Factors influencing colour variation and oxidative stability of South African game meat species

by

Nikki E Neethling

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Promoter: Prof L.C. Hoffman (Department of Animal Sciences, Faculty of AgriSciences, Stellenbosch University)

Co-Promoter: Prof S.P. Suman (Department of Animal and Food Sciences, University of Kentucky)

Co-Promoter: Dr G.O. Sigge (Department of Food Science, Faculty of AgriSciences, Stellenbosch University)

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DECLARATION

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ABSTRACT

It is believed by many that the future of South African game farming depends on the development of a sustainable game meat industry. To develop such an industry, game meat products of consistently high quality must be supplied to consumers. To ensure the quality and consistency of these meat products, standard processing guidelines are required. No such guidelines are currently available and research on meat quality of South African game meat is thus required to establish these guidelines. There is currently only limited research available on the meat quality of game meat.

Meat colour is important as it is the sole quality factor which consumers can use at the time of purchasing to select meat. Consumers prefer meat which is bright red in colour as they perceive it to be fresher, more wholesome and of higher quality than discoloured meat. Discoloured meat is often discounted, resulting in a loss of profits. Thus, maintaining the bright red colour of meat is essential in ensuring maximum profits. Colour stability is thus an important meat quality attribute which must be examined. Currently no research exists regarding the colour stability of South African game species.

The colour stability of three major South African game species, blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) and fallow deer (*Dama dama*) were evaluated by measuring surface colour attributes (*L**, *a**, *b**, hue, chroma and R (630/580)), surface myoglobin redox forms (percentage deoxymyoglobin, percentage oxymyoglobin and percentage metmyoglobin) and various biochemical attributes (pH, metmyoglobin reducing activity, oxygen consumption, thiobarbituric acid reactive substances, total, heme and non heme iron and total myoglobin) of three muscles, the *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL) and *biceps femoris* (BF) over an eight day colour stability trial at 2°C.

The data indicated that the IS was the most colour stable of the three muscles for all the game species. For both the blesbok and fallow deer, the LTL was observed to be marginally more colour stable than the BF, whereas the LTL and BF for the springbok were observed to have similar colour stabilities. Overall the colour stability of the IS was determined to be eight days or more and that of the LTL and BF only one day for all three game species. Although significant gender differences were observed for the colour stability data, these were disregarded as no gender differences were visually perceived. Despite the similarities in colour stabilities noted for the muscles of the three game species, species differences were observed for various of the surface and biochemical attributes highlighting the need for both muscle and species specific processing strategies to improve colour stability of game meat.

This study provided baseline data for the colour stability of game meat, specifically springbok, blesbok and fallow deer. It also highlighted the vast amount of research that is still

required to ensure that the colour stability of game meat is optimised to ensure maximum colour stability.

UITTREKSEL

Daar word deur baie geglo dat Suid-Afrikaanse wildboerdery se toekoms van die ontwikkeling van ‘n volhoubare wildsvleis mark afhang. Om so ‘n mark te ontwikkel, moet vleisprodukte van hoë gehalte konsekwent aan verbruikers voorsien word. In orde om die kwaliteit en konsekwentheid van sulke vleisprodukte te verseker, word standaard prosesseringsriglyne benodig. Geen sulke riglyne is tans beskikbaar nie en navorsing op die kwaliteit van Suid-Afrikaanse wildsvleis word dus benodig om hierdie riglyne van stapel te stuur. Slegs ‘n beperkte hoeveelheid navorsing is tans op die vleiskwaliteit van wildvleis beskikbaar.

Vleiskleur is baie belangrik aangesien dit die enigste kwaliteitsfaktor is wat verbruikers tydens aankoop kan benut om vleis te kies. Verbruikers verkies vleis wat helder rooi van kleur is aangesien hulle dit as varser, meer voedsaam en van hoër kwaliteit as bruinverkleurde vleis ag. Die prys van verkleurde vleis word dikwels deur handelaars afgemerk wat tot ‘n verlies in wins lei en daarom speel die behoud van vleis se helder rooi kleur ‘n sleutel rol in die maksimering van winste. Kleurstabiliteit is dus ‘n belangrike vleiskwaliteit kenmerk wat ondersoek moet word. Geen navorsing is tans op die kleurstabiliteit van Suid-Afrikaanse wildspesies beskikbaar nie.

Die kleurstabiliteit van drie gewilde Suid-Afrikaanse wildspesies, blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) en takbok (*Dama dama*), was ge-evalueer deur die eienskappe van oppervlak kleur (L*, a*, b*, hue, chroma en R (630/580)), oppervlak mioglobien redoks staat (persentasie deoksimioglobien, persentasie oksimioglobien en persentasie metmioglobien) sowel as verskeie biochemiese eienskappe (pH, metmioglobien verminderings aktiwiteit, verbruik koers van suurstof, Tiobarbitiensuur suur reaktiewe stowwe, totale heme en nie-heme yster en totale mioglobien) van drie spiere, die *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL) en *biceps femoris* (BF), oor ‘n agt dae kleurstabiliteits proefloop by 2°C te meet.

Die data het getoon dat die kleurstabiliteit van die IS die beste van die drie spiere vir al die spesies was. Vir beide blesbok en takbok is dit waargeneem dat die LTL effens meer kleurstabiel as die BF was. Vir springbok daarenteen is dit waargeneem dat beide die LTL en BF soortgelyke kleurstabiliteit getoon het. Vir al drie wildspesies is dit vasgestel dat die kleurstabiliteit vir die IS agt dae of langer en vir die LTL en BF slegs een dag was. Hoewel beduidende geslagsverskille in die kleurstabiliteit data waargeneem is, is dit verontagsaam aangesien dit nie visueel waargeneem kon word nie. Ongeag die ooreenkomste opgemerk in die kleurstabiliteit van die spiere vir die drie wildspesies, is verskille vir verskeie oppervlak en biochemiese eienskappe tussen die verskillende spesies opgemerk wat die noodsaaklikheid vir beide spier en spesie spesifieke prosesserings strategië aandui om die kleurstabiliteit van wildsvleis te verbeter.

Riglyn data vir kleurstabiliteit van wildsvleis, spesifiek springbok, blesbok en takbok, is in hierdie studie voorsien. Dit het ook die groot hoeveelheid navorsing wat steeds benodig word om te verseker dat kleurstabiliteit van wildsvleis geoptimeer word om maksimum kleurstabiliteit te verseker beklemtoon.

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NOTES

This thesis is presented in the format prescribed by the Department of Food Science, Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusions. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Results from this dissertation that have been submitted for publication in the following journal:

- Neethling, N.N., Suman, S.P., Sigge, G.O. & Hoffman, L.C. (under review). Factors which affect the colour and colour stability of ungulate meat: A review. *Meat Science.*

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CHAPTER 1

INTRODUCTION

The South African game farming industry has developed into a multi-billion rand industry over the last couple of decades. It currently ranks as South Africa's sixth largest agricultural sector, employing over 100 000 people (Cloete *et al.,* 2015). The industry is based on several consumable and non-consumable divisions, including: live game trade; leisure, trophy and biltong hunting; game meat; and eco-tourism; with hunting and ecotourism being the most profitable (Cloete *et al.,* 2007). Furthermore, various researchers have noted that the great potential exists in the wildlife sector to contribute to economic growth and development (Van der Merwe *et al.,* 2007; Booth, 2010; Musengezi, 2010, Saayman, 2011a, 2011b; Child *et al.,* 2012). In particular it has been noted that the local game meat industry is relatively undeveloped and demonstrates great potential for growth (Hofmeyr, 2014; Cloete *et al.,* 2015). The lack of development in this industry is mainly attributed to, amongst other things, consumers' perceptions, regulations and the focus on the wildlife breeding. However, many believe that the future of the game industry rests on the production of game meat, with some arguing that a sustainable local and international game meat market is required for the future of the game farming industry as a whole (Cloete *et al.,* 2015). Not only is the development of sustainable game meat industry important to the game farming industry and the economy, but it also has the potential to play an integral role in food security in South Africa (Hofmeyr, 2014).

To develop a sustainable game meat industry in South Africa, the industry must be able to deliver products of a consistently high quality (Hutchison *et al.,* 2010). In order to achieve this, standardised processing procedures need to be used when handling of carcasses and meat (North & Hoffman, 2015). Currently, the South African game meat industry functions as a free-market enterprise. Although this creates opportunities for individual game farmers and game meat producers, it also leads to several problems. For example, no standard cuts or quality standards have been implemented for game meat (Hoffman & Bigalke, 1999) and thus any type of game meat cut of any quality can be sold (Hoffman *et al.,* 2005). As a result, one of the major challenges facing the game meat industry in South Africa is to develop and implement standard guidelines and procedures for the processing of game meat to ensure that a consistently high quality product is produced. However, only limited research is available on the meat quality of game meat species and thus there is not much information from which guidelines and procedures can be developed.

In addition, another potential problem exists regarding the terminology used to refer to meat derived from game species. Venison is currently the terminology commonly used to refer to meat derived from game species. This, however, indirectly implies that the meat

quality from all these species is uniform. Hoffman and Wiklund (2006) attempted to address this issue by suggesting that game meat derived from animals in Australia, New Zealand, Europe and America be differentiated from game meat derived from animals in South Africa by referring to the former as venison and the latter as game meat. The reason for this differentiation is attributed to the differences in rearing systems, which could lead to meat quality differences (Priolo *et al.*, 2001; Suman *et al.,* 2014); meat derived from game animals in Australia, New Zealand, Europe and America is increasingly being obtained from domesticated animals (intensive), whereas meat derived from game animals in South Africa is still obtained from wild, free-roaming animals (Hoffman & Wiklund, 2006). Despite this differentiation, meat derived from different species is still grouped under two generic terms, which again implies uniform meat quality amongst these species. It should be remembered that venison and game meat can be derived from several different species, which could be as different in meat quality as that of the common domestic red meat species, beef, pork, mutton and chevon. In fact, terminology even exists for many of these traditionally farmed species to differentiate when the meat is derived from younger animals, veal (beef), lamb (mutton) and capretto/kid (chevon). These terms are linked to certain quality traits, such as colour, flavour, tenderness and juiciness. As such, consumers know, to a certain degree, what to expect when purchasing these meats. It thus begs the questions, “If domesticated species are individuated by specific terminology, why is the same not done for venison and game meat?” Grouping meat from different species under a common generic term could result in misconceptions regarding the quality of meat from different game species by consumers. For example, if a consumer were to consume game meat derived from springbok but which is market under the generic term “game meat” and have a negative eating experience, they may avoid game meat from all species in the future. Furthermore, grouping meat derived from various game species under a generic term could also lead to uniform processing procedures for these species, which could potentially result in a reduction in the meat quality and shelf-life. Thus, to ensure that game meat is correctly processed and marketed, all quality aspects relating to the various individual game species should be investigated. One important meat quality aspect which should be investigated is the colour stability of various game meat species.

The colour stability of meat is potentially a limiting factor in the shelf-life of meat as the consumer purchasing intent is largely based on the colour of meat (Faustman & Cassens, 1990; Risvik, 1994; Mancini & Hunt, 2005; Hoffman *et al.,* 2007; Yin *et al.,* 2011). Consumers prefer meat which has a bright red appearance, as it is perceived as being indicative of wholesome, high quality, fresh meat (Kropf, 1980; Faustman & Cassens, 1990; Mancini & Hunt, 2005). Colour is also the only quality factor consumers can use at the time of purchasing meat (Suman *et al.,* 2014). Meat which is discoloured is often sold at reduced prices or

reworked into lower value processed products, which result in a reduction in revenue (Kropf, 1980). Thus, the longer the meat remains colour stable, the longer the product can remain on the shelf, provided there is no microbial spoilage, and still fetch a premium price.

The colour of meat principally results from the presence of myoglobin (Mb) (Faustman & Cassens, 1990; Yin *et al.,* 2011). The perceived colour of meat is determined by the quantity and chemical state of the Mb pigment present (Faustman & Cassens, 1990; Mancini & Hunt, 2005). In addition, the chemical and physical state of other components within the meat also affects the perceived meat colour (Lawrie & Ledward, 2006). In turn, these components can be affected by a variety of intrinsic and extrinsic factors, which includes, amongst others, species, breed, animal age, sex, season, feeding regime, storage temperature, ultimate pH, aging, muscle fibre type and lipid oxidation (Mancini & Hunt, 2005; Suman *et al.,* 2014). Meat colour and meat colour stability is thus very complex because, not only is meat itself a complex biological system, but the factors affecting the colour stability of meat are not mutually exclusive and act together to influence the perceived meat colour.

No investigations have ever been conducted on colour stability of game meat species and as such no baseline data is available for the colour stability of meat derived from various game meat species. The objective of this study was thus to investigate the colour stability of three popular South African game species, springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus pygargus phillipsi*) and fallow deer (*Dama dama*) to determine the differences in colour stability between these species, if any, and establish baseline data for future research.

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CHAPTER 2

FACTORS WHICH AFFECT THE COLOUR AND COLOUR STABILITY OF UNGULATE MEAT: A REVIEW

ABSTRACT

The purchasing intent of consumer is largely based on meat colour as this is the only quality factor they can evaluate at time of purchase. Consumers prefer the bright red colour associated with fresh meat as they assume the colour is indicative of wholesomeness and eating quality. Similarly, discoloured (brown) meat is assumed by consumers to be of poorer quality. Consequently, discolouration result in revenue losses and improving meat colour and colour stability is thus of great value to the meat industry. Vast amounts of research and literature is available on the colour and colour stability of domestic species, whereas literature on wild ungulates is notably lacking. With an increasing demand for meat from these species, it is pertinent that the gaps in knowledge regarding the colour and colour stability be identified. The aim of the review was to evaluate the literature on the factors influencing meat colour and meat colour stability to identify where research is lacking, in particular with regard to wild ungulates. From this review it was clear that although the principles of meat science remain the same, differences exist between species, breeds and muscle. Furthermore, while prodigious quantities of literature are available for domestic species, research for most of the intrinsic and extrinsic factors which affect meat colour is notable lacking for wild ungulates. As a result, there is scope for research into this colour and colour stability of wild ungulates.

Keywords: Venison; Game meat; Myoglobin; Muscle specificity; Fibre type

IMPORTANCE OF MEAT COLOUR TO MARKETABILITY

The importance of meat colour with regards to consumer acceptance and purchasing intent has been extensively noted in literature (Faustman & Cassens, 1990; Risvik, 1994; Mancini & Hunt, 2005; Hoffman *et al.,* 2007; Yin *et al.,* 2011). In red meat, a bright red colour is preferred by consumer whereas brown, discoloured meat is not (Mancini & Hunt, 2005). The reason for these differences in preference is due to consumer perception. Consumers perceive bright red meat as being fresher, more wholesome and, as having a better eating quality in comparison to discoloured meat (Kropf, 1980; Faustman & Cassens, 1990; Mancini & Hunt, 2005). The notion that meat colour is indicative of its freshness, wholesomeness and eating quality is somewhat of a common misconception amongst consumers. Although meat discolouration may be indicative of its quality, this is not always the case. In fact, discoloured meat may be of better quality than meat which is bright red in colour. Meat colour can be

affected by numerous intrinsic and extrinsic factors and is thus colour is not necessarily a good measure of meat quality. However, the reality is that consumers will still use meat colour to gauge meat quality as it is the only quality factor they can evaluate at the time of purchasing (Suman *et al.,* 2014). Thus continual research will always need to be done to investigate the factors which influence meat colour and meat colour stability to ensure optimal colour and shelf-life of fresh meats.

In the global market, where the demand for meat from exotic species is increasing (Hoffman & Cawthorn, 2012), it is pertinent that industry stays current by examining the meat quality parameters of exotic species which show marketing potential. Meat from wild ungulates such as deer and various South African game species, has shown great marketing potential (Hoffman & Wiklund, 2006). Thus the various quality aspects of meat from wild ungulates should be examined to establish base-line quality parameters for these species. Since meat colour is the primary determinant of consumer purchasing intent, it is pertinent that it be investigated. While vast amounts of research has been done on the meat colour of various domestic red meat species (SCOPUS 3320), limited information is available on venison (SCOPUS 36) and South African game species (SCOPUS 14). With regard to research into the colour stability of meat from South African species, no literature currently exists.

