the Charolais meat colour is affected by ageing during early days when aged in vacuum bags. The other breeds maintain meat colour for longer periods, for the steaks aged in vacuum bags.

When comparing objective with instrumental colour measurements, similarities in meat colour were observed between the visual panel results and instrumental colour analysis. Visual colour was negatively correlated to *L** (*r*=-0.809, *P*<0.0001**)**, *b** (*r*= -0.698; *P*<0.0001) and Chroma (*r*=-0.428; *P*<0.0001) and positively correlated to Hue angle values (*r*= 0.797; *P*< 0.0001) (Table 5.26 and 4.23). This implies that as the panel colour rating increases (meat becomes darker), the meat became lighter, its redness became more intense, and as the rating became higher, meat discoloration increased. The visual panel was highly successful in analysing meat colour, where visual analysis results corresponded very well with instrumental colour results. Xie *et al*. (2012) also reported a very good correlation between *L** values and visual colour. The authors reported that *L** and *a** values could explain 69% of the variations in colour, this corresponds with results of this study whereby *L** and *a** values explained about 68% of the variations in visual colour. However, the combination of *L** and *b** values explained most of the differences in visual colour. Similar results were also observed for Phase 1, with lower correlations, but still good and significant (Table 4.23).

As was observed with visual colour measurements, Nguni breed produced meat with the lowest *L** values compared to other breeds, as highlighted in Table 4.5 and 5.5. Several studies have reported this breed as producing the darkest meat. Studies by Muchenje *et al*. (2008a) showed that compared to other breeds, Nguni produced darker meat. The authors reported lower *L** values for meat from Nguni breeds, with higher *L** values for Bonsmara and Angus. This is in line with our study, where Nguni produced meat with lower *L** values and Bonsmara and Angus produced meat with the same *L** values, but higher than Nguni. Brahman is the one, which produced meat with the highest *L** values, where Charolais was similar with Angus and Bonsmara. The breeds also showed differences in *a**, *b**, Chroma and Hue angle. Unlike in this study, where differences could be observed between *a**, *b**, Chroma and Hue angle between breeds, Muchenje *et al*. (2008a), Chambaz *et al*. (2003), and Revilla and Vivar-Quintana (2006) reported no differences in these meat colour characteristics between breeds (some studied breeds were similar with the ones in this study). Differences that arise in meat colour between breeds are not fully understood. According to (O'Neill, 2016) as cited by Muchenje *et al*. (2008a), Nguni cattle were found to release more catecholamines during pre-slaughter period, causing the depletion of glycogen and resulting in the increase in ultimate pH, which is unfavourable for the conversion of muscle to meat. However, in 2009, Muchenje and others reported lower levels of catecholamines in the Nguni breed. There is still some controversy as to what really causes dark colour of meat from Nguni breed. But for Phase 2 of

this study, Nguni and Charolais breeds showed faster depletion of glycogen compared to other breeds (Table 5.22). These breeds had the lowest glycogen levels at one hour *post mortem*, showing that they could have been stressed prior to slaughter. If the animal is stressed before and during slaughter, glycogen is used up, and the lactic acid level that develops in the meat after slaughter is reduced (FAO, nd. *www.fao.org/docrep/003/x6909e/x6909e04*), the low lactate levels of these breeds also supports the findings. The pH values for these breeds dropped more slowly than other breeds between three and 24 hours (Figure 5.1). Ultimate pH levels for these breeds were 5.70 and 5.78 respectively, which can explain the higher L^* values recorded for Charolais and Nguni by the Minolta meter and the darker meat, which was observed by the sensory panel for Phase 2 of the study. Phase 1 results told a different story about pH decline between the breeds, where there were no significant differences between the breeds. The ultimate pH for the Charolais and Nguni was 5.49 and 5.50 respectively, and this was lower compared to what was obtained in Phase 2. Even though Nguni breed had similar ultimate pH as the other breeds, the meat colour was still darker and similar to Phase 2. It seems as though it is a specific characteristic of Nguni to produce darker meat. On the other hand, Charolais breed gave the lightest meat colour in Phase 1. It is therefore not always the case that higher pH values give rise to darker meat colour (O' Neill, 2016). This therefore seems to be a breed-dependent factor for some breeds like the Charolais, but not for Nguni. Other breeds, Angus, Bonsmara and Brahman were not affected by environmental conditions. In spite of the harsh conditions, these breeds produced similar meat colour for both Phase 1 and Phase 2. For Phase 2 of the study, it was found that pH and both pH and temperature interaction correlated with visual colour (r=-0.361, P<.0001; r=0.409, P<.0001 respectively) (Table 5.29). This means that the higher the pH and temperature interaction, the darker/redder the meat colour, but as mentioned above, this seems to be a breed-dependent factor. The amount of lactate in muscle does not seem to affect colour, as the pH and pH and temperature interaction. Studies have reported that a decrease in muscle pH *post mortem* was a result of the amount of lactate produced during glycolysis. If this was the case, the amount of lactate was also expected to affect meat colour in this study. The lack of correlation between colour and lactate concentration could reveal that lactate concertation on its own is actually not responsible for the pH decrease, it is actually the accumulation of H^+ and lactate, which is as a result of glycolysis (Ferguson and Gerrard, 2014; Scheffler *et al*., 2011). In support of this, Monin and Sellier (1985) observed similar lactate values in Hampshire versus Large White pigs, despite different ultimate pH values.

Dark red meat colour has been associated with more myoglobin content. The more myoglobin meat contains, the darker it will appear. Myoglobin has three natural colors depending on its exposure to oxygen and the chemical state of the iron. The three chemical states of myoglobin were investigated in this study (oxymyoglobin, metmyoglobin and deoxymyoglobin). Results revealed that oxymyoglobin and metmyoglobin concentrations showed significantly low correlations with visual colour. The concentration of oxymyoglobin and metmyoglobin showed a significant but very low relationship with visual colour (Table 5.26) ($P<0.0001$, r =-0.203; $P=0.0078$; $r=0.134$ respectively). Angus and Nguni breeds, which showed the lowest oxymyoglobin concentration, also showed the highest metmyoglobin concentration (Table 5.5). Hue angle (meat discoloration) for these breeds were higher even though the Charolais was similar to the Angus and Bonsmara. Chroma also showed that the red colour in these breeds was less intense. For Phase 1 of the study, there were no significant differences between the breeds in deoxymyoglobin, oxymyoglobin and metmyoglobin concentrations. Deoxymyoglobin showed a non-significant correlation with visual colour ($r=0.378$; $P=-0.045$). Deoxymyoglobin results in a dark purplish-red or purplish-pink colour typical colour for vacuum packaged meat. This typical colour was not observed, as it is usually a result of vacuum packaged meat immediately after cutting. Studies by Moss, (1992) as cited by (Pethick *et al*., 1992) reported that high pH and dark colour of meat could be due to higher surface deoxymyoglobin. In this study this was not obvious from the measured level of deoxymyoglobin. Phase 1 results showed no differences between breeds, and Phase 2 results showed lower surface deoxymyoglobin for the Charolais, Nguni and Brahman breeds compared to Angus and Bonsmara breeds. This does not correspond with the higher pH values and darker Phase 2 Minolta colour measurements on Nguni and Charolais. Oxymyoglobin and metmyoglobin may have a greater influence on meat colour differences than does deoxymyoglobin.

Storage period and atmosphere around the meat can determine the proportion of the different states of myoglobin, and this may determine the shelf life of meat (Beriain *et al*., 2009). Exposure of meat to oxygen leads to slow and continuous oxidation of myoglobin in fresh meat. Even though meat aged for three days *post mortem* in polystyrene trays was exposed to oxygen, the accumulation of metmyoglobin on the surface of these steaks was lower than meat that was aged in vacuum bags and had no access to oxygen for 14 and 20 days. Beef aged three days probably had the most favourable oxygen partial pressure for myoglobin oxygenation processes compared to 14 and 20 days *post mortem*, due to the fact that the