Mancini and Hunt (2005) reviewed the applied strategies of colour and colour stability in beef and pork from 1999-2004 while, Suman and Joseph (2013) reviewed the chemistry of meat colour and colour stability, including information about both domestic and wild game species. Recently, Suman *et al.,* (2014) reviewed the practical strategies for improving beef colour. This review evaluates the main factors which influence the meat colour and meat colour stability of both domestic and wild ungulates. The aim is to use the literature for domestic ungulate species to identify the short-comings within the literature with regard to wild ungulates. These short-comings will help identify potential areas for further research into the colour and colour stability of meat from wild ungulates.

For the purposes of this review, game meat will be used to refer to meat from game animals in Africa and venison will be used to refer to meat from game animals, particularly deer, originating elsewhere. The reason for this distinction is that meat from game animals in Africa generally originates from wild, free roaming animals whereas venison is increasingly being used to refer to farmed/domesticated animals (Hoffman & Wiklund 2006).

MEAT COLOUR AND MYOGLOBIN CHEMISTRY

Meat obtained from ungulates is red in colour. This red colour primarily results from the presence of the protein myoglobin (Mb) (Faustman & Cassens, 1990; Yin *et al.,* 2011). Other

haem proteins (haemoglobin and cytochromes) may also contribute to the colour of red meat but to a far lesser extent (Mancini & Hunt, 2005). The colour of the muscle tissue is influenced by the amount and chemical state of the Mb pigment present (Faustman & Cassens, 1990; Mancini & Hunt, 2005) and, by the superficial structure of the meat, which is directly related to its ultimate pH (pH_u) (Insausti *et al.,* 1999). Understanding the structure and chemistry of Mb as well as the factors which influence muscle colour is thus essential to understanding meat colour.

Myoglobin structure and function

It is important to remember, when trying to understand meat colour that Mb is a protein and, as with all proteins, it is susceptible to changes in its environment. A change in pH or temperature for instance, could cause a protein to denature changing the structure and functionality of the protein. These changes could have a dramatic effect on the ultimate perceived colour of the meat.

Myoglobin is an intercellular, iron containing, monomeric globular protein made up of 153 amino acids (in mammals) (Renerre, 2000) found in cardiac and skeletal muscle (Livingston & Brown, 1981). It is water-soluble and consists of eight right-handed alpha helices (designated A to H) with a central hydrophobic core (Mancini & Hunt, 2005; AMSA, 2012). Inside the hydrophobic core there is a prosthetic heme group which consist of a porphyrin ring with a central iron atom (Mancini & Hunt, 2005; AMSA, 2012). The iron has six available valence electrons; four of which are bound to the porphyrin ring via the nitrogen's of pyrroles, one which is bound to an imidazole ring from a histidine residue (proximal histidine-93) on the protein and one which is available to bind reversibly to various ligands (Mancini & Hunt, 2005; AMSA, 2012). A distal histidine-64 is also present within the porphyrin ring. This distal histidine is not bound to the iron but is available to interact with oxygen, promoting the binding thereof (Livingston & Brown, 1981).

Myoglobin binds oxygen reversibly, provides oxygen storage and can enhance oxygen availability particularly when oxygen partial pressure is low (Bailey *et al.,* 1990). In the living cell, Mb facilitates the diffusion of oxygen from the extracellular space to the mitochondria (Wittenberg & Wittenberg, 1989). Myoglobin thus supplies the oxygen necessary for various biological processes and has an affinity for binding oxygen.

Myoglobin redox forms

Mb is the main protein responsible for the perceived colour of fresh meat. As haemoglobin is the oxygen transport protein in the blood, Mb is the protein responsible for transporting oxygen in living muscle (Livingston *et al.,* 1983; Renerre, 2000). It is this predisposition of the iron in

Mb to bind to oxygen, which causes it to oxidise so readily post-mortem, resulting in various colour changes. The binding of various other ligands (carbon monoxide and nitric oxide) to the iron in Mb can also cause colour changes (Mancini & Hunt, 2005; AMSA, 2012) but will not be discussed in the review. It is not only the binding of ligands to the iron, but also the redox form of the iron (ferrous or ferric), which influences the perceived muscle colour (Mancini & Hunt, 2005). The three major redox forms of Mb are deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) (Bekhit & Faustman, 2005).

Deoxymyoglobin is the redox state of Mb where no ligand is bound to the sixth binding site on the iron and the iron is in its reduced state (Fe^{2+}) (Fig. 1) (Faustman & Cassens, 1990; Mancini & Hunt, 2005; AMSA, 2012). This state can only be maintained under conditions where very low oxygen tension (<1.4 mm Hg) occurs such as vacuum packaging and the interior of muscle (AMSA, 2012). Thus it is indicative of freshly cut meat prior to blooming or meat which is vacuum packed (Renerre, 2000; Mancini & Hunt; 2005; AMSA, 2012). In this state the meat is perceived as being purple/purplish-red in colour (Faustman & Cassens, 1990; Renerre, 2000; AMSA, 2012). It has been noted that consumer perception to the purple/purplish-red colour of DMb (Warriss, 2000; Carpenter *et al.,* 2001) is low and that displaying meat in vacuum packaging may negatively influence meat sales (Carpenter *et al.,* 2001).

When DMb is exposed to oxygen, in a process known as blooming, a bright red Mb redox state is formed known as OMb (Fig. 1) (Faustman & Cassens, 1990; Mancini & Hunt, 2005; AMSA, 2012). In this state, diatomic oxygen is bound to the sixth binding site of the iron which remains in the reduced state (Fe^{2+}) (Mancini & Hunt, 2005; AMSA, 2012). The binding of the oxygen to the iron is stabilised by the distal histidine-64 resulting in a more compact protein structure than compared to DMb (Mancini & Hunt, 2005; AMSA, 2012). This binding also makes OMb less liable to oxidation than DMb (O'Keeffe & Hood, 1982). As the exposure of the meat surface to oxygen increases, the depth of the oxygen penetration increases and therewith the thickness of the OMb layer. The thickness of this layer is highly dependent on various factors, including the pH and temperature of the meat, the oxygen partial pressure in the surrounding environment, and the competition for oxygen by various other respiratory processes still active within the muscle (Mancini & Hunt, 2005). With the increase in the thickness of this layer, there is a concurrent increase in saturation of the bright red colour (O'Keeffe & Hood, 1982; Mancini & Hunt, 2005). It is this bright red colour that consumers find aesthetically desirable and which is assumed, by most, to be indicative of fresher more wholesome meat (Stevenson *et al.,* 1989; Young & West, 2001; Mancini & Hunt, 2005).

As the oxidation of the muscle's surface progresses, with ever increasing exposure to oxygen, the surface of the meat will change from bright red in colour to brown. This brown colour is caused by the Mb redox state known as MMb (Fig. 1) (Faustman & Cassens, 1990;

Mancini & Hunt, 2005; AMSA, 2012). In this state the iron is oxidised (Fe^{3+}) and there is no ligand bound to iron but rather the binding site is occupied by water (Faustman & Cassens, 1990). Consumers find the brown colour of MMb to be undesirable and indicative of poorer quality meat (Kropf, 1980; Faustman & Cassens, 1990; Rosenvold & Andersen, 2003a; Mancini & Hunt, 2005). It has been noted that even very low concentrations of MMb (<20%) on the surface of meat can influence consumer perception and reduce sales of meat (Warriss, 2000). Metmyoglobin formation depends on various factors including the oxygen partial pressure of the environment, the temperature and pH of the meat, the available reducing capacity of the meat and in some cases microbial growth (Mancini & Hunt, 2005).

Mechanism of meat discolouration

The chemistry of meat colour oxidation in meat has been discussed in the sections above (*2.2 Myoglobin redox forms*) but the mechanism of colour change has only been eluded to. The mechanism of oxidation will be discussed for fresh meat which is exposed to atmospheric oxygen at 2-4°C.

In freshly cut meat all the Mb is in the DMb redox state (Fig. 1) (Warriss, 2000). This changes rapidly when the muscle is exposed to oxygen. As the muscle blooms, the surface DMb will oxidise to OMb (Mancini & Hunt, 2005; AMSA, 2012) (Fig. 1). This oxidization will cause the perceived surface colour of the meat to change from purple/purplish red to bright red in colour. With the increase in the thickness of this layer, there is a concurrent increase in saturation of the bright red colour (Warriss, 2000). A cross-section of the muscle at this point would show that below the OMb layer, DMb is present where the oxygen partial pressure is very low (<1.4 mm Hg) (AMSA, 2012). After one to three days, a thin band of MMb will become apparent between the oxy- and DMb layers (AMSA, 2012). This layer of MMb occurs where the oxygen partial pressure (<7 mm Hg) is too low for the formation of OMb but not low enough for the formation of DMb (Ledward, 1971). Although it may seem contradictory that MMb should occur in fresh meat, it is due to oxidation being favoured over oxygenation at low partial pressures (Warriss, 2000; AMSA, 2012). At this point both the MMb and DMb layers are not visible at the surface as they are overshadowed by the OMb layer. As time progresses the underlying MMb layer will begin to thicken resulting in a concurrent thinning of the OMb layer. As the thickening of the MMb layer progresses, the surface of the meat will change from bright red to brown-red and eventually discolour to brown completely. The time taken for meat to discolour is dependent on the temperature, pH, antioxidant capacity and reducing activity of the meat (AMSA, 2012).

It is important to note that OMb does not convert directly to MMb as this process is thermodynamically unlikely (AMSA, 2012). The OMb will first revert back to DMb and then be

oxidised to MMb (Fig. 1). This is not visually observed as the conversion happens very rapidly (stimulated by metal ions (iron and copper) and is usually concealed by either the layer of OMb or later by the layer of MMb (AMSA, 2012). Stated simply, but not completely accurately in terms of Mb chemistry, the surface of meat changes from purple/purplish red in colour to bright red and then to brown over time.

FACTORS INFLUENCING MEAT COLOUR

There are numerous factors which affect meat colour. It is important to remember that meat is a highly complex biological system and that the factors which affect meat colour do not function independently. The complexity of meat and the interactions between factors makes understanding meat colour very challenging. The factors can be divided into extrinsic and intrinsic factors.

Extrinsic factors

Season

Seasonal variation in physical and chemical meat quality has been noted by several authors (Kim *et al.,* 2003; Hoffman *et al.,* 2009a; Węglarz, 2010; Wiklund *et al.,* 2010; Neethling *et al.,* 2014). With regard to differences in meat colour it can often be attributed to differences in physical activity, stress and/or differences in diet between seasons which result in differences in the muscle composition (e.g. differences in intramuscular fat (IMF) concentration and ultimate pH (pH_u).

In Hanwoo (Korean native cattle) *L** values were lowest during winter and highest in Autumn and Spring, *a** and chroma values were highest for spring and summer and lowest in winter, *b** values were highest in summer and autumn and lowest in winter and hue angle values were highest in summer and lowest in winter. Possible reasons for these difference were not provided (Kim *et al.,* 2003). Contradictory to these results Węglarz (2010) found that the cattle slaughtered in winter had significantly higher *L** (all except the cows which had slightly higher *L** values in summer), *a**, *b**, hue angle and chroma values (it should be borne in mind that any instrumental colour analysis values may differ in magnitude depending on the illuminate used in that study) than those slaughtered in summer. Similar results were reported

by Kadim *et al.* (2004) for the *L**, *a** and *b** values of cattle where differences in muscle colour were investigated between summer and winter.

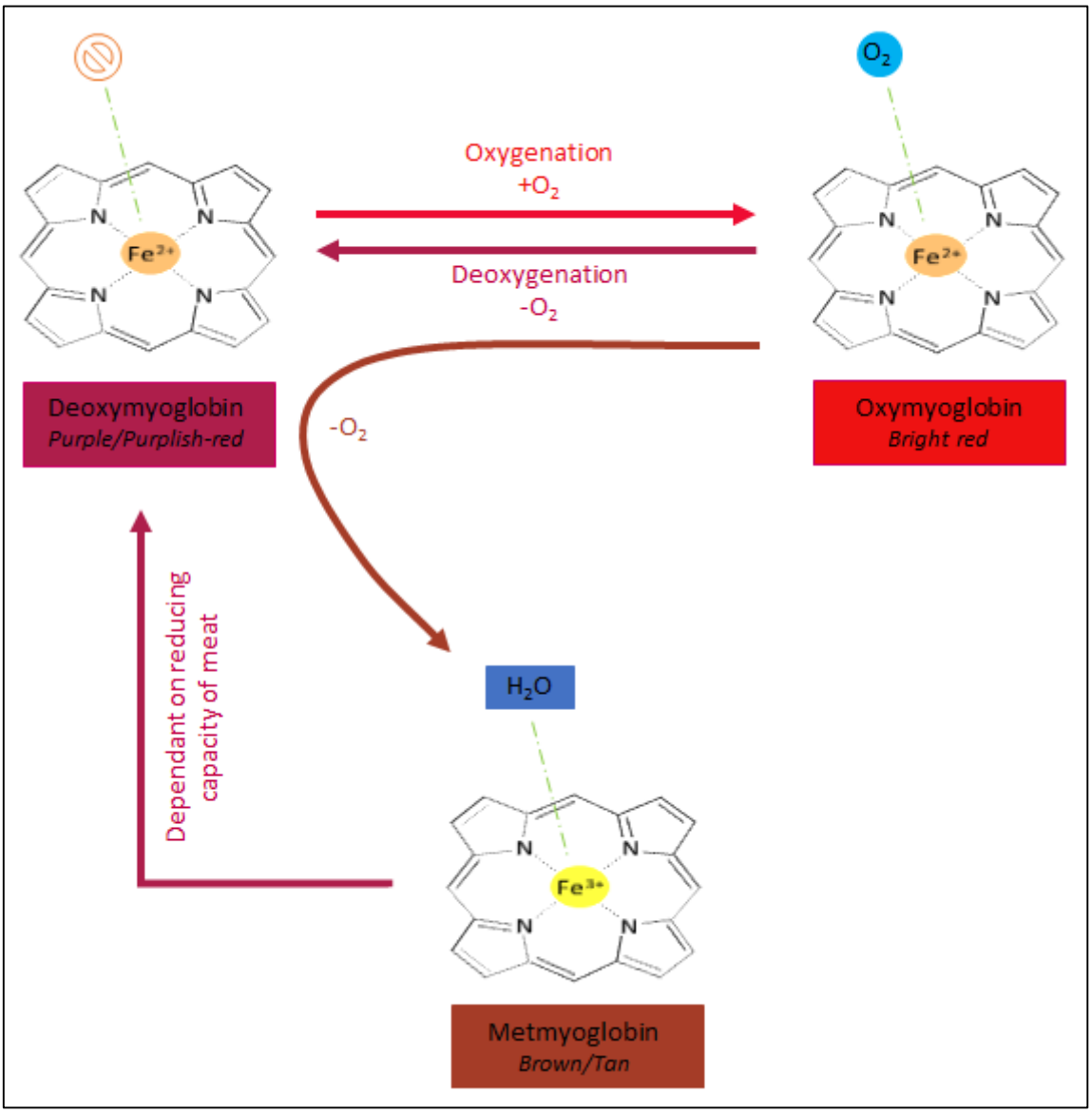

Figure 1 Myoglobin redox forms and their corresponding meat colour.

Neethling *et al.* (2014) noted that the meat from male blesbok (*Damaliscus pygargus phillipsi)* harvested in winter had higher *a** and chroma values. This was attributed to increased physical activity as the males were harvested during the rutting season. Males tend to eat less and fight more during the rutting season (Kohn *et al.,* 2005). This increased physical activity leads to increased Mb content and consequently higher *a** and chroma values. Hoffman *et al.* (2009a) observed darker meat (lower *L** values and higher hue angle) in black wildebeest (*Connochaetus gnou*) harvested in winter compared to those harvested in spring or autumn. Possible reasons for these differences were not provided.

Differences in the colour stability of red deer *longissimus dorsi* (LD) was noted between seasons, with venison from deer harvested in summer having the poorest colour stability and spring the best colour stability. This was attributed to the variance in pH as well as differences in diet. The venison from animals harvested during summer and spring had lower and higher pH values, respectively (Wiklund *et al.,* 2010). Lower pH values are known to lead to poorer colour stability in meat and higher pH values to improved colour stability (Faustman & Cassens, 1990). It was also suggested that the better quality pasture in spring, which leads to higher levels of antioxidants in the muscle, also contributed to the improved colour stability of animals harvested during this period (Wiklund *et al.,* 2006).