mitochondrial activity is decreased, and the protein structure is more degraded, allowing a higher oxygen penetration in meat aged for longer. The accumulation of metmyoglobin during 14 and 20 days of ageing could mean that during storage, the co-factor NAD(H) decreases in muscles, causing the loss in reducing activity of the muscle. NAD(H) is used in the reduction process of metmyoglobin to deoxymyoglobin, consequently maintaining meat colour stability of muscles. When looking at results for nine, 14 and 20 days *post mortem* (metmyoglobin reductase activity), there is indeed decreased reducing ability in the muscle (Table 5.11). Very high differences were seen between Phase 1 and Phase 2 results during the day 9 ageing period. Metmyoglobin levels were higher on steaks that were aged for 9 days during Phase 1. This is because day 9 steaks were aged in polypropylene cling wrap during this phase. This contributed to the steaks' unacceptability. As a result, higher metmyoglobin has resulted in darker meat, and reduced shelf life in this study (Table 4.10). Oxidation of oxymyoglobin to metmyoglobin generates reactive intermediates capable of enhancing further oxidation of oxymyoglobin and unsaturated fatty acids. Superoxide anions are then formed, which dismutates rapidly to hydrogen peroxide that subsequently reacts with metmyoglobin generated in the oxidation process. An activated metmyoglobin complex is formed and is capable of enhancing lipid oxidation. In this study, significant lipid oxidation was obvious during day 9 ageing of Phase 1. Day 9 ageing experienced a very high concentration of metmyoglobin, while the amount of lipid oxidation was also very high, as can be seen by measurements of thiobarbituric acid reactive substances (TBARS) (Table 4.9) (0.210 mg/kg for Phase 1 and 0.021 mg/kg for Phase 2) (Faustman *et al*., 2010). This further shows that steaks aged in display cabinet for 9 days were unacceptable. This is what led to changing day 9 packaging to vacuum packaging, as it was noticed that the steaks aged in polystyrene trays could only be acceptable for at least up to seven days *post mortem*. Changing day 9 to day 7 ageing was not an option for our study, because it was overlapping with other project activities; therefore the solution was to change the nine days *post mortem* packaging to vacuum packaging.

Myoglobin oxidation has also been reported to be dependent on muscle fibre type. Hood (1980) reported muscle type to be the major factor controlling the rate of discolouration of beef muscle on exposure to oxygen, accounting for almost half of the variances in colour stability. The relative proportions of muscle red fibres have been associated with meat colour (Gil *et al*., 2001). These fibres are known to contain a great deal of myoglobin, and many mitochondria. In this study, both the area and percentage of the Red type I, Intermediate type IIA and White type IIB were evaluated. Only the percentage of White type IIB fibres were found to be related

to visual meat colour (Table 5.27). The more red the meat is the lower the white type IIB fibres (r=-0.300, P=0.0361). The negative correlation could be because these muscle fibres are characterised by a lack of myoglobin. Studies have reported that abundant Red type 1 fibres have been associated with decrease in the rate and extent of *post mortem* pH decline and lightness and improved WHC (Choi *et al*., 2006, Gil *et al*., 2003, Larzul *et al*., 1997, Ryu and Kim, 2005). Again, a higher proportion of Red type 1 fibres has been associated with decreased colour stability, with a possible shift to brownish pigment (Renerre, 1984). These muscle fibre types have actually been associated with a rich content of myoglobin, which is the oxygen carrier and pigment responsible for the red colour (Listrat *et al*., 2016). Nevertheless, this study did not show any relationship between Red type 1 fibres and visual meat colour (r=-0.031, P=0.833 for area and r=0.206, P=0.156 for percentage). Breed differences were not observed in this study according to Table 5.7, only the size (area) of these fibre types differed amongst breeds and the percentages were similar. Strydom *et al*. (2000) reported differences between breeds in Type I, Type IIA and intermediate fibre type percentages and size (area).