Increased incidences of dark, firm dry (DFD) meat have also been reported for beef slaughtered during the summer (Kreikemeier *et al.,* 1998; Mitlöhner *et al.,* 2002; Kadim *et al.,* 2004). These increased incidences could possibly be due to animals slaughtered during summer being more prone to physiological stress (heat stress) and that these animals have lower glycogen reserves than animals slaughtered during winter (Kadim *et al.,* 2004). In contrast Miranda-de la Lama *et al.* (2009) observed higher incidences of DFD in lamb meat during winter. Their results were similar to observations by Knowles *et al.* (1998) and, Zähner *et al.* (2004) who noted higher stress levels in lambs and dairy cows during winter, respectively.

The effect of season on meat colour can be the result of differences in behaviour of the animal (e.g. mating vs. non-mating season), differences in the quality of the grazing (diet) and differences in susceptibility to physiological stress. Discrepancies in observations also exist and it seems that seasonal effects may be confounded by other factors and/or be country/area specific and/or be species/breed specific. No research was found on the effect of season on the colour stability of game species. Although hunting of game species is typically restricted to a season (typically winter when the breeding and rutting seasons are finished), there are some species, such as the springbok (*Antidorcas marsupialis*), that are hunted throughout the year. Research is thus required to quantify the effect of season on the meat colour stability of game species, particularly those which are hunted year-round.

Feeding systems

Colour and colour stability can be affected by the type of feeding system employed; feeding systems commonly include either extensive (grass/pasture/forage) or intensive (concentrate/grain/feedlot) systems. Feeding system with higher levels of antioxidants (e.g. vitamin E) could lead to an increase in the colour stability of meat. It has also been noted that diet can influence the concentration of volatile fatty acids (VFA) in the rumen. Different VFA have different metabolisms, which could influence the glycogen deposition in the muscle and

subsequently the pH_u and colour of the meat (Daly *et al.,* 1999; Priolo *et al.,* 2001). The pH_u is very important in meat colour and may be the reason for differences noted between pasture and grain-based systems. It has been observed that some pasture reared animals may have an insufficient energy intake leading to elevated pH_u values (Daly *et al.,* 1999). The higher pH_u in pasture reared animals could also be attributed to the differences between the production systems. Animals reared on extensive pasture systems have minimal human contact and handling in comparison to feedlot reared animals. Thus, pasture reared animals could be more susceptible to pre-slaughter stress, which in turn could lead to a decrease in glycogen pre-slaughter and low pH_u in the meat (Daly *et al.,* 1999). Furthermore, feeding systems can also influence fat colour particularly the subcutaneous fat, with pasture diets often resulting in more yellow fat compared to grain-based diets. The increase in yellowness is due to an increase in carotene deposits in the fat from green leaf tissue (Yang *et al.,* 1992). Most game species do not have thick layers of subcutaneous fat. It has however been noted that female game animals that do not conceive within a given year/season are known to exhibit thicker layers of subcutaneous fat (Hoffman & Wiklund, 2006).

Priolo *et al.* (2001) compiled a review on the effects of pasture and concentrate feeding on meat colour and flavour in ruminants. The review revealed that the meat from animals finished on pasture diets was darker (lower *L** values) than meat from animals finished on concentrate diets. Several factors were noted to be responsible for the observed difference, with the pH_u and intramuscular fat appearing to have the largest influence.

Díaz *et al.* (2002) observed no differences in lamb meat colour measurements for the *rectus abdominis* but observed a differences in *L** values for the LD. The LD of the pasture animals was darker than that of the concentrate animals. The darker colour of the pasture lambs was attributed to higher Mb concentrations due to higher amounts of physical activity (Vestergaard *et al.,* 2000). Differences in subcutaneous fat colour was also observed, with the pasture animals having darker (lower *L** value), yellower (higher *b** value) fat. Luciano *et al.* (2012) observed no differences for the initial bloomed meat colour of pasture versus concentrate animals. However, the colour stability of meat from pasture animals was greater than that from concentrate animals. This difference in colour stability was attributed to the higher concentration of antioxidants in the meat of pasture animals, imparted to the meat from the green herbage of which pasture is comprised (Wood *et al.,* 1997; Faustman *et al.,* 2010). Interestingly, the study observed that the time on pasture (4 h versus 8 h) did not have an effect on the colour stability.

Significant differences in meat and subcutaneous fat colour have been observed for beef fed either concentrate or forage diets. In agreement with Priolo *et al.* (2001), Avilés *et al.* (2015) noted that forage diets led to darker (lower *L** values) meat in comparison to concentrate diets. The *a** was not affected by the feeding system but *b** values were, which

is congruent with the observations of Daza *et al.* (2014). Concentrate diets resulted in lower *b** values in comparison to forage diets. Several authors have noted higher *b** values (more yellow) for subcutaneous fat of cattle fed forage based diets (Cooke *et al.,* 2004; Duckett *et al.,* 2013; Avilés *et al.,* 2015). The yellowing is attributed to a higher carotenoid content in forage diets (Casasús *et al.,* 2012).

Vestergaard *et al.* (2000) investigated the influence of feeding system (pasture vs. concentrates) on muscle fibre type and the consequent effect of muscle fibre type on colour. The effect of the production system on muscle fibre type was attributed to differences in physical activity and feeding level, and to a lesser extent the diet (pasture vs. concentrates). Overall the results showed that extensive rearing led to higher levels of physical activity in comparison to intensive rearing, resulting in meat from extensively reared animals having more slow-contracting fibres, higher oxidative metabolic potential and darker coloured meat.

Studies on venison have shown that feeding systems can affect colour stability, with improved colour stability mainly being attributed to higher concentrations of antioxidants in the meat (Wiklund *et al.,* 2006, 2010). Similar finding have been reported for lamb (Díaz *et al.,* 2002; Perlo *et al.,* 2008) and beef (Lanari *et al.,* 2002). Wiklund *et al.* (2006) found that deer fed pellets had a lower pH_u than grazing animals, which was implicated in the higher *a** values observed in meat from pellet-fed deer. The lower pH_u results in lower mitochondrial activity and oxygen consumption (Faustman & Cassens, 1990) which subsequently leads to faster oxygenation, more efficient blooming (Lawrie & Ledward, 2006) and deeper penetration of the OMb layer (Feldhusen *et al.,* 1995). Furthermore, the pellet-fed carcasses had a thicker fat covering and thus cooled more slowly than the carcasses of grazing animals (Wiklund *et al.,* 2006). This high temperature, low pH_u combination can lead to protein denaturation and a subsequent decrease in enzyme activity (Lawrie & Ledward, 2006). Reduced enzyme activity leads to lower oxygen consumption, resulting in deeper penetration of oxygen and faster blooming (Lawrie & Ledward, 2006).

Grazing generally results in higher levels of polyunsaturated fatty acids (PUFAs) (Wood *et al.,* 2003) which are more prone to oxidation (Morrissey *et al.*, 1998). A positive correlation between Mb and lipid oxidation have been noted (Faustman & Cassens, 1990). Thus increased oxidation could lead to increased discolouration. However, meat from grazing animals also generally contains higher concentrations of antioxidants which can protect against lipid and Mb oxidation (Faustman & Cassens, 1990). In fact, Wiklund *et al.* (2006) found that the meat from grazing animals had better colour stability than meat from pellet-fed animals despite the later having higher levels of PUFAs. This contradiction was attributed to higher levels of vitamin E in the meat. Although other authors have implicated colour and colour stability differences on differences in Mb content in animals fed different diets (Gatellier *et al.,* 2005), Wiklund *et al.* (2006) observed no differences in Mb content between meat from

pellet-fed and grazing animals. In contrast to the findings of Wiklund *et al.* (2006), other studies done on venison noted no differences in meat colour between concentrate and pasture diets (Hutchison *et al.,* 2012; Volpelli *et al.,* 2003).

It seems evident that in some cases it is not the feeding system (i.e. type of feed) directly that affects meat colour and colour stability, but rather the nature of the production system. Furthermore, the effect of antioxidants, specifically vitamin E, on colour stability is species-specific. The effect of vitamin E also seems to counteract lipid oxidation and consequently improves the colour stability of meat. In Africa, game species are only reared in intensive production systems, although some species are known to inhabit small territories (Hoffman *et al.,* 2008), and thus no literature is available on the effect of feeding systems on the meat colour and colour stability of game species. Similarly, no research has been conducted on the antioxidant levels in game meat.

Ante-mortem stress

Ante-mortem stress can be divided into two sub-categories, prolonged ante-mortem stress and acute ante-mortem stress. Animals subjected to prolonged ante-mortem stress often produce meat which is dark, firm and dry, a condition commonly referred to as DFD (Warriss, 2000). DFD meat is undesirable from a marketability perspective (Faustman & Cassens, 1990) and has poor processing characteristics (Warriss, 2000). There are two possible reasons why DFD meat is perceived as dark, both of which are related to the characteristic high pH_u of DFD meat (pH>6). Firstly, the high pH_u results in the meat having a higher water-holding-capacity (WHC). The increased WHC results in meat with a more compact structure. This compact structure prevents oxygen diffusion into the muscle, decreasing the amount of OMb formed and reduces the amount of light reflected from the surface of the meat resulting in a perceived darker colour (Lawrie & Ledward, 2006). Secondly, mitochondria function better and their activity post-mortem is prolonged at a high pH_u, resulting in increased oxygen consumption (OC) which consequently leads to lowered OMb production. The DMb form thus prevails in the meat and at the meat's surface, giving DFD meat a darker appearance (Bendall, 1972; Bendall & Taylor, 1972). The increased WHC and elevated oxygen consumption are not mutually exclusive and act together to give DFD meat its dark appearance (Faustman & Cassens, 1990). The high pH_u results from depleted glycogen stores in the muscle caused by prolonged ante-mortem stress. The low glycogen concentration leads to decreased production of lactic acid (anaerobic glycolysis) in the muscles. Thus, not enough lactic acid is produced to reduce the muscle pH to the normal pH_u of 5.5. The susceptibility of muscle to DFD differs and is determined mainly by difference in muscle fibre type composition of the muscle, with red muscle fibres being more susceptible than white (Warriss, 2000).

On the other hand, acute ante-mortem stress can lead to a pale soft and exudative meat (Warriss, 2000). This meat defect, referred to as PSE, most commonly occurs in pigs with either the halothane (Hal^n) or Rendement Napole (RN^-) gene but has been reported in pigs without these genes (Rosenvold & Andersen, 2003a) and other ungulates (Aalhus *et al.,* 1998; Hoffman, 2001a). PSE occurs either in meat with a rapid pH decline post-mortem (Hal^n) or meat with a low pH_u (RN^-) (below 5.3) (Warriss, 2000). The rapid pH decline as well as the low pH_u leads to denaturation of the sarcoplasmic proteins on the myofibril. The denaturation results in a reduction in the WHC of the muscle (Warriss, 2000). This causes Mb to leach out of the muscle and the structure of the muscle to become more "open". The "open" structure of the muscles causes light hitting the surface of the meat to be scattered (Warriss, 2000; Lawrie & Ledward, 2006). In both cases (rapid pH decline and low pH_u) the Mb is exposed to conditions which promote its oxidation to MMb which has a low colour intensity (Lawrie & Ledward, 2006). The decrease Mb concentration and increased light scattering at the surface of the meat contribute to PSE meat being perceived as pale (Lawrie & Ledward, 2006). As with DFD the susceptibility of muscles to PSE differs, with white, glycolytic fibres being more prone to PSE than red, oxidative fibres (Warriss, 2000).

Both DFD and PSE can occur in all species but DFD is more common in dark fleshed species such as beef and venison and PSE is more common in pigs (Lawrie & Ledward, 2006). Various studies have been published on DFD in beef (Bartoš *et al.,* 1993; Viljoen *et al.,* 2002; Wulf *et al.,* 2002; Holdstock *et al.,* 2014). Studies have also shown the occurrence of DFD in mutton (Newton & Gill, 1978), chevon (Simela, 2005) and pork (Lewis *et al.,* 1987; O'Neill *et al.,* 2003; Guàrdia *et al.,* 2005).

Hoffman (2001a) reported that many game animals tend to produce DFD meat due to prolonged stress during the cropping process. Numerous studies on pre-slaughter handling processes of red deer have shown that these animals were also prone to DFD (Wiklund *et al.,* 1995; Malmfors & Wiklund, 1996; Wiklund *et al.,* 1996; Wiklund *et al.,* 2001; Wiklund & Malmfors, 2004).

Various studies have investigated the occurrence of PSE in pork (Lewis *et al.,* 1987; Aalhus *et al.,* 1998; Bowker *et al.,* 2000; O'Neill *et al.,* 2003; Rosenvold & Andersen, 2003a, 2003b; Guàrdia *et al.,* 2005). PSE meat has also been observed in other domestic ungulates such as beef (Aalhus *et al.,* 1998; Warriss, 2000; Lawrie & Ledward, 2006). Hoffman (2001a) reported incidences of PSE in buffalo killed using scoline. These incidences where similar to a phenomenon referred to as white muscle capture myopathy or white muscle disease which is often seen in live capture of game (Hoffman, 2001b). Warthog meat has also been observed as being prone to PSE (Hoffman, 2001b).

Ante-mortem stress does not only affect the colour of meat but also the colour stability of meat. The high pH_u of DFD meat reduces the oxidation of Mb leading to an increase in

colour stability (Gotoh & Shikama, 1974; Ledward, 1985; Faustman & Cassens, 1990). It should however be noted that despite the increased colour stability, the dark colour is undesirable to consumers (Faustman & Cassens, 1990; Viljoen *et al.,* 2002; Lawrie & Ledward, 2006). Furthermore, the increased colour stability is counteracted by a decrease in shelf-life as the high pH_u encourages the proliferation of microorganisms (Lawrie & Ledward, 2006; Webb & Casey, 2010; Holdstock *et al.,* 2014). On the contrary, the low pH_u of PSE meat increases the rate of OMb oxidation leading to a reduction in colour stability (Gotoh & Shikama, 1974; Ledward, 1985; Faustman & Cassens, 1990). Research on colour stability of PSE and DFD pork found that DFD meat exhibited the highest colour stability in comparison to normal and PSE meat, with PSE having the lowest colour stability (Zhu & Brewer, 1998). The difference in colour stability were attributed to a higher MMb reducing activity observed for the DFD meat, with the high pH values of the meat maintaining the reducing enzyme activity (Faustman & Cassens, 1990).

Ante-mortem stress can thus have profoundly detrimental effects on meat colour and meat colour stability. Ante-mortem stress should thus be kept to a minimum or negated by implementation of preventative procedures during the transport, lairage and slaughtering/hunting of animals.

Storage temperature

As with all proteins, Mb is sensitive to temperature fluctuations. The rate at which Mb is oxidised is accelerated with increased temperatures (Brown & Mebine, 1969). Temperature is thus very important in terms of discolouration of meat. Inversely, low temperatures delay discolouration in meat (Lanier *et al.,* 1977; Hood, 1980; O'Keeffe & Hood, 1982; Nortjie *et al.,* 1986). One of the reasons for this delay is that the distance from the meat surface at which MMb forms is increased due to increased solubility of oxygen in the water present in the meat tissue (Urbin & Wilson, 1958).

Myoglobin oxidises more readily at higher temperatures for several reasons. Firstly, higher storage temperatures result in increased reaction rates of pro-oxidants inherently present within the muscle (Faustman & Cassens, 1990). Secondly, the dissociation of oxygen from OMb is favoured due to the decreased solubility of oxygen in meat at higher temperatures (Urbin & Wilson, 1958). This leads to higher concentrations of DMb which is less stable than OMb and more prone to oxidation (O'Keeffe & Hood, 1982). High temperature storage also increases the OC of the meat (increase in enzyme activity at higher temperatures) (Ashmore *et al.,* 1972; Bendall, 1972; Bendall & Taylor, 1972), enhances microbial growth (Lawrie & Ledward, 2006) and accelerates lipid oxidation (Chaijan, 2008) all of which play a synergistic role in enhancing the discolouration of meat (Faustman & Cassens, 1990). It was also noted

by Hood (1980) that that the degree of discolouration of muscles subjected to the same temperature was muscle dependant.