Myoglobin is referred to as a sarcoplasmic protein due to its presence in the sarcoplasm. Sarcoplasmic protein solubility can therefore be expected to be related to meat colour. This study shows that sarcoplasmic protein solubility and total protein solubility had positive significant correlations with meat colour, as visualised by the trained panel (r=0.304, P<0.0001; r=0.309, P<0.0001 respectively). Myofibrillar protein solubility had a very low relationship with visual colour (r=0.265, P<0.0001) (Table 4.25). Joo *et al*. (1999) have reported that sarcoplasmic protein solubility is closely related to meat lightness in pork. It seems that in beef, sarcoplasmic protein solubility is similarly related to meat colour. Joo *et al*. (1999) observed that as the lightness of meat increases, the solubility of sarcoplasmic proteins also increased. According to correlations results of the study, sarcoplasmic protein solubility and total protein solubility increases as the meat gets darker. Between breeds, protein solubility did not show any clear pattern when compared to meat lightness (Table 4.4 and 5.4).

Sarcoplasmic protein solubility seems to increase with ageing, with day 20 having highest sarcoplasmic protein solubility, showing that as the meat gets darker, protein solubility increases (Table 4.9 and 5.10).

According to Lawrie and Ledward (2006), and Muchenje *et al*. (2008b) another explanation for variations in muscle colour are the differences in the intramuscular fat content (IMF), with higher percentage IMF values resulting in lighter muscles. Table 4.28 shows that visual colour measurements did not have significant correlations or relationship with

intramuscular fat (r=0.015; P=0.916). In this study, although differences were observed in meat colour between breeds, intramuscular fat content did not show any breed differences or the pattern did not follow that of the meat colour differences (Table 4.7 and 5.8).

Post mortem glycolic changes have an influence on the physical and sensory features of beef, which in turn determine the successive processes and impact on beef quality traits such as colour.

Correlation results showed that the concentration of glycogen had very good negative correlations with visual colour. When these metabolites increases, as was reported above, the meat colour redness decreases (Table 5.29) (r=-0.548; P<.0001). Glycogen levels have been reported to be related to meat colour (Pethick *et al*., 1992). High levels of glycogen at slaughter assure the production of lactic acid during the *post mortem* phase so that ultimate pH of meat reaches normal levels (5.5-5.8, according to some studies). Normal ultimate meat pH is a guide of high quality, since it is associated with bright red colour, increased tenderness, as well as increased flavour and shelf life. Studies have shown that meat pH decreases as the concentration of lactate increases. The denaturation of actin and myosin in the muscle caused by the decrease in pH will alter the muscle protein matrix, leading to changes in meat colour, toughness and water holding capacity. However, lactate concentration was found to have no relation with meat colour (r=-0.183, P=0.0102). Again, as mentioned above, it is actually the accumulation of H^+ and lactate which is responsible for pH decrease, and not lactate on its own (Ferguson and Gerrard, 2014; Scheffler *et al*., 2011). Unfortunately, muscle energy status results for Phase 1 are not available for comparison. During Phase 1, muscle energy samples were taken at 1 hour and 24 hours only It was realised that there was a big difference between 1 hour and 24 hours *post mortem,* and therefore to improve our results during Phase 2, samples were taken at one, three, six and 24 hours *post mortem*.

Concentrations of the metabolites, glucose, glycogen, glucose-6-phosphate, ATP and creatine phosphate were all affected by time *post mortem* for all the breeds (also shown in, Table 4.22 and Figure 4.5). Nguni and Charolais, which had similar and lower muscle energy behaviour *post mortem*, also showed similar muscle energy profiles for glycogen decline and glucose, glucose-6-phosphate and lactate increase. On the other hand, Bonsmara, Angus and Brahman had similar higher muscle energy activity profiles for glycogen decline, glucose, glucose-6-phosphate and lactate concentration increase (Figure 4.5). The increase in glucose is due to its release during the debranching of glycogen. The amount of ATP is depleted with time *post mortem,* because ATP is used up in amongst others the formation of glucose-6-

phosphate. Therefore, as seen in this study, glucose accumulates and does not enter the glycolytic pathway. Charolais and Nguni carcasses had lower concentrations of lactate, which could mean that these breeds entered *rigor* slower than carcasses from the Angus, Bonsmara and Brahman. This means that higher lactate concentrations could be associated with higher ultimate pH (Scheffler *et al*., 2011), although other studies disagree.