In ground beef it was observed that the colour of samples stored at a higher temperature (2.8°C) discoloured more rapidly than those stored at a lower temperature (-1.7°C) (Martin *et al.,* 2013). This was attributed to the higher temperatures, which resulted in increased microbial growth and lipid oxidation. Both microbial growth and lipid oxidation increase myoglobin oxidation and produce undesirable by-products which affected the meat colour and led to noticeable colour changes (Martin *et al.,* 2013). Rosenvold and Wiklund (2011) found that higher storage temperatures significantly reduced the colour stability of lamb loins due to an increased rate of MMb formation. Various other studies have also shown that increased temperature decreased the colour stability of fresh meat (O'Keeffe & Hood, 1980-81; Ledward *et al.,* 1968; Jacobsen & Bertelsen, 2000; Lanier *et al.,* 1977; Hood, 1980).

The development of bloom is also greatly affected by temperature. Warmer meat will bloom less as enzyme systems present in the meat will compete with Mb for oxygen whereas enzyme systems in colder meat are less active and will thus be less competitive for oxygen. Blooming in cooler meat will thus be more rapid and extensive (AMSA, 2012).

The literature clearly demonstrates the importance of storage temperature with regard to colour development and colour stability of meat. To the authors' knowledge no research has been done on the effect of different storage temperatures on the colour and colour stability of venison and game meat. This lack of research is most likely due to the assumption that temperature would affect all red meat in a similar way. Furthermore, normal retail display temperatures are also used to mimic retail conditions. Low temperatures (-1.5 to 2°C) are thus commonly used in red meat studies, including studies of game and venison, to maximise blooming, colour stability and microbial shelf-life.

Intrinsic factors

Ultimate pH

When evaluating the influence of pH on muscle colour and colour stability two factors should be considered, the rate of pH decline and the pH_u reached in the meat. The pH of muscle in the living animals is approximately 7.2 (Lawrie & Ledward, 2006). The post rigor pH_u of meat is approximately 5.4-5.8 (Lawrie & Ledward, 2006). In general, high pH_u results in a darker meat colour and a lower pH lead to lighter meat colour (Lawrie & Ledward, 2006). This is, at least in part, due to the effect that pH has on the WHC of muscle and with high and low WHCs having darker and lighter colours, respectively (Lawrie & Ledward, 2006).

It has been shown in beef that oxidation of Mb is accelerated at lower pH values (Gotoh & Shikama, 1976; Ledward, 1985) leading to a decrease in colour stability. This accelerated

Mb oxidation may be due to accelerated protonation of bound oxygen which enhances the release of superoxide anions, a known pro-oxidant in meat (Livingston & Brown, 1981). Metmyoglobin reducing activity (MRA) is also influenced by pH, with an increase in pH leading to increased MRA (Ledward, 1971; Stewart *et al.,* 1965). Increased MRA has been linked to an increased colour stability (AMSA, 2012), thus meat with a higher pH_u should be more colour stable than meat with a lower pH_u. Furthermore, the formation of MbO is reduced at higher pH_u as a result of increased and prolonged mitochondrial activity post-mortem (increased OC) (Bendall, 1972; Bendall & Taylor, 1972).

The effect of pH_u on colour is illustrated in a study on the evolution of beef colour as a function of its pH_u (Abril *et al.,* 2001). The study investigated the change in instrumental colour (*L**, *a**, *b**, chroma and hue) and surface reflectance over nine days, for meat of two different pH groupings, pH<6.1 and pH≥6.1 (DFD meat). The results indicated that the pH_u had a significant effected on all the colour variables. The *L** values where decreased (meat became darker) as pH values increased. Meat with lower pH values were observed to have higher *b** and hue values. The reflectance spectrum of the beef from the higher pH group (pH≥6.1) was always below that of the lower pH group (pH<6.1). This trend has also been observed by other researchers (Guignot *et al.,* 2004). The lower *L** values observed for the higher pH group is attributed to lower amounts of light reflectance and higher amounts of absorption at all wavelengths. The reflectance results indicated that more MbO was formed at the surface of beef with pH<6.1 (better blooming), resulting from decreased mitochondrial function at lower pH values (Bendall, 1972; Bendall & Taylor, 1972). Furthermore, the reflectance results indicated that beef with pH<6.1 had earlier onset of MMb formation in comparison to the pH≥6.1. This, along with the higher *b** and hue of beef from the lower pH group, confirms the observations of other researchers, who found that the rate of Mb auto-oxidation increases and the rate of reduction decreases at decreasing pH values (Bendall & Taylor, 1972; Ledward, 1985).

The effect of extreme pH_u deviations, as seen with PSE and DFD, on the colour and colour stability of meat are discussed in section *3.1.3 Ante-mortem stress*.

The pH_u of meat is very important in determining the colour and colour stability in fresh meat. The majority of the research regarding pH_u of muscle has been done with regard to DFD and PSE in beef and pork. Although some research has been done on the effect of pH_u on the colour of venison (MacDougall *et al.,* 1979; Dhanda *et al.,* 2002, Dhanda *et al.,* 2003; Rincker *et al.,* 2006; Wiklund *et al.,* 2006; Farouk *et al.,* 2007; Dahlan & Norfarizan Hanoon, 2008) and game meat (Hoffman *et al.,* 2009b; Hoffman & Laubscher, 2010), there is only limited literature with regard to its effect on colour stability. Furthermore, the studies which included results regarding the relationship of colour and pH_u in game meat and venison have generally been part of a broader study on meat quality and where not specifically focussed on

this relationship. Thus ample scope exits for more detailed research into this area, particularly since both venison and game meat are prone to DFD (Hoffman, 2001a).

Animal-to-animal variation (influence of genetics)

Very little research has been conducted on the effect of animal-to-animal variation on colour stability, with most research being focused on ante- and post-mortem factors, and differences between muscles (King *et al.,* 2010). It has been suggested that the effect of ante- and post-mortem factors, and differences between muscles would be greater than the animal-to-animal variation (Mancini & Hunt, 2005). Nevertheless, various inherent biochemical and metabolic differences within muscles have been implicated in influencing colour stability (Faustman & Cassens, 1990; Mancini & Hunt, 2005). Since genetics has been shown to influence growth rate, muscularity and leanness in animals (Casas *et al.,* 2003, 2005; White *et al.,* 2007), it seems likely that genetics could influence the variation in colour stability between animals (King *et al.,* 2010). King *et al.* (2010) found that there was substantial animal-to-animal variation and, that animal-to-animal differences played a larger role in the capacity of the muscle to maintain its colour, rather than in its initial colour. Furthermore, they found that there was significant opportunity to improve colour stability through genetic selection. In contrast, Newcom *et al.* (2004) found that in pork, genetics played a larger role in the initial colour values than those reported by King *et al.* (2010) for beef. It has been demonstrated that initial OC and reducing capacity contributed to variation in colour stability between animals (King *et al.,* 2011). However, earlier research on beef found that muscle type (Hood, 1980; Renerre & Labas, 1987) and storage temperature (Hood, 1980) had a greater effect on colour stability than the animal-to-animal variation. The greater influence in variation between muscles in comparison to animal variation was observed in a study by King *et al.* (2011). Interestingly, the results from this study showed that the influence of animal-to-animal variation was consistent across muscles. Thus strategies to improve the colour stability of lean would thus be consistent across all muscles.

In an attempt to ascertain which factors influence animal-to-animal variation Canto *et al.* (2015) evaluated differences in the sarcoplasmic proteome of LD steaks which demonstrated variations in colour stability and correlated colour stability attributes to variably abundant proteome components. The results indicated that animal-to-animal variation could be explained by differences in the sarcoplasmic proteome profile. An excess of glycolytic enzymes in colour-stable muscles was found to contribute to the improved colour stability of these muscles, most likely due to the regeneration of NADH post-mortem. Furthermore, in colour-liable muscles, the decrease in colour stability was attribute to situ post-translational modification of Mb.

Due to the lack of research on the influence of animal-to-animal variation on colour stability, and the evidence that it may have some effect, there is a great deal of scope for research in this area. Animal-to-animal variation may also have a greater effect on colour stability in wild ungulates due to greater genetic variation compared to that of domestic species.

Species

The colour that consumers perceive as desirable is species dependant (Faustman & Cassens, 1990). In pork, lighter flesh, which is greyish-pink in colour is considered acceptable to consumers. Meat from other domestic ungulates (beef, lamb and chevon) is darker than that found in pigs, and a bright red colour is deemed acceptable (Bekhit & Faustman, 2005). Venison and game meat is darker red in appearance than meat from domestic ungulates (Hoffman *et al.,* 2000; Daszkiewicz *et al.,* 2011). It has been noted that the dark colour of game meat may negatively influence the purchasing decision of consumers who prefer red meat that is neither too dark nor too pale (Jeremiah *et al.,* 1972). The differences in muscle colour perceived between species is largely due to differences in Mb content (Warriss *et al.,* 1990; Kranen *et al.,* 1999; Gatellier *et al.,* 2001), proportion of muscle fibre type (Vestergaard *et al.,* 2000; Lawrie & Ledward, 2006) and IMF content (Lawrie & Ledward, 2006). The darker colour of venison and game meat is attributed to a higher Mb content (Vestergaard *et al.,* 2000; Díaz *et al.,* 2002; Kritzinger *et al.,* 2003; Daszkiewicz *et al.,* 2011), differences in muscle fibre types (Curry *et al.,* 2012; North & Hoffman, 2015) and lower levels of IMF (Hoffman *et al.,* 2005) in comparison to many domestic species. Furthermore, a higher Mb content in meat leads to higher iron concentrations which promotes oxidation leading to a reduction in colour stability (Farouk *et al.,* 2007; Purchas *et al.,* 2010).

Chevon has been observed to be darker and more red compared to lamb with the differences mainly being attributed to the lower IMF content of chevon (Babiker *et al.,* 1990). Farouk *et al.* (2007) noted that venison has a poorer colour in comparisons to beef. The instrumental colour measurements indicated that beef had higher *L**, *a** and chroma values (lighter, redder and more saturated) in comparison to venison when stored at -1.5°C for 4 weeks. The poorer colour and colour stability of the venison was attributed to its higher concentration of Mb (Young & West, 2001) and pro-oxidants (Drew & Seman 1987; Stevenson-Barry *et al.,* 1999) in comparison to beef. Similar differences in colour were observed between beef, caribou and reindeer (Rincker *et al.,* 2006). The *L**, *a** and *b** values for the caribou and reindeer were significantly lower than those of the beef but did not differ from each other. The lower colour values of the reindeer and caribou indicated that the meat was darker, less red and yellow in comparison to the beef. Differences were also observed

for the Mb concentrations of the three species, with beef (7.29 mg/g) having a significantly lower concentration than the reindeer (9.79 mg/g) and the caribou (8.59 mg/g) concentration not differing from either. Thus, although not expressly stated in the article, the Mb concentration may, to some extent, explain the differences in colour between the species.

Impala (*Aepyceros melampus*) meat was observed to be darker and redder (*L** = 29.22, *a** = 11.2) (Hoffman, 2000) than corresponding values for pork (*L** = 43.7, *a** = 5.44) (Fisher *et al.,* 2000). Another study on impala found that the Mb content (7.25-7.50 $mg.g^{-1}$) was higher than that found in beef (5.80 $mg.g^{-1}$) and was given as the reason for the dark colour observed in game meat (Hoffman *et al.,* 2005). A comparison between the meat quality of beef and eland found that beef was lighter by comparison (Bartoň *et al.,* 2014). These findings were similar to other studies which found that beef was lighter in comparison to bison (Koch *et al.,* 1995), reindeer and caribou (Rincker *et al.,* 2006) and, red deer (Farouk *et al.,* 2007). An investigation into the meat quality of impala and kudu (*Tragelaphus strepsiceros*) found that the meat from kudu has significantly higher *L**, *a**, *b** and chroma values in comparison to the impala. The higher *a** values were not attributed to the Mb content as no significant differences were observed between the two species. Interestingly, the hue angle did not differ significantly but was higher than values reported for springbok (Hoffman *et al.,* 2007) and black and blue wildebeest (Van Schalkwyk, 2004). Volpelli *et al.,* (2002) noted that venison meat is characterised by low *L** values below 40, high *a** values and low *b** values which are indicative of the dark red colour. It has also been suggested that the darker colour of game animals may be attributed to stress from poor cropping methods resulting in the dark, firm, dry (DFD) condition (Von La Chevallerie & Van Zyl, 1971; Von La Chevallerie, 1972; Scanga Belk *et al.,* 1998; Hoffman, 2001a). The higher Mb content and resulting darker meat found in game meat and venison is most likely due to higher levels of physical activity (Vestergaard *et al.,* 2000; Díaz *et al.,* 2002; Lawrie & Ledward, 2006), with wild ungulates being more active than domestic ungulates (Hoffman, 2000). However, this is not the case for all wild ungulates. The meat of mountain reedbuck (*Redunca fulvorufula*) was observed to be only slightly darker than that of beef. It was postulated that this was due to the behaviour of the mountain reedbuck, which only frequents small territories and is relatively inactive (Hoffman *et al.,* 2008).

It is evident that differences in meat colour and colour stability between species can largely be attributed to differences in their activity which influences the muscle fibre type, Mb concentration and IMF content of the meat, which in turn influences muscle colour and colour stability. Although literature is available on the colour differences between various species, limited research is available comparing the colour stabilities of various species, especially with regard to venison and game species.

Breed

It has been noted that breed is one of the main production factors which influences meat quality (O'Keeffe & Hood, 1982; Renerre & Labas, 1987; Insausti *et al.,* 1999). As with different species, breeds within a species will also differ in colour and colour stability due to differences in the biochemistry of their muscles.

Lanari and Cassens (1991) found that meat from Holstein cattle was less colour stable than meat from crossbreeds. They attributed the differences to differences in muscle enzyme reducing activities among breeds; those with the highest reducing activities (Holstein) were the most colour liable. These results are consistent with other research (O'Keeffe & Hood, 1982; Renerre & Labas, 1987; Echevarne *et al.,* 1990). Similarly, meat from Parda de Montaña cattle breeds was observed to be more colour stable than meat from Pirenaica breeds, although no reasons for the difference was given (Ripoll *et al.,* 2014). Differences in initial colour were observed for five different Spanish cattle breeds (Insausti *et al.,* 1999). The Morucha breed was observed to differ greatly from the others, with this breed having significantly redder muscle. The redder muscle was postulated to be due to a higher Mb content (Demos & Mandigo, 1996) resulting from earlier development (Renerre & Valin, 1979 as cited in Insausti *et al.,* 1999) and higher physical activity associated with this breed (Kempster, 1981). The Morucha breed was also found to be the least colour stable of the breeds, most likely also due its higher Mb concentration (Insausti *et al.,* 1999). Other authors also noted differences in Mb content and reflectance for different cattle breeds, with lower Mb content and higher light reflectance for Limousine, Charolais, Romangola and Blonde d'Aquitane cattle than for Simmental, White Cattle, Hereford and Chianina crossbreeds (Liboriussen *et al.,* 1977). It was suggested that CIE Lab values and Mb content may be a good method to characterise beef from different breeds (Insausti *et al.,* 1999). Conversely, no significant difference were observed for the meat colour or colour stability between Limousine and Retinta breeds (Avilés *et al.,* 2015).

Kadim *et al.* (2003) investigate the meat quality differences between four muscles (LD*, biceps femoris* (BF)*, semitendinosus* (ST) *and semimembranosus* (SM)) of three different Omani goat breeds, Batina, Dhofari and Jabal Khaddar. No significant differences were observed between the *a** and *b** values amongst breeds for any of the muscles but differences in *L** were observed, with the LD of the Jabal Khaddar goats being lighter than that of the Batina and Dhofari and the SM of the Jabal Khaddar and Dhofari being lighter than that of the Batina. Thus both breed and muscle type influenced the colour of meat. Other authors have also noted differences in muscle colour amongst goat breeds (Dhanda *et al.,* 1999).

Differences in colour among pig breeds has also been noted. A study which investigated the colour differences between the LD and BF of pure bred Hampshire, Swedish

Landrace and Swedish Yorkshire pigs found that the LD of the Hampshire breed was redder in comparison to the other two (Lindahl *et al.,* 2001). The LD and BF of the Hampshire breed were also more yellow by comparison. These differences in colour were attributed to difference in glycogen content in the muscle of different breeds. Previous studies noted that Hampshire pigs had higher levels of glycogen in their muscles resulting in low protein content and higher water to protein ratios compared to other breeds, which may influence the colour of their meat (Sellier & Monin, 1994). Meat colour differences were also noted for Celta pigs (C) cross-bred with Duroc (D) and Landrace (L) breeds (Franco & Lorenzo, 2014). The results showed that he *L** value for C was lower than for CxL. *L** values for CxD did not differ from either of the other two breeds. The *a** values for C was the highest, with the *a** values for CxL and CxD not differing significantly form one another. The *b** values for C was higher than those for CxD, with the values for CxL not differing from either. The differences in *a** were attributed to differences in Mb content between the breeds, with higher Mb contents linked to higher *a** values. Explanations for the differences in *L** and *b** values between breeds was not given. Other studies have also noted difference in instrumental colour analysis amongst different pig breeds (Gjerlaug-Enger *et al.,* 2010; Li *et al.,* 2013; Shen *et al.,* 2014)

Differences in colour between lamb species has been noted by various authors (Hopkins *&* Fogarty, 1998; Martínez-Cerezo *et al.,* 2005; Teixeira *et al.,* 2005). Martínez-Cerezo *et al.* (2005) compared the surface colour of three different Spanish breeds (Rasa Aragonesa, Churra and Spanish merino) and found that the *L** values for the Churra were significantly higher. Furthermore, they noted that the Spanish merino had the highest *a** value and the Rasa Aragonesa had the highest *b** value. No explanation for the differences was provided.

Unlike domestic ungulates, where several different breeds exist within each species, no breeds exist for wild ungulates only sub-species. Breed differences definitely plays a crucial role in muscle colour and colour stability in domestic ungulate species and should be brought into considered as a production parameter. It may also be viable option to crossbreed for better muscle colour and colour stability.

Gender differences

It has been postulated that meat from male animals is darker than meat from females. This is attributed to differences in Mb concentration, with males having higher concentrations due to higher amounts of physical activity (Seideman *et al.,* 1982). The higher Mb concentrations found in males could contribute to a decrease in colour stability (Insausti *et al.,* 1999). However, observations regarding gender effects are inconclusive.

Higher *L**, *a**, *b** and chroma values for steers in comparison to bulls and castrates haven been reported by Kim *et al.* (2003). In the same study, no significant differences were reported for *a**, *b** and chroma between cows and bulls. Węglarz (2010) noted differences in meat colour between cows and bulls, with cows having higher *b** and lower hue values.

In pork, differences in meat colour between gilts and castrates have been noted by several authors (Warriss *et al.,* 1990; Jeremiah *et al.,* 1999; Franco & Lorenzo, 2014), while other found no differences (Unruh *et al.,* 1996; Lindahl *et al.,* 2001; Li *et al.,* 2013). Warriss *et al.* (1990) observed higher *a** values and lower hue values in gilts compared to castrates but no differences in *L**, *b** and chroma values. Conversely Franco & Lorenzo (2014) found higher *L** values in castrates compared to entire females but found no differences in *a** and *b** values.

Simela *et al.* (2004) noted differences in meat colour from female, male and castrated goats; intact males had lower *a** values than females and castrates, and the chroma value of the intact males and females was lower than that of the castrates.

Differences in meat colour between male and female lambs was noted by Teixeira *et al.* (2005). Although no significant differences were observed between *a** and *b** values, *L** was significantly higher in males than females.

No colour differences were observed between the LD of male and female mountain (*Redunca fulvorufula*) reedbuck (Hoffman *et al.,* 2008). These findings are congruent to those for impala (*Aepyceros melampus*) and kudu (*Tragelaphus strepsiceros*) where no colour differences were observed between male and female animals (Hoffman *et al.,* 2009b). The lack of significant differences in colour between male and female impala and kudu meat may be due to no significant differences being observed between the Mb content of male and female impala and kudu meat (Hoffman *et al.,* 2009b). This could also possibly suggest that the male and female muscles of impala and kudu would have similar colour stabilities. Similarly, no colour differences were observed for between male and female roe deer (Daszkiewicz *et al.,* 2009; Purchas *et al.,* 2010; Daszkiewicz *et al.,* 2012). However, differences in meat colour have been observed for gemsbok (*Oryx gazella*), with females having lower *L**, *b**, hue and chroma values (Hoffman & Laubscher, 2010). These findings contradict the theory that males have darker muscle than females. Furthermore, differences in gender were observed for springbok (*Antidorcas marsupialis*), with females having higher *a** and chroma values due to significantly higher pH_u values (Hoffman *et al.,* 2007).

Although differences in gender have been noted for various species, the findings are disseminated and no clear trend is apparent. Gender should thus be included in colour and colour stability research to elucidate possible effects, if any.

Animal age

It has been noted that the concentration of Mb in the muscle increases with the age of the animal (Onyango *et al.,* 1998; Kim *et al.,* 2012; Humada *et al.,* 2014; Cho *et al.,* 2015). Since Mb and its concentration in the muscle can affect the perceived muscle colour (Warriss *et al.,* 1990; Kranen *et al.,* 1999; Gatellier *et al.,* 2001), an increase in Mb with age could result in older animals having darker (lower *L** values) and redder (higher *a** values) meat. The increase in Mb may also lead to a decrease in colour stability with age (Insausti *et al.,* 1999). Furthermore, fat deposits also tend to become more yellow in colour over time due to an increase in carotenoid deposits (Lawrie & Ledward, 2006), which may affect perceived muscle colour depending on the quantity of IMF.

A comparison between kid and goat meat found that there was an increase in Mb concentration with an increase in age (Dhanda *et al.,* 1999). A decrease in *L** and *b** and an increase in *a** values was observed with an increase in age, indicating that the meat became darker, more red and less yellow with age. Although an increase in fat yellowness was observed in older animals, these measurements were done on subcutaneous fat and would thus not necessarily give an indication of the colour change on lean meat colour. Other researchers have also noted an increase in subcutaneous fat yellowing with age in goats (Rao *et al.,* 1988)

Differences in *L** values between foals aged 8 and 11 months has been observed (Domiguez *et al.,* 2015); older foals had lower *L** values. This differences could not be attributed to the Mb concentration as no significant differences were observed between the 8 and 11 month old foals. Similarly, no significant differences were noted for the *a** and *b** values. Conversely, a comparison of instrumental colour and heme iron content of foals aged 9 and 12 months found no significant differences (Franco *et al.,* 2011)

Jacob *et al.* (2007) found a decrease in colour stability in lamb with age. This was attributed to an increase in Mb concentration with age which suggested that the older lamb meat was more aerobic in nature than that of the younger lambs. The increase in Mb with age in lamb meat was consistent with findings from other studies (Pethick *et al.,* 2005; Kim *et al.,* 2012). Kim *et al.* (2012) noted higher *L** and lower *a** values for younger lambs (3-4 months) in comparison to older lambs (10-11 months). These differences were attributed to the higher Mb concentrations found in the older lambs.

A study conducted on three different muscles (LD, SM and ST) of male deer found differences in meat colour between animals of different ages (Sookhareea *et al.,* 1995). It was found that, with age, *L** and *a** values increased and *b** values decreased for all muscles. The increase in *L** values with age is contradictory to what would be expected. This increase was attributed to a lower pH_u and slower cooling of heavier carcasses. Although no explanation

was given for the increase in *a** values, this is most likely due to an increase in Mb concentration in the meat with age. Differences in colour were also noted for sub-adult and adult male kudu and impala (Hoffman *et al.,* 2009). Sub-adult males had higher *L** values and correspondingly lower Mb concentrations in comparison to adult males. Similarly, colour differences between adult and sub-adult springbok have also been noted (Hoffman *et al.,* 2007). Adult springbok had lower *L**, *a** and chroma values and, higher hue angle values in comparison to sub-adults. No differences in *b** values were observed. In beef, similar results have been noted. Tang *et al.* (2010) observed lower *L**, *a** and chroma values for older (9-10 years) Chinese yellow cattle compared to younger animals (1.5-6 years). Cho *et al.* (2015), observed lower *L**, *a**, *b** and chroma values and decreased colour stability for older Korean Hanwoo (*Bos taurus coreanae*) animals. In the latter study, higher Mb concentrations and increased lipid oxidation with age were given as the reasons for a lower *L** values and decreased colour stability, respectively. Conversely, Volpelli *et al.* (2003) noted no differences in colour between male deer aged 18 and 30 months.

It would seem evident that, in most cases, colour and colour stability was affected by animal age. Increases in Mb concentrations with age and to a lesser extent lipid oxidation where implicated in colour changes and decreased colour stability. Thus, age is an important factor to take into consideration when harvesting/slaughtering animals as it may affect the colour, colour stability and ultimately the consumer perception and shelf-life of the product.

Muscle specific variation

Variation in muscle colour and colour stability have been noted by several authors (Ledward, 1971; O'Keeffe & Hood, 1982; Renerre & Labas, 1987; McKenna *et al.,* 2005; Von Seggern *et al.,* 2005; Jeong *et al.,* 2009; Kim *et al.,* 2009; Joseph *et al.,* 2012). It has been suggested that the intermuscular variability is the most important factor affecting the shelf-life of pre-packaged beef (Hood, 1980). The differences in muscle colour and colour stability is related to the differences in the relative proportions of muscle fibres types present in the muscle (Faustman & Cassens, 1990). The fibre type influences the amount of Mb present in the muscle, the oxidative capacity of the muscle and the pH_u, all of which influence muscle colour and muscle colour stability.

Muscle fibre type

In mammals, muscle fibre types are divided into four groups type I and type IIA, IIX and IIB. The fibre types are divided according to the energy source utilised, metabolic pathway and contraction rate (Lawrie & Ledward, 2006; Curry *et al.,* 2012). Type I fibres also referred to as slow-twitch oxidative fibres. These fibres are slow contracting (Schiaffino & Reggiani, 1996;

Bottinelli, 2001), contain large numbers of mitochondria and aerobically metabolise fat, glucose and glycogen to produce ATP and, are highly resistant to fatigue (Pette, 1985). Type IIA also referred to as fast-twitch oxidative fibres. These fibres are fast contracting, contain large numbers of mitochondria and can aerobically and anaerobically produce ATP. The ability to use both aerobic and anaerobic metabolism to produce ATP makes these fibres resistant to fatigue (Pette, 1985; Schiaffino & Reggiani, 1996). Type IIX fibres also known as fast-twitch glycolytic fibres. These fibres are fast contracting (faster than type IIA), contain few mitochondria, primarily metabolise glucose and glycogen anaerobically for ATP production and, fatigue rapidly (Pette, 1985). Type IIB fibres contain low levels of mitochondria, are mostly anaerobic and extremely susceptible to fatigue (Warriss, 2000). They also have a lower oxidative capacity than type IIX fibres (Greenwood *et al.,* 2007; Lefaucheur, 2010; Curry *et al.,* 2012).

As mentioned, the colour and colour stability of muscles is influences by the relative proportions of the different muscle fibre types. Muscles with higher quantities of oxidative fibres (type I) are a darker, deep red colour than glycolytic fibres (type IIX) due to a higher Mb content. Oxidative muscles (majority oxidative fibres) (e.g. SM) are more prone to oxidation and thus have a faster rate of discolouration than glycolytic muscles (majority glycolytic fibres) (e.g. ST) (O'Keeffe & Hood 1982; Renerre & Labas, 1987). Oxidative muscles are thus, in general, darker and less colour stable than glycolytic muscles.

A study on the effect of the diet/production system (intensive vs. extensive) on the muscle fibre characteristics and meat colour of beef concluded that the difference in physical activity had a greater effect on the muscle colour than did the diet associated with that production system (intensive = feedlot; extensive = grazing) (Vestergaard *et al.,* 2000). In extensive production systems, animals have higher levels of physical activity than intensive production systems (Vestergaard *et al.,* 2000), which leads to higher ratios of slow-twitch muscle fibres (Aalhus & Price, 1991). Increased physical activity leads to an increase in type IIA (fast-twitch, oxidative and glycolytic) and a decrease in type IIB (fast-twitch, glycolytic) muscle fibres in young bulls (Van Vooren *et al.,* 1992). Whereas in lambs and pigs, type I (slow-twitch, glycolytic) muscle increases with increased physical activity (Aalhus & Price, 1991; Petersen *et al.,* 1998). The results indicated that the ST and LD had higher proportions of type IIB muscle fibres in comparison to type I and IIA and the *supraspinatus* (SS) had higher proportions of type I muscle fibres. Correspondingly, the SS had the highest Mb concentration followed by the LD and then the ST. Interestingly, the SS did not have the highest *a** value despite having the highest percentage of type I muscle fibres and Mb content. The ST has a similar *a** value to that of the SS, with the LD having the highest *a** value. Furthermore, only a significant increase in type IIA muscle fibres was observed for the ST with an increase in muscle activity (Vestergaard *et al.,* 2000).

Renerre and Labas (1987) investigated the colour stability of three bovine muscles, *tensor fasciae lataeI* (TFL), *diaphragm medialis* (DM) and *psoas major* (PM). The results indicated that the TFL was the most colour stable, the DM the most unstable and the PM had an intermediate colour stability. The high colour stability of the TFL was also noted by McKenna *et al.* (2005). Furthermore, the results alluded to a relationship between muscle fibre type composition of the different muscle and colour stability (Renerre & Labas, 1987). The TFL muscle is mainly composed of fast-twitch white muscle fibres (glycolytic), the DM muscle mainly slow-twitch red (oxidative) and PM fast-twitch red (intermediate). The muscles with the highest oxidative activity/most oxidative muscle fibre types had the poorest colour stability. Several studies have noted the difference between the colour stabilities of the beef LD and PM muscles (Madhavi & Carpenter, 2003; McKenna *et al.,* 2005; Seyfert *et al.,* 2006; Seyfert *et al.,* 2007; Kim *et al.,* 2009; Joseph *et al.,* 2012). This difference has been attributed to variations in the proportions of muscle fibres present in these muscles (Seyfert *et al.,* 2006). The LL has been noted as having higher proportion of type IIA fibres than type I fibres and the PM muscle being composed primarily of type I fibres, thus explaining the variance in colour stability between theses muscles. It has been suggested that the differences in colour stabilities between these two muscles makes them ideal models for more in-depth biochemical research into the reasons behind their differing colour stabilities (Joseph *et al.,* 2012)

Although the influence muscle fibre type on the colour and colour stability of venison and game meat has not been investigated, research regarding the muscle fibre types present in various muscles of these species has been conducted. Studies done on venison (deer and reindeer) (Kiessling & Kiessling, 1984; Essén-Gustavsson & Rehbinder, 1985) and game meat (blesbok, kudu, springbok, black and blue wildebeest) (Kohn *et al.,* 2011; Curry *et al.,* 2012; North, 2014) have found that muscles from these species contained proportions of muscle fibre types in descending order of type IIX, IIA, I. These findings are contradictory to what would be expected. The dark red colour associated with these species would suggest high proportions of type I muscle fibres (Curry *et al.,* 2012).

From the results above, it is clear that the muscle fibre type compositions differ between muscles of different species. The fact that the muscle fibre type of venison and game meat is different to what is expected from the perceived colour of the muscle, creates the need for further research into this relationship.