6.7 Effect of electrical stimulation on objective and instrumental meat colour

Electrical stimulation has been reported to affect meat colour and other meat quality attributes. The process has been reported to accelerate *post mortem* glycolysis so that when muscle enter *rigor,* it is prevented from shortening excessively (Hwang *et al*., 2003; Young *et al*., 1999; Simmons *et al*., 2008). Electrically stimulated carcasses had a significantly higher chilling rate than NS treated carcasses, because they were put in chillers quicker than NS carcasses. This shows the effect of ES and NS post-slaughter treatments on temperature and pH decline profiles (Figure 5.3 and 4.3). The pH/temperature decline rate differed (P <0.001) between ES and NS treated carcasses. Nevertheless, there were no significant differences at 24 hours *post mortem.*

Electrical stimulation has been reported to influence meat colour and water holding capacity. The process has been reported to result in paler meat with an improved bright red colour on cut meat surfaces at 24 hours *post mortem* (Eikelenboom *et al*. 1985, Vergara & Gallego, 2000; Wiklund *et al*., 2001; Davel *et al*., 2003; King *et al*., 2004). Other studies have only reported negative effects of ES on drip loss and no effect on meat colour (Strydom *et al*., 2005). In this study, ES has shown positive influence on drip loss and meat colour as the effect was similar to NS. Results of colour analysis by the visual panel did not show any colour differences between steaks from stimulated and non-stimulated carcasses for both Phase 1 and Phase 2 (Table 4.13 and 5.14).

Electrical stimulation had a positive effects also on the water holding capacity, total protein solubility, TBARS, free thiol groups, Minolta colour characteristics (*L**, *a**, *b**, Chroma, hue), glycogen and creatine phosphate for Phase 2. There were no significant differences in these measurements between ES and NS. Significant differences were only observed for metmyoglobin reductase activity, sarcoplasmic protein solubility, lactate, glucose, and ATP. Electrically stimulated steaks had higher metmyoglobin reductase activity, which is related to lower myoglobin oxidation levels, and this results in improved meat colour stability (Luciano *et al*., 2011) (Table 5.15). Sarcoplasmic protein solubility was higher in steaks from carcasses that were not electrically stimulated and lower in steaks from carcasses that were

elastically stimulated. As mentioned above, Joo *et al*. (2013) reported that as the lightness of the meat increases, solubility of sarcoplasmic proteins also increased. This shows that ES steaks could have had darker colour and higher myoglobin content, but according to colour results, this was not the case when looking at results of this study (Table 5.14).

Lactate and glucose levels were higher for steaks from ES carcasses; this could be due to the faster glycolysis rate. The level of ATP was also lower in ES because of its accelerated depletion during the process.

A different effect was observed during Phase 1, electrical stimulation has shown more beneficial effects on the meat colour compared to non-stimulation. Table 4.15 showed lighter steaks from electrically stimulated carcasses; according to Eikelenboom *et al*. (1985), this could be due to a greater amount of light being reflected from the muscle as a result of a looser structure occurring, also allowing deeper oxygen penetration. Eikelenboom *et al*. (1985) found that electrically stimulated beef at 24 hours was classically more red, and that this was reported to be due to damage to the enzyme systems responsible for oxygen consumption, reduced oxygen consumption rate, and hence, higher concentrations of oxymyoglobin in the surface meat layer. According to Rees *et al*. (2003), protein denaturation does not result in higher L^* values, but this is due to lower muscle pH that increases free water at the cell surface, resulting in an increased reflectance, giving the meat a lighter appearance. Results of Phase 1 showed improved L^*, a^*, b^* colour values for electrically stimulated carcasses (Table 4.15) and the level of oxymyoglobin was higher (although there were no significant differences). Roeber *et al*. (2000) also found L^*, a^*,b^* values to be higher due to electrical stimulation. However, quite a number of studies have reported inconsistent results around electrical stimulation. Martin *et al*. (1983), McKenna *et al*. (2005), Cetin *et al*. (2012) and Nazli *et al*. (2010) reported improvements in meat colour when carcasses were subjected to electrical stimulation. On the other hand, results from Ledward *et al*. (1986) showed that electrically stimulated bovine *semimembranosus* muscle indicated distinct development of metmyoglobin, which resulted in colour loss. Hector *et al*. (1992) reported that low voltage ES causes pH decrease (<6) in beef, which in combination with *in vivo* temperature, was responsible for colour loss, partly due to myosin denaturation. Drip loss was also increased in electrically stimulated carcasses, the increased drip loss coincided with decreased sarcoplasmic protein solubility (Table 4.14). There seems to be inconsistency between our Phase 1 and Phase 2 results when electrical stimulation was applied, again, the harsh climatic conditions experienced during Phase 2 of the study might have played a big role. Although no significant differences were observed due to