Oxidative and reductive capacity

The oxidative and reductive capacity of a muscle is important in meat colour and meat colour stability. The oxidative capacity in muscle is generally measured by the OC and reductive capacity by MMb reducing activity (MRA). The OC is determined by the residual mitochondrial

respiration activity within the meat. The mitochondria will compete with Mb for available oxygen, decreasing the quantity available to bind to Mb to form OMb. The depth to which OMb can penetrate into the meat is also decreased (Bendall & Taylor, 1972). Since OC is dependent on residual mitochondrial activity, it is linked to muscle fibre type. Oxidative fibres contain more mitochondria and would thus have higher OC than glycolytic fibres (Beecher *et al.,* 1965; Beecher *et al.,* 1969; Jeong *et al.,* 2009). This may, at least in part, explain the reduced colour stability of muscles with higher proportions of oxidative fibres. Several authors have shown that the relationship between OC and oxygen depth penetration was greatly influenced by muscle type (Bendall & Taylor, 1972; MacDougall & Taylor, 1975; O'Keeffe & Hood, 1982; Renerre & Labas, 1987; Lanari & Cassens, 1991; Madhavi & Carpenter, 1993; McKenna *et al.,* 2005). Conversely, MRA refers to the ability within meat to reduce MMb back to DMb. The resulting DMb can subsequently be re-oxygenated to OMb thereby sustaining the colour stability of the meat (Madhavi & Carpenter, 1993). Higher levels of MRA in meat thus results in better colour stability. It has been suggested that the pathway responsible for this reduction process is primarily enzymatic in nature with NADH as cofactor. These systems are controlled by specific MMb reductases and it is thought that mitochondria and sub-mitochondrial particles may also play a role in MMb reduction (Giddings, 1974). Functional mitochondria and sub-mitochondrial particles have been known to occur in meat for a considerable time post-mortem (Cheah & Cheah, 1971). As time post-mortem increases, MRA is reduced and eventually lost completely. This loss in MRA is due to the fall in pH post-mortem, depletion of substrates and co-factors and loss of function and structural integrity of mitochondria (Ledward, 1985). Non-enzymatic reduction of MMb can occur via NADH and NADPH (Brown & Snyder, 1969) or ascorbic acid in the presence of substituted pyridines (Fox *et al.,* 1975). Differences in MRA between different types of muscles has been noted (O'Keeffe & Hood, 1982; Echevarne *et al.,* 1990; McKenna *et al.,* 2005). In addition, various factors such as storage time, lipid oxidation, oxygen, light, diet and exercise can influence MRA (Bekhit & Faustman, 2005), with an increase in both temperature (Cutaia & Ordal, 1964; Stewart *et al.,* 1965; Hutchins *et al.,* 1967; Zimmerman & Snyder, 1969) and pH (Ledward, 1970; Stewart *et al.,* 1965) resulting in increased MRA (Bekhit & Faustman, 2005).

The importance of the OC of muscles in colour development and colour stability has been noted (Faustman & Cassens, 1990; Madhavi & Carpenter, 1993; Bekhit & Faustman, 2005; Mancini & Hunt, 2005). However, the relative contribution of MRA and OC to the colour stability of muscles had been greatly disputed. While some researchers argue that MRA within the muscle is the principal determinant of muscle colour stability (Ledward, 1971), others have observed that the reducing activity is of little consequence and that OC has more influence on muscle colour stability (Atkinson & Follett, 1973; O'Keeffe & Hood, 1982; Renerre & Labas, 1987). Furthermore, evidence has shown that both reducing activity and OC have an influence

on the colour stability of muscles (Madhavi & Carpenter, 1993). In contrast, other researchers have suggested that that low OCs negatively affect colour stability as mitochondrial respiration is required to replenish the NADH required for MRA (Sammel *et al.,* 2002). McKenna *et al.* (2005) and, Atkinson and Follett (1973) proposed a relationship between reducing activity and OC which could explain the differences in colour stability between muscles. They theorised that the OC influenced colour stability relative to the reducing activity within a muscle. In muscles with low OC and low MRA (e.g. *adductor*), the reducing activity cannot compete with the oxidative stress imposed by the OC and the colours ability in the muscle is reduced. Muscles with high MRA relative to low OC (e.g. TFL) have higher colour stabilities as the reducing activity can compensate for the oxidative stress imposed by the OC.

Renerre and Labas (1987) investigated the colour stability of three bovine muscles, *tensor fasciae lataeI* (TFL), *diaphragm medialis* (DM) and *psoas major* (PM). The results indicated that the TFL was the most colour stable, the DM the most unstable and the PM had an intermediate colour stability. The high colour stability of the TFL was also noted by McKenna *et al.* (2005). Furthermore, the results alluded to a relationship between muscle fibre type composition of the different muscle and colour stability (Renerre & Labas, 1987). The TFL muscle is mainly composed of fast-twitch white muscle fibres (glycolytic), the DM muscle mainly slow-twitch red (oxidative) and PM fast-twitch red (intermediate). The muscles with the highest oxidative activity/most oxidative muscle fibre types had the poorest colour stability. The authors also noted that variability in meat colour was primarily due to variation in OC and not MRA. Other researchers have also noted that OC plays a more prominent role in muscle colour variation than does MRA (Lanari & Cassens, 1991). These results are contradictory to those of Ledward (1985) who noted the importance of MRA in colour stability of muscles. Seyfert *et al.* (2006) investigated the colour stability of bovine muscles (LL, ST, SM and PM) and observed that the colour stabilities of the muscles ranked as follows: LL (most stable)>ST>SM>PM (least stable). The differences in colour stability was linked to differences in muscle fibre type, OC and MRA. The most colour stable muscle were found to have high proportions of white, glycolytic fibre types, lower OC and higher MRA whereas the least stable muscles had high proportion or red, oxidative fibre types.

As mentioned, the varying colour stabilities of the beef LD (colour stable) and PM (colour liable) means that these to muscles are ideal models to investigate the biochemical basis for colour stability. A study on the colour stability of bovine muscles found that the PM was the least colour stable and the LD the most (O'Keeffe & Hood, 1982). The results indicated that the OC in the PM was higher and the MRA lower in comparison to the LD. The differences in the colour stabilities between the muscles was attributed to the differences in the demand for oxygen by the various respiratory systems present in the muscle, with those of the PM being higher than that of the LD. Other researchers have also noted that the

differences in colour stabilities between these muscles was attributed to differences in OC and/or MRA activity (Jeong *et al.,* 2009; Kim *et al.,* 2009).

A decrease in OC during storage has been observed (O'Keeffe & Hood, 1982; Madhavi & Carpenter, 1993; McKenna *et al.,* 2005; Seyfert *et al.,* 2006; King *et al.,* 2011) and is attributed to decrease in mitochondrial activity. It has been noted that meat aged for three days was less colour stable than meat aged for seven days. It was concluded that that the high OC of the three day aged meat relative to the seven day aged meat, reduced the thickness of the OMb layer leading to more rapid discolouration of the meat (Hood, 1980). Similar findings have been noted by other authors (Young *et al.,* 1999; Lee *et al.,* 2008; Lindahl, 2011; Mancini & Ramanathan, 2014)

The rapid discolouration of venison post-mortem (Stevenson-Barry *et al.,* 1999) makes it an ideal model for investigating MMb reducing activity in meat (Bekhit *et al.,* 2007). MRA was shown to be present in venison which had been displayed aerobically at 4°C for 6 days, to produce total discolouration, and subsequently vacuum packed (Bekhit *et al.,* 2007). The reducing activity was present whether the venison was stored 1 day, 3 or 6 weeks post-mortem and then subjected to the retail display. Interestingly, it was noted that visible effects of MRA was only notable under anaerobic conditions as the pro-oxidant activity present in meat is favoured during aerobic storage. Thus, the elimination of oxygen, by vacuum packaging, allowed MRA to be favoured. Despite a method for the regeneration of the red colour of venison being found, the practical and economic viability of this method was questioned as the regeneration of the colour required repackaging and was short lived (approximately 30 hrs).

Although some contradiction exists in literature regarding the relative contributions of OC and MRA to the colour stability of muscle it seems evident that both contribute. Literature on the influence of OC and MRA on the colour stability of venison and game species is notably lacking and should be investigated to provide better insight into the rapid discolouration of venison and game meat.

Lipid oxidation

Correlations have been noted between lipid oxidation and Mb oxidation in meat (Faustman & Cassens, 1990; Faustman *et al.,* 2010). The exact mechanism by which lipid oxidation affects Mb oxidation (colour stability) in meat is not yet fully understood. It is unclear whether Mb oxidation initiates/accelerates lipid oxidation or vice versa and scenarios for both have been proposed (Chaijan, 2008). In the case of lipid oxidation induced Mb oxidation, it is thought that the highly reactive free-radical or by-products generated by lipid oxidation initiate/accelerate Mb oxidation. The by-products of lipid oxidation include α,β unsaturated

aldehydes, which are able to form adducts with Mb, accelerating meat discolouration (Alderton *et al.,* 2003; Suman *et al.,* 2007; Faustman *et al.,* 1999, Faustman *et al.,* 2010; Suman *et al.,* 2013). Alternatively, Mb has the ability to initiate lipid oxidation when oxygen is released from OMb resulting in the production of ferric (Fe^{3+}) MMb and super oxide anion radicals. Subsequently the MMb can be oxidised to the highly reactive ferryl (Fe^{4+}) Mb (Richards & Hultin, 2002). McKenna *et al.* (2005) evaluated the lipid oxidation of 19 bovine muscles and found that the least colour stable muscles also had the highest lipid oxidation reiterating the relationship between lipid oxidation and colour stability. Conversely, Jeong *et al.* (2009) found no differences in lipid oxidation between bovine muscles of varying colour stabilities. Various factors affect the rate of lipid oxidation including the degree of unsaturation of the fatty acids, light, oxygen concentration, temperature, anti- and pro-oxidants (naturally present or added to the diet), and the presence of enzymes (Chaijan, 2008).

Meat from animals with high levels of PUFAs is more prone to lipid oxidation and discolouration (Morrissey *et al.,* 1998; Nute *et al.,* 2007). It has been noted that 100 g meat from antelope (960.9 mg), deer (967.4 mg) and elk (707.5 mg) had much higher phospholipid concentrations than beef (502.3 mg) (Williams *et al.,* 1983). The higher PUFA concentrations noted in venison/wild game could contribute to the rapid discolouration of venison post-mortem (Stevenson-Barry *et al.,* 1999). The level of PUFAs in meat can be affected by diet. Grazing/pasture diets lead to higher levels of PUFAs than concentrate diets (Wood *et al.,* 2003). It would thus be expected that meat from animals reared on grazing/pasture would be more prone to lipid oxidation and discolouration. However, Wiklund *et al.* (2006) observed better colour stability in red deer meat from grazing animals compared to concentrate fed animals despite the grazing animals having higher levels of PUFAs. This discrepancy was attributed to the higher levels of vitamin E in the meat of grazing animals which results from their diet. This argument is strengthened by the work of Ponnampalam *et al.* (2012) who investigated the joint relationship between vitamin E, heme iron and PUFAs on colour stability of lamb. The results showed that the level of vitamin E and heme iron in the meat influenced colour stability more than the level of PUFAs. Thus, investigating the relationship between colour stability and lipid oxidation, without measuring the antioxidant and heme iron content may cause misleading result and conclusions. It should be noted that vitamin E is not equally effective in meat from all species (Suman *et al.,* 2013). It has been noted that the effect of vitamin E supplementation on colour stability is more distinct in beef compared to lamb and pork (Faustman & Wang, 2000). A possible explanation for the differences in susceptibilities was provided by Suman *et al.* (2007) who noted that pork Mb is less liable to alkylation by α,β unsaturated aldehydes than beef due to fewer nucleophilic histidine residues being present in pork. Thus, pork Mb is less susceptible to the lipid oxidation than beef Mb (Suman & Joseph, 2013). Other research has also noted the higher susceptibility of beef to lipid oxidation in

comparison to pork (Rhee & Ziprin 1987; Rhee *et al.,* 1996; Kim *et al.,* 2002; Min *et al.,* 2008; Ramanathan *et al.,* 2009). Interestingly, other authors have noted that differences in in Mb biochemistry did not explain the differences in colour stabilities observed between species (Joseph *et al.,* 2010; Yin *et al.,* 2011). Further research is required to elucidate the reasons for these differences.

Antioxidants have the ability to delay or inhibit lipid oxidation and thus increase colour stability in meat. It has been noted that the differences observed that the differences observed for colour stable (LD) and colour liable (PM) muscles in beef (McKenna *et al.* 2005, Seyfert *et al.* 2007) is attributed to higher levels of anti-oxidant proteins present in colour stable muscles (Joseph *et al.,* 2012). The anti-oxidant which has received the most attention with regards to meat and lipid oxidation is vitamin E. Numerous researchers have investigated its effect of lipid oxidation and colour stability in meat (Faustman *et al.,* 1998; Jacobsen & Bertelsen, 2000; Gatellier *et al.,* 2001; Luciano *et al.,* 2009). It has been noted that vitamin E is affective at different concentrations in muscles from different species. Vitamin E levels of 2.95-3.0 mg/kg (Jose *et al.,* 2008; Ponnampalam *et al.,* 2012) were reported to reduce lipid oxidation in lamb whereas 7-9 mg/kg was reported for venison (Okabe *et al.,* 2002)

Conversely, pro-oxidants initiate/accelerate lipid oxidation (Chaijan, 2008). There are several naturally occurring pro-oxidants in red meat including iron and copper (Lawrie & Ledward, 2006). Iron is the most notable of these and occurs as either heme (associated with Mb) or non-heme iron. Liu and Watts (1970) noted that both heme iron and non-heme iron accelerate lipid oxidation. Non-heme iron, in particular, has been implicated as a catalyst for lipid oxidation (Chen *et al.,* 1984) and thus muscles with higher levels non-heme iron could lead to reduced colour stability. High levels of heme iron have been note in deer (24 µg $Fe.g^{-1}$) (Wiklund *et al.,* 2006) in comparison to beef (19-29 µg $Fe.g^{-1}$) (Berge *et al.,* 1997). As with PUFAs, the higher heme iron concentrations noted in venison/wild game could contribute to the rapid discolouration of venison post-mortem (Stevenson-Barry *et al.,* 1999).

The high PUFA and pro-oxidant concentrations in venison and game meat could explain their susceptibility to discolouration. Processing methods which inhibit/reduce lipid oxidation may thus contribute to increasing the colour stability and marketability of venison and game meat. Although prodigious quantities of literature are available on the effect of lipid oxidation on colour stability, research is notably lacking for venison and game species.

CONCLUSION

Colour and colour stability of meat are important to the meat industry as they determine the purchase intent, marketability and shelf-life of meat products. This review reiterates the complexity of meat biochemistry and that the factors affecting colour and colour stability are

not mutually exclusive but are all inter-linked. Furthermore, many of the factors seem to be species, breed and muscle specific and thus highlight the need for species, breed and muscle specific research into colour and colour stability. For many of the factors, literature on game meat and venison is notably lacking. Researchers also often group wild ungulate species together, assuming that they will have similar colour and colour stabilities when, in fact, they may have colour and colour stabilities as varying as those noted between domestic species. Thus research into the differences between the colour and colour stabilities of various venison and game species warrants further research.

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CHAPTER 3

COLOUR STABILITY OF FIVE BLESBOK (*DAMALISCUS PYGARGUS PHILLIPSI*) MUSCLES

ABSTRACT

No research is currently available on the colour stability of meat from South African game species. This study investigated the colour stability of five blesbok muscles, *infraspinatus* (IS), *supraspinatus* (SS), *biceps femoris* (BF), *semimembranosus* (SM) and *semitendinosus* (ST) stored under aerobic conditions at 2±0.60°C for ten days. Surface colour attributes (*L**, *a**, *b**, hue and chroma), surface myoglobin redox forms (deoxymyoglobin, oxymyoglobin and metmyoglobin), ultimate pH, and lipid oxidation were measured at various time intervals. The results indicated that the surface colour attributes were not indicative of visual colour changes and that surface myoglobin redox forms were more representative. The colour stability of the five blesbok muscles was determined to be IS>SS>BF=SM=ST. The results were inconsistent with those observed for domestic red meat species and emphasises the need for further research into the colour stability of game meat. In addition, similar trends in temporal colour stability were observed for the IS and SS, and BF, SM and ST muscles, indicating that muscles in these groups could be processed, packaged and market together or in a similar manner.

Keywords Game meat; Venison; Myoglobin; Meat colour

INTRODUCTION

Despite South African game meat being regarded as a high-value product (Hofmeyr, 2014), limited research has been done on its quality and none on its colour stability. Considering its potential role in food security, and its value to the South African game industry and the economy (Hofmeyr, 2014), research into the quality of game meat is critical to growing this market.

While large quantities of research have been done regarding the colour stability of red meat derived from various domestic species, little to no research has been done on venison and game meat. Furthermore, meat from all game animals is often grouped together and referred to as venison (Hoffman & Wiklund, 2006). However, it is recommended that meat originating from game animals in Africa be distinguished from that originating from other countries as the latter is progressively being replaced by farmed, domestic animals, whereas the former is still obtained from wild, free-roaming animals (Hoffman & Wiklund, 2006). Thus, it is advisable to use the term game meat rather than venison, when referring to meat

originating from game animals in Africa (Hoffman & Wiklund, 2006). The grouping of meat from game animals under one umbrella term (game meat or venison) also leads to the assumption that the quality of these species is uniform when in fact, they may be as different as that of the various popular domestic red meat species (beef, mutton and pork).

In addition to the species effect on colour stability, muscle type and gender have also been known to effect colour stability. Muscle-specific colour stability has been reported by several authors, which is related to various biochemical differences between the muscles (Ledward, 1971; O'Keeffe & Hood, 1982; Renerre & Labas, 1987; McKenna *et al.,* 2005; Von Seggern *et al.,* 2005; Jeong *et al.,* 2009; Kim *et al.,* 2009; Joseph *et al.,* 2012). With regard to gender, it has been suggested male animals may have darker meat than females due to higher Mb concentrations, resulting from higher amounts of physical activity (Seideman *et al.,* 1982). The higher Mb concentrations suggested in meat from males could contribute to a decrease in colour stability due to the pro-oxidant effect of the iron present in the Mb (Insausti *et al.,* 1999).

The objective of this study was to investigate the of colour stability of five blesbok muscles, two from the forequarter (*infraspinatus*; IS and *supraspinatus*; SS) and three from the hindquarter (*biceps femoris*; BF, *semimembranosus*; SM and *semitendinosus*; ST), during refrigerated storage to gain some insight into the colour stability of game meat.

MATERIALS AND METHODS

Harvesting and dressing

Twelve (six male and six female) mature blesbok (live weight, bled 53.6 ± 7.2 kg) were harvested during winter (July 2013) on the farm Brakkekuil (34°18'24.0"S and 20°49'3.9"E) in the Western Province of South Africa. The animals were harvested according to the standard operating procedure approved by the Stellenbosch University Animal Care and Use Committee (SU-ACUM14-001SOP). To reduce stress, the animals were harvested at night. A .308 calibre rifle was used for harvesting and the animals were shot in the head. The animals were exsanguinated in the field within two to three minutes after being shot. Subsequently, the carcasses were dressed (head, legs and skin were removed and the carcass eviscerated) at the abattoir facilities on Brakkekuil. Once dressed, the animals were hung by both hind legs in a cold room (2±0.60°C) for 24 h to allow for the development of rigor mortis.

Muscle removal

The IS, SS, SM, BF and ST were removed from the left and right sides of the chilled carcass 24 h post-mortem. Each muscle pair (left and right) was considered an experimental unit (variation between the left and right muscles was considered negligible). The muscles were vacuum packed in a composite plastic (70 µm polyethylene and nylon; moisture vapour transfer rate of 2.2 $g.m^{-2}.24\ h^{-1}.1\ atm^{-1}$, O_2 permeability of 30 $cm^3.m^{-2}.24\ h^{-1}.1\ atm^{-1}$ and a CO_2 permeability of 105 $cm^3.m^{-2}.24\ h^{-1}.1\ atm^{-1}$) bag with a residual pressure of 5 mb (as per the machine gauge pressure reading) (Multivac, Model C200; Sepp Haggenmuller, Wolfertschwenden, Germany). The muscles were transported to the Department of Animal Sciences at Stellenbosch University and placed into a cold room (2±0.60°C). The samples were arranged in a single layer to ensure uniform cooling of the samples.

Muscle fabrication

The muscles were stored at 2±0.60°C for 48 hours prior to the commencement of the colour stability trial. The muscles were removed from the vacuum packaging and fabricated into 2.5 cm steaks; the muscles were cut through the centre, perpendicular to the long axis of each muscle, and steaks were cut from either side of the central line, radiating towards the anterior and posterior ends of the muscle. Twelve steaks were portioned from each experimental unit (six from each muscle). The steaks were packed in a single layer by muscle type (IS, SS, SM, BF and ST) and carcass in polystyrene trays and overwrapped with low-density polyethylene wrap (LDPE) (moisture vapour transfer rate of 585 $g.m^{-2}.24\ h^{-1}.1\ atm^{-1}$, O_2 permeability of 25 000 $cm^3.m^{-2}.24\ h^{-1}.1\ atm^{-1}$ and a CO_2 permeability of 180 000 $cm^{-3}.m^{-2}\ 24\ h^{-1}\ 1\ atm^{-1}$). The trays were randomly packed in a single layer on shelfs in the cold room (2±0.60°C) to reduce variation between samples in terms of light exposure, temperature and gas permeation through the LDPE film.

Colour stability trial

The colour stability trial was conducted over a ten day period with samples being taken at six time periods: day zero (D0); day two (D2); day four (D4); day six (D6); day eight (D8); and day ten (D10). For the duration of the trial, the samples were stored in a cold room at 2±0.60°C under fluorescent lights (OSRAM L58W/640, Energy saver, Cool White, 4600 Lumen, 4000°K, 65 CRI).

Instrumental colour measurement

The surface colour of the steaks was measured at each time point. Surface colour was measured using a colour-guide 45°/0° colorimeter (aperture size 11 mm; illuminant/observer of D65/10°) (Catalogue number 6801; BYK-Gardner, Geretsried, Germany). Calibration of the colorimeter was done using the standards provided (BYK-Gardner). Prior to colour measurement, the overwrap was removed and the colour was measured directly on the surface of the meat, perpendicular to the muscle fibre orientation; after the measurement the specific sample was removed for further analyses. On D0 the steaks were bloomed for 60 min at 2±0.60°C (covered in overwrap to prevent surface desiccation) prior to measurement. On subsequent days, blooming was not done as the overwrap was oxygen permeable which negated the need for blooming. Measurements were taken at five different locations on each steak. The measurement comprised of L^* (lightness), a^* (redness) and b^* (yellowness) values. From these a^* and b^*values the hue angle and chroma were calculated using equation 1 & 2, respectively (AMSA, 2012).

Equation 1: Hue angle= arctan (b^*/a^*)

Equation 2: Chroma = $(a^{*2} + b^{*2})^{1/2}$

The average of the five measurements for each attribute was calculated and used for statistical analysis.

Surface myoglobin redox forms

Concurrent to the surface colour measurements, the colorimeter also measures surface reflectance. The reflectance is measured from 400-700 nm at 10 nm increments. The reflectance data can be used to calculate surface myoglobin (Mb) redox forms (deoxymyoglobin; DMb, oxymyoglobin; OMb and metmyoglobin; MMb) using the equations proposed by Tang *et al.* (2004). The reflectance was measured at the isobestic wavelengths of Mb (473, 525, 572 and 730 nm). It has been noted that, if the instrument used does not measure up to 730 nm, a wavelength of 700 nm can be used, as was the case in this study. Wavelengths which were not specifically measured but which were required for the calculations (473, 525 and 572 nm), were calculated using integration (AMSA, 2012). For example, the reflectance at 473 nm was calculated using the reflectance values measured at 470 nm and 480 nm. This method is based on reflex attenuance and as such, all reflectance values required must first be converted to their corresponding reflex attenuance values (A) (Equation 3a). Reflex attenuance is the logarithm of the reciprocal of reflectance. The reflex attenuance values are subsequently used to calculate the percentages of the various Mb redox forms, present at the surface of fresh meat, using equations 3b-d (Tang *et al.,* 2004).

Equation 3a: $A = \log \frac{1}{R}$

where *R* is the reflectance at a specific wavelength

Equation 3b: $\%MMb = \{1.395 - [\frac{(A572-A700)}{(A525-A700)}]\} \times 100$

where *A525*, *A572* and *A700* is the reflex attenuance at 525, 572 and 700 nm, respectively

Equation 3c: $\%DMb = \{2.375 - [\frac{(A473-A700)}{(A525-A700)}]\} \times 100$

where *A473*, *A525* and *A700* is the reflex attenuance at 473, 525 and 700 nm, respectively

Equation 3d: %OMb = 100 – (%MMb + %DMb)

pH

The pH of the steaks was measured at each time point. The pH was measured at using a calibrated (standard buffers at pH 4.0 and pH 7.0) Crison 506 portable pH meter with a knife electrode (Lasec SA, Cape Town, South Africa).

Lipid oxidation

Lipid oxidation was measured for all time periods with measurements performed in duplicate using a modified version of the 2-thiobarbituric acid (TBARS) extraction method described by Lynch and Frei (1993). Briefly, a 1 g sample, taken from both the surface and subsurface, was homogenised (P-8; Kinematica, Littau, Switzerland) in 10 mL of 0.15 mol L^{-1} KCl. An aliquot (0.5 mL) of the homogenate was added to equal parts (0.25 mL) of 50 mmol L^{-1} NaOH with 1% (w/v) 2-thiobarbituric acid and 2.8% (w/v) trichloroacetic acid. The homogenate was incubated in a waterbath (95°C) for 60 min (Marcuse & Johansson, 1973) and subsequently produced a pink chromogen. The chromogen was extracted using n-butanol. The pink n-butanol extraction was measured at 532 nm (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). The TBARS concentration was calculated from a standard curve of 1,1,3,3-tetramethoxypropane and expressed as mg malondialdehyde (MDA) kg^{-1} of meat.

Statistical analysis

Mixed model repeated measures ANOVA was used to investigate the differences of measurements between muscles taking into account gender and time effects. Thus gender, muscle and time were treated as fixed effects, and animal nested in gender as random effect.

RESULTS

The significant interactions between the main effects for the various attributes are shown in Table 1. Gender as an individual main effect had no significant effect on any of the attributes measured. Therefore, where applicable, only the significant interactions and significant individual main effects will be stated and discussed further.

The interaction between muscle and time (MxT) was observed to be the most prevalent for the various attributes, suggesting that the type of muscle and the time for which the muscles were stored had the most significant effect in the colour stability of the blesbok meat.

A MxT interaction was observed for the *L*, a*,* chroma and hue values (Table 1). Furthermore, similar temporal trends were observed for the five muscles for each of these attributes (Fig. 1). A temporal decrease ($P \leq 0.05$) was observed for the *L*, a** and chroma values (Fig. 1a-c) whereas a temporal increase ($P \leq 0.05$) was observed for the hue values (Fig. 1d).

Initially (D0), the *L** values for the SM differed ($P \leq 0.05$) from all the other muscles and the ST differed from the BF and IS ($P \leq 0.05$) (Fig. 1a). The SM initially had the lowest ($P \leq 0.05$) values and the ST the highest. The SM and SS both indicated an initial increase ($P \leq 0.05$) (D0 to D2) whereas the other muscles showed no change ($P > 0.05$). The IS, SM and ST and, the BF and SS showed a decrease ($P \leq 0.05$) after D4 and D6, respectively. On D10, the SS differed from the IS, BF and SM and, the ST differed from the IS ($P \leq 0.05$).

For the *a** values, the SS and ST initially (D0) differed significantly from all the muscles, with the SS having the highest value ($P \leq 0.05$) and the ST the lowest ($P \leq 0.05$) (Fig. 1b). On D10, the BF and SM differed significantly from all the muscles, including each other, with the IS, SS and ST not differing ($P > 0.05$) from each other; the BF ($P \leq 0.05$) had the highest values ($P \leq 0.05$) and the ST the lowest.

On D0, the chroma values for the SS differed ($P \leq 0.05$) from those of the BF and IS, and the ST values differed ($P \leq 0.05$) from those of the SM and SS (Fig. 1c). The SS had the highest values and the ST the lowest. An initial increase ($P \leq 0.05$) was observed for all the muscles from D0 to D2. Subsequently, the values decreased ($P \leq 0.05$) for the IS and SS, the SM and ST, and the BF after D2, D4 and D6, respectively. On D10, the BF had the highest ($P \leq 0.05$) chroma values, whereas no differences (($P > 0.05$) were observed between the other muscles.

Table 1 The *P*-values[1] indicating the impact of gender, muscle and time on the various attributes measured for blesbok meat

Attributes	GxMxT[2]	MxT[3]	GxT[4]	GxM[5]	Gender	Muscle	Time
*L**	0.720	**0.020**	0.494	0.465	0.315	**0.000**	**0.000**
*a**	0.326	**0.000**	0.963	0.499	0.286	**0.000**	**0.000**
*b**	0.728	0.061	0.837	0.981	0.751	0.261	**0.000**
Chroma	0.715	**0.000**	0.915	0.852	0.253	**0.000**	**0.000**
Hue	0.147	**0.000**	0.972	0.723	0.536	**0.000**	**0.000**
Deoxymyoglobin (%)	0.958	**0.000**	0.560	0.463	0.844	**0.000**	**0.000**
Oxymyoglobin (%)	0.334	**0.000**	**0.026**	**0.032**	0.334	**0.000**	**0.000**
Metmyoglobin (%)	0.716	**0.000**	0.714	0.970	0.365	**0.000**	**0.000**
pH	-	-	-	0.244	0.070	**0.001**	-
Lipid oxidation	0.799	0.748	0.670	0.811	0.520	0.186	**0.002**

[1]*P*-values in bold indicate a significant interaction/difference at $P \leq 0.05$.
[2]Interaction between gender, muscle and time.
[3]Interaction between muscle and time.
[4]Interaction between gender and time.
[5]Interaction between gender and muscle.

Initially (D0), the hue values of the ST differed ($P \leq 0.05$) from all the other muscles, having the highest values (Fig. 1d). Subsequently, a sharp increase (D0 to D2) in hue values was observed for all muscles. The BF, SM and ST and, the IS and SS followed similar trends from D2 to D10; the BF, SM and ST had a trough-like trend from D2 to D8, followed by a decrease (D8 to D10), which was significant for the BF and SM but not for the ST. The IS and SS had an arc-like trend despite no temporal differences ($P > 0.05$) being observed and thus no overall change ($P > 0.05$) from D2 to D10. The ST had the highest ($P \leq 0.05$) value on D10 and differed from all the other muscles. The IS and SS, and the BF and SM did not differ ($P > 0.05$) from each other on D10, with the BF having the lowest value.

Only the main effect of time had a significant temporal effect on the *b** values of the muscles (Table 1). The trend for the *b** values was characterised by a sharp initial increase ($P \leq 0.05$) (D0 to D2), followed by a gradual temporal decrease ($P \leq 0.05$) (Fig. 2).

A MxT interaction was observed for both the %DMb and %MMb (Table 1). On D0, the ST had the highest %DMb and differed ($P \leq 0.05$) from the BF, SM and SS (Fig. 3a). A decrease ($P \leq 0.05$) for all the muscles was observed from D0 to D2, followed by a plateau (no change) ($P > 0.05$) to D8. From D8 to D10, an increase ($P \leq 0.05$) in %DMb was observed for all muscles except for the BF. On D10, the IS and SS had the highest values ($P \leq 0.05$), and did not differ ($P > 0.05$) from each other but did differ ($P \leq 0.05$) from all the other muscles. Similarly, the BF and SM had the lowest values ($P \leq 0.05$) on D10, and did not differ ($P > 0.05$)

from each other but did differ ($P \leq 0.05$) from all the other muscles. Furthermore, the ST differed ($P \leq 0.05$) from all the muscles on D10. An overall decrease ($P \leq 0.05$) was observed for the BF, SM and ST, whereas no overall change ($P > 0.05$) was observed for the IS and SS.