electrical stimulation during Phase 2, stimulation had a favourable effect on carcasses resulting in a faster pH decline immediately after slaughter for both Phase 1 and Phase 2 (Figures 4.3 and 5.3).

6.8 Association between objective colour and instrumental tenderness

The use of objective colour measurements could serve as a cost effective and non-invasive technology in predicting meat tenderness. This could be used as a new technology in classifying carcasses in to palatable and unpalatable, respectively. Several studies have tried to find an association between colour and meat tenderness, where some studies revealed positive results, while others revealed negative results (Woerner & Belk. 2008; Wulf and Page, 2000; Wulf *et al*., 1997; Hilton *et al*., 1998; Hodgson *et al*., 1992). Studies by Hodgson *et al.* (1992) and Hilton *et al.* (1998) found that cooked beef palatability was related to instrumental lean and fat colour scores for mature cow carcasses. Wulf *et al.* (1997) found that (CIE) *L*a*b** values were highly related to beef carcass palatability. Additionally, Wulf and Page (2000) found that *L** and *b** values effectively differentiated beef that was low tenderness and flavour. According to Woerner and Belk (2008), the use of colour in combination with other carcass characteristics effectively explains variation in beef palatability. Results of this study shows that there is a very low association between the objective colour analysis and objective tenderness related measurements, especially the WBSF (Table 5.28). Very low negative significant correlations were found between shear force and visual colour (r=-0.243, P<0.0001). The only other association that existed was between the visual colour and sarcomere length, but in this study, SL was found not have any influence on the shear force for Phase 2. Longer sarcomeres were found to be related to lighter meat colour (r=-0.461, P<0.0001) (Table 5.28). Some reports have actually associated heat shortened muscles with a paler colour (Meat technology update, 2011). Fibre separation and structure integrity did show significant potential to predict meat tenderness. Visual marbling, could also have potential in predicting meat tenderness (r=-0.312, P<0.0001**)**, but surface texture showed no potential at all. According to Wulf *et al.* (1997), objective colour scores were more highly related to WBSF and sensory panel ratings, and showed a greater ability to a differentiate carcasses in to tenderness groups than marbling scores.

CHAPTER 7
Conclusion

7.1 General conclusion

The main aim of this study was to assess the possibility of predicting meat tenderness through evaluating meat colour characteristics and certain structural properties on the surface of the meat, objectively and instrumentally. Visual measurements included visual colour, marbling (flecks of fat on the surface of the steak), fibre separation (degree of separation of fibres on the surface of the meat), texture (the degree of meat fineness or coarseness), and integrity structural (how intact the structure when pressed lightly with a finger). Based on results of this study, it can be concluded that fibre separation, structural integrity and marbling could be used to visually predict meat tenderness, but only where training is provided. It is believed that in an experiment where treatments and conditions were chosen to give more significant differences in terms of tough and tender meat, these technologies could correlate on a higher level. Visual texture did not show any potential as a reliable visual tenderness attribute.

Although marbling was found to have very good correlations with WBSF, similarly to Dinh (2008) the amount of marbling was found to indirectly influence meat tenderness, and should be used with caution to predict tenderness.

The two phases of the project gave different results regarding which breeds produce, the most tender meat. During Phase 2, the Angus and Nguni produced the most tender meat, whereas during Phase 1, Brahman, Angus and Nguni produced the least tender meat. Bonsmara produced the least tender meat during Phase 2 and the most tender meat during Phase 1. It can therefore be concluded that, depending on certain uncontrollable conditions prior to slaughter (such as environmental climate conditions) similar beef cattle breeds can produce meat with variable tenderness. On the other hand, extended ageing of up to at least 20 days *post mortem* eliminates most of the meat tenderness differences between breeds, and then compares favourably. This study has shown that many factors can affect meat tenderness. Based on reaction of breeds during Phase 1 and Phase 2, it can be concluded that meat tenderness is dependent on breed, but the differences between the breeds is very slight. Apart from breed as a factor affecting tenderness, other factors play a major role, as was experienced during these two phases.

Electrical stimulation showed a positive effect on tenderness during both Phase 1 and Phase 2, as was reported by certain studies (Mombeni *et al*., 2013; Warner *et al*., 2010; Strydom *et al*., 2005; Ferguson *et al*., 2000). Results of this study showed that electrically stimulated

carcasses produced more tender steaks than non-electrically stimulated carcasses during Phase 1. However, during Phase 2, there were no significant differences between electrical stimulation and non-stimulation in combination with step-wise chilling. Nevertheless, electrical stimulation did have some beneficial effect on tenderness in both studies. It is therefore evident that environmental conditions play a crucial role where *post mortem* treatments are concerned. Electrical stimulation also led to a more beneficial effect on other meat quality characteristics in Phase 2 than Phase 1. Although the effect of electrical stimulation during Phase 1 did not compromise meat quality, it caused higher drip loss, TBARS, L^* values, and Chroma compared to non-electrical stimulation. Electrical stimulation during Phase 2 gave the same effect as non-stimulation. There is actually quite a lot of controversies around electrical stimulation, where some studies have reported beneficial effects and some, detrimental effects. It is important that electrical stimulation conditions be carefully monitored and not over or under done. The availability of results from both Phase 1 and 2 has shown that certain factors do affect the effect of electrical stimulation. In this study, although no negative effect was observed, the differences in effect were believed to be due to the harsh climate conditions experienced during Phase 2. It is believed that chilling temperatures were probably higher due to environmental conditions during Phase 2.

Visual analysis of colour by the panel was very successful. The visual panel were able to accurately determine different meat colours from the different breeds. The panel was also able to differentiate meat colour from different ageing periods. Therefore, visual analysis of meat colour can be taken to be an actual indicator of the colour quality of meat. According to AMSA (Hunt *et al*., 2012), visual evaluations of colour are closely related to consumer evaluations and set the standard for assessment of instrumental colour.

Breed differences in meat colour were observed in both Phase 1 and Phase 2. Nguni breed was found to produce darker meat than Angus, Bonsmara, Brahman, and Charolais. Charolais breed meat colour on the other hand was dependent on the stress level of the animals prior to slaughter. This study has shown that during Phase 1, where maximum environmental temperatures were around 27ºC, Charolais breed produced meat with a lighter colour. During Phase 2, where several heat waves were experienced during several slaughter days, Charolais breed produced darker meat colour. It seems that the Charolais breed is naturally more sensitive to stress that could have been caused by heat in this study. Meat colour from Nguni breed was darker and similar for both Phase 1 and Phase 2. Therefore, Nguni has natural darker colour meat characteristic, where Charolais has a natural light meat colour characteristic, but the meat

becomes dark if animals become stressed, which is a sign of DFD. Nguni has naturally lower glycogen in muscle which could be attributed to its adaptability to harsher tropical climates, but if Charolais glycogen in muscle is low, it is not natural, but an indication of stress. It is also therefore necessary to inform consumers to be able to differentiate between DFD (dark, firm and dry) and naturally dark meat, because uninformed consumers might discriminate against meat from certain breeds due to its dark colour. Ageing and breed results have shown that meat from Nguni does not change with ageing, although other breeds become darker with ageing, and eventually at day 20, they become more or less similar in colour to the meat obtained from Nguni.

Different packaging methods, which were used in this study, showed that it was impractical to age meat for nine days *post mortem* in a display cabinet. Meat can only be aged for a maximum of seven days *post mortem* under these conditions.

Evaluation of the association between visual meat colour and tenderness, where correlations were evaluated between visual meat colour and instrumental tenderness (WBSF) showed no potential at all. This association could have assisted as a non-invasive technology to classify carcasses into tender and tough by using visual colour, but unfortunately, no association was found.

7.2 Recommendations

According to the South African Red Meat Carcass Classification system, carcasses are classified according to animal age, fatness class, confirmation class and damage of the carcass (RSA Government Notice no. R55 of 2015). This system does not clearly indicate to the consumer the physical, compositional and sensory characteristics of the meat (Naude *et al*., 1990). The system also has limitations in classifying carcasses into most tender, tender, less tender and least tender (Strydom *et al*., 2015). The results of this study were aimed at assisting in classifying meat into tender and tough using visual structural measurements, as researched in this study. The use of different breeds and different ageing periods did yield meat with different tenderness levels, even though tenderness differences were significant, the values were still too close. Nevertheless, the visual panel was able to differentiate tenderness levels between days 3, 9, 14 and 20. This shows that if different classes of meat were to be studied, the visual methods could result in greater success than was achieved in this study. Therefore, according to the results obtained, it is assumed that an experienced classifier can be able to use these visual attributes to characterise meat tenderness. This technology could therefore be used

to classify meat into tender and tough by using the fibre separation and structure integrity. The amount of marbling could also be used as an additional attribute.

Aspects of this study were presented at the 12th meat symposium and at 58th, 59th, 60th, 61st and 63rd International Congress of Meat Science and Technology.

1. Modika, K. Y., Frylinck, L., Snyman, J.D. & Strydom, P.E., 2017. The influence of *post mortem* pH and temperature decline on meat colour of five South African beef breeds. 63rd International Congress of Meat Science and Technology. Cork (Ireland). Pp. 228.
2. Modika, K. Y., Frylinck, L., Anderson, J. & Strydom, P.E., 2017. The influence of *post mortem* muscle energy status on meat colour and tenderness of five South African beef breeds. 63rd International Congress of Meat Science and Technology. Cork (Ireland). Pp. 236.
3. Modika, K. Y., Frylinck, and Strydom. P.E. 2015. The effect of breed and ageing on the shear force, protein solubility and myofibril hydrophobicity. 61st International Congress of Meat Science and Technology. France (Clermont-Ferrand). Full paper available at: http://icomst-proceedings.helsinki.fi/index.php?year=2015.
4. Modika, K.Y., Frylinck, L., Moloto, K.W., Strydom, P.E., Pitse, T.M. & Webb, E.C., 2014. Visual evaluation of beef tenderness by using surface structural observations and its relationship to meat colour. 12th Meat Symposium: Relevance of the South African Carcass Classification Systems. South Africa (Pretoria). Abstract available at: http://www.rmrdsa.co.za/Portals/0/Documents/12th%20MEAT%20SYMPOSIUM%202014.pdf.
5. Modika, K.Y., Frylinck, L., Moloto, K.W., Strydom, P.E., Pitse, T.M. & Webb, E.C. 2014. Visual evaluation of beef tenderness by using surface structural observations and its relationship to meat colour. 60th International Congress of Meat Science and Technology, Uruguay (Punta Del Este) Full paper available at: http://icomst- proceedings.helsinki.fi/index.php?year=2014.
6. Modika, K.Y., Frylinck, L., Heinze, P.H & Strydom, P.E., 2013. Meat colour stability as influenced by genotype, post slaughter treatment, and ageing/packaging combination. 59th International Congress of Meat Science and Technology, Turkey (Izmir). Full paper available at: http://icomst-proceedings.helsinki.fi/index.php?year=2013.
7. Frylinck, L., Modika, K.Y., Heinze, P.H. & Strydom. P.E. 2012. Effects of genomics and post-slaughter treatment on ageing beef colour. 58th International Congress of Meat Science

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8. Frylinck, L., Moloto, K.W., Modika, K.Y. & Strydom, P.E., 2012. Beef sarcomere length after applying two “ideal” slaughter procedures. 58th International Congress of Meat Science and Technology. Canada (Montréal). Full paper available at: http://icomst- proceedings.helsinki.fi/index.php?year=2012.

CHAPTER 8

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