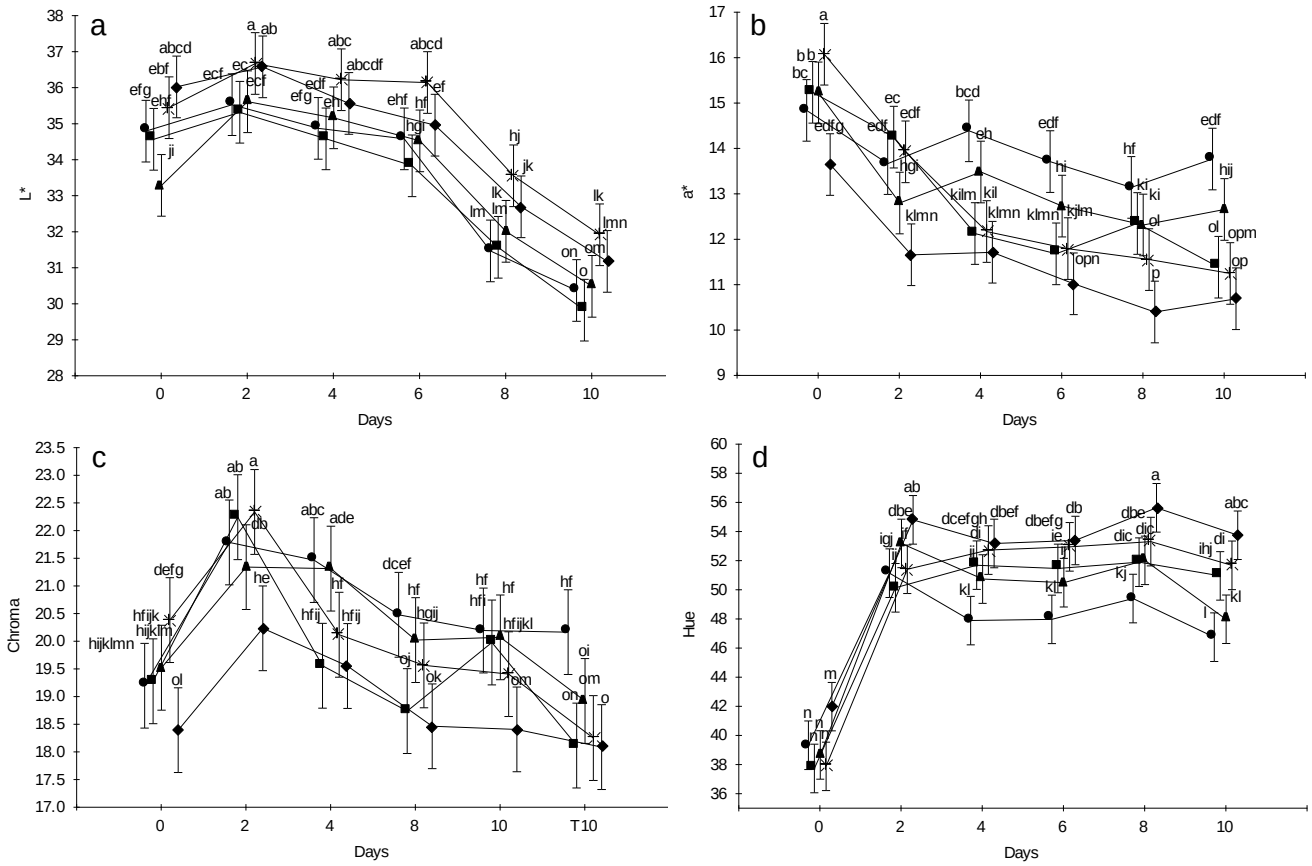

Figure 1 The temporal changes in (a) *L**, (b) *a**, (c) chroma and (d) hue (with standard error bars) for five blesbok muscles, (●) *biceps femoris,* (■) *infraspinatus,* (▲) *semimembranosus*, (✳) *supraspinatus* and (◆) *semitendinosus*, stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

Initially (D0), no differences ($P > 0.05$) in %MMb were observed between the muscles (Fig. 3b). From D0 to D2, a sharp increase ($P \leq 0.05$) was observed for all the muscles, where after an arc-like trend was observed. For the BF an SM an increase ($P \leq 0.05$) was noted from D2 to D8, with no overall increase ($P > 0.05$) from D2 to D10. A decrease ($P \leq 0.05$) was observed for the SS and ST from D2 to D10 but no change ($P > 0.05$) was observed for the IS. Overall, a temporal increase ($P \leq 0.05$) was observed for all the muscles.

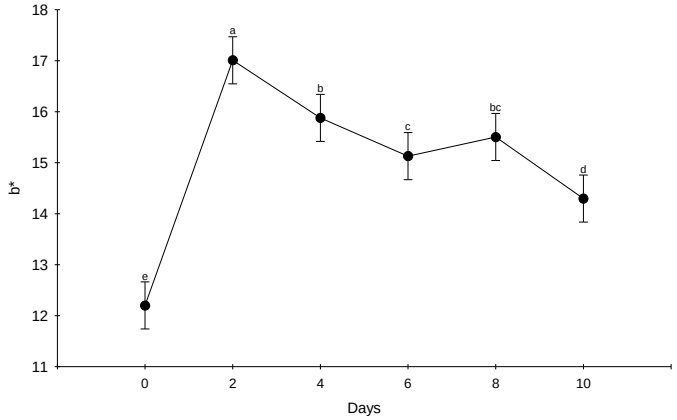

Figure 2 The overall temporal change in *b** (with standard error bars) for the blesbok muscles stored at 2±0.60°C. Means with different letters differ significantly ($P \leq 0.05$).

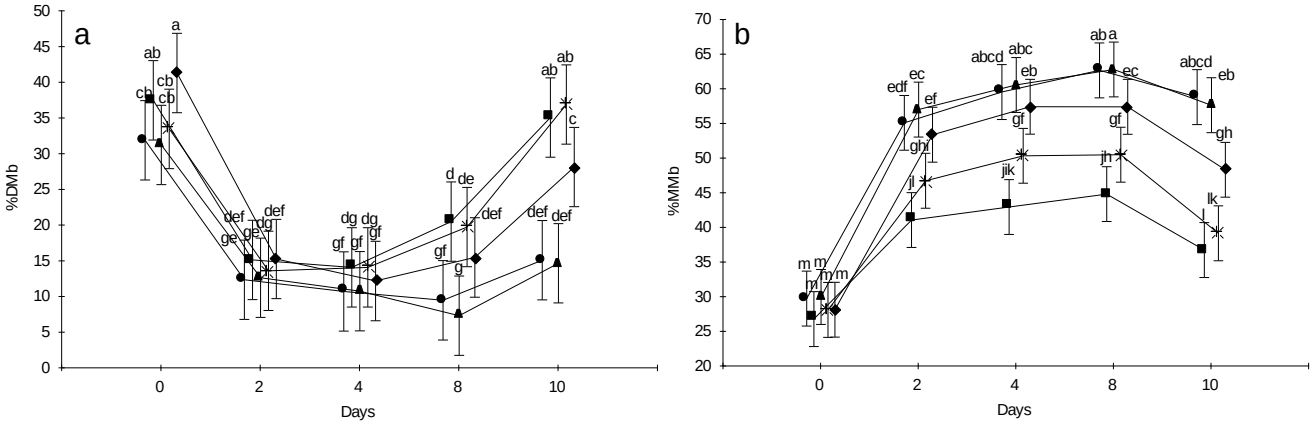

Figure 3 The temporal change in (a) percentage deoxymyoglobin (%DMb) and (b) percentage metmyoglobin (%MMb) (with standard error bars) for five blesbok muscles, (●) *biceps femoris,* (■) *infraspinatus,* (▲) *semimembranosus*, (✳) *supraspinatus* and (◆) *semitendinosus*, stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A GxM, GxT and MxT interaction was observed for the %OMb content (Table 1). The GxM interaction (Fig. 4a) showed that the IS of the male blesbok had significantly higher %OMb in comparison to the female IS, with none of the other muscles differing significantly from each other. The GxT interaction was not highly significant (Table 1) but a difference in the temporal trend was observed between the male and female %OMb values, with the female data decreasing in a straight line, while the male data had an arc-like trend (Fig. 4b). On D0, for the MxT interaction (Fig. 4c), the ST had the lowest %OMb ($P \leq 0.05$) and differed significantly from all the other muscles, which did not differ ($P > 0.05$) from each other. The IS was the only muscle for which an increase ($P \leq 0.05$) was observed from D0 to D2 resulting in the IS having the highest ($P \leq 0.05$) %OMb values from D2 to D8. The SS had higher values ($P \leq 0.05$) on D2 to D4 in comparison to the BF SM and ST. The BF, SM and ST have a similar temporal trend

and did not differ (P>0.05) from each from D2 to D8. The IS differed from the SS and ST, and the SM differed from the ST on D10. Overall, a decrease (P≤0.05) in %OMb was observed for all muscles.

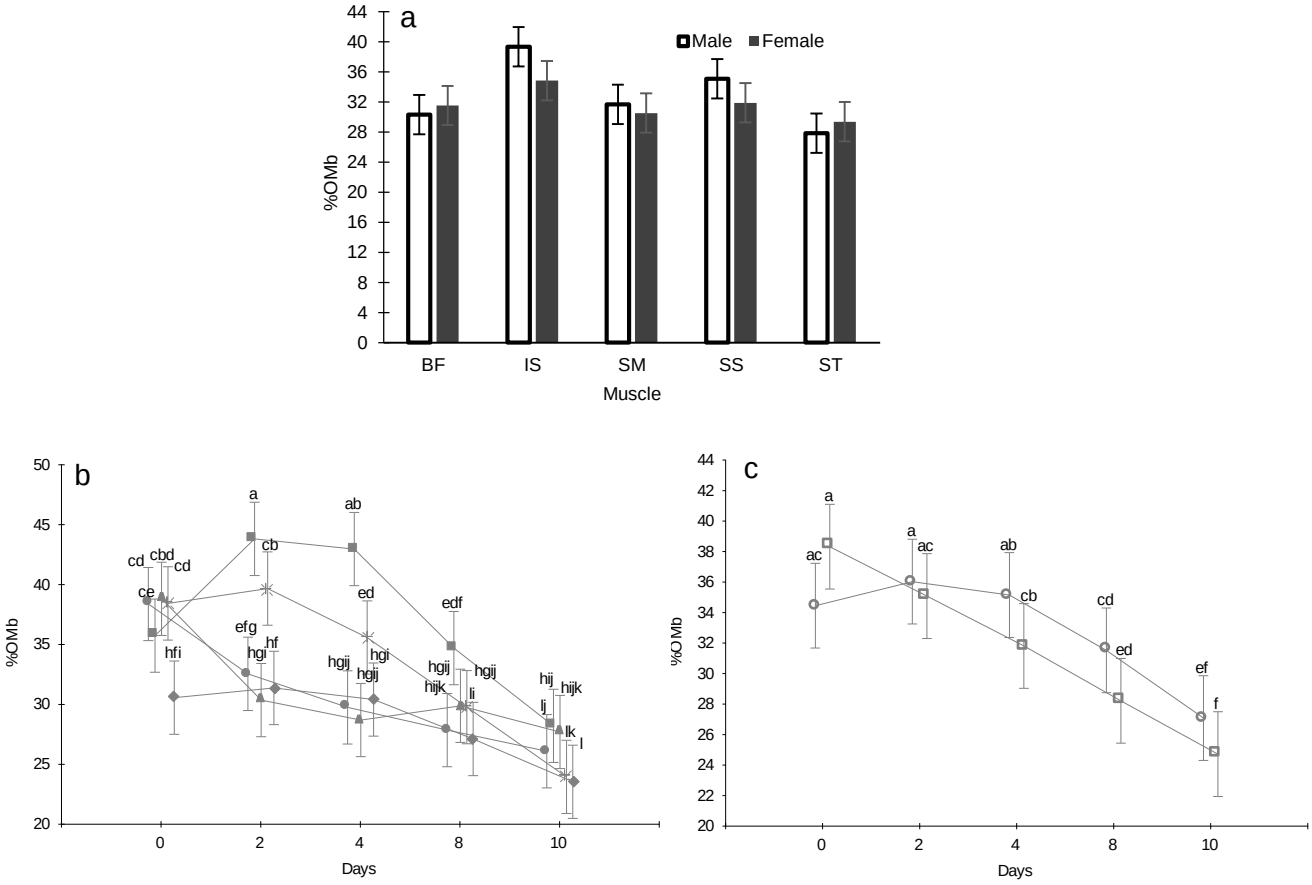

Figure 4 (a) The average percentage oxymyoglobin (%OMb) values (with standard error bars) for the various (O) male and (□) female blesbok muscles, (b) the overall temporal change in percentage oxymyoglobin (with standard error bars) for (O) male and (□) female blesbok muscles (with standard error bars) and (c) temporal change in percentage oxymyoglobin (with standard error bars) for five blesbok muscles, (●) *biceps femoris,* (■) *infraspinatus,* (▲) *semimembranosus*, (✱) *supraspinatus* and (◆) *semitendinosus*, stored at 2±0.60°C. Means in figures with different letters differ significantly (P≤0.05).

The main effect of muscle had a significant effect on the pH values (Table 1). The IS has the highest (P≤0.05) pH and differed from all the other muscles, which did not differ (P>0.05) from each other (Fig. 5).

Only the main effect of time had a significant temporal effect on the TBARS values (Table 1). The TBARS values showed a significant temporal increase (Fig. 6).

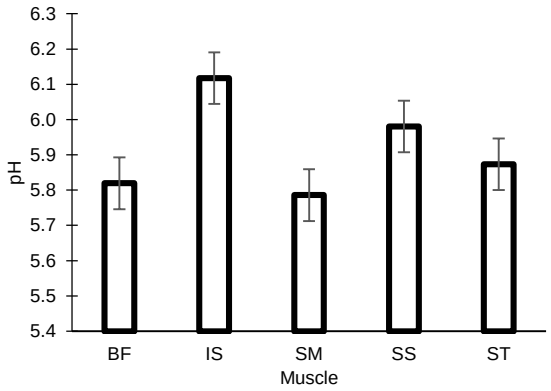

Figure 5 The average ultimate pH values (with standard error bars) for five (BF=biceps femoris; IS=*infraspinatus*, SM=*semimembranosus*, SS=*supraspinatus*; and ST=*semitendinosus*) blesbok muscles stored at 2±0.60°C. Means with different letters differ significantly ($P \leq 0.05$).

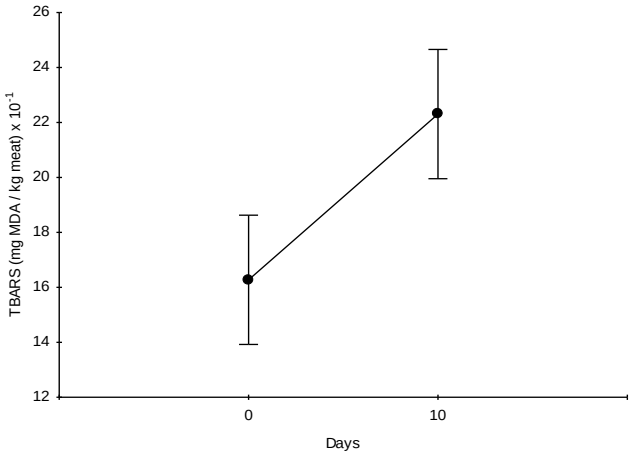

Figure 6 The temporal change in TBARS (with standard error bars) for the blesbok muscles, stored at 2±0.60°C. Means with different letters differ significantly ($P \leq 0.05$).

DISCUSSION

Interestingly, a trend can be observed between the muscles for all the MxT interactions (Figs. 1b-d, 3a and b, and 4c). It appears that the IS and SS, and the BF, SM and ST have similar trends. Furthermore, these muscle also appear to group together (have similar values) for some, but not all of the attributes. These groupings are consistent with the anatomical locations of the muscles within the carcass; the IS and SS are located in the forequarter/anterior of the carcass, and the BF, SM and ST are located in the hindquarter/posterior of the carcass. This would suggest that the there is a relationship between muscle location/function and muscle colour stability. This relationship could possibly be attributed to the relative differences in muscle fibre type found in these groups due to their

functional differences (Hunt & Hedrick, 1977; Klont *et al.,* 1998; Lefaucheur, 2010). Muscle fibre type was, however, beyond the scope of this study and not investigated but does warrant further research.

The *L** values are indicative of the brightness of the muscle colour (AMSA, 2012). The results indicate that the brightness of all the muscles decrease over time (Fig. 1a). This decrease is consistent with observations made by other authors (McKenna *et al.,* 2005; Seyfert *et al.,* 2006; Kim *et al.,* 2011; King *et al.,* 2011). It would appear that the lightness of the muscles in this study are in descending order of SS>ST>SM>BF>IS. These results are not consistent to those of McKenna *et al.* (2005) or King *et al.* (2011). McKenna *et al.* (2005) noted that the ST was the lightest (highest *L**) of all 19 bovine muscles assessed, but these authors did not report its lightness values relative to the other muscles measured. In agreement with McKenna *et al.* (2005), King *et al.* (2011) also noted that the ST was lightest of the muscles. In addition, King *et al.* (2011) noted the lightness of the muscles in descending order of ST>IS>BF>SM (SS was not measured), which is almost in complete contradiction to the results from this study. These differences may be attributed to species and processing differences between the studies. However, the importance of *L** in assessing red meat colour stability is questionable. Many studies pertaining to red meat colour stability do not report their findings for *L*.* Furthermore, McKenna *et al.* (2005) has noted that *L** appeared to play only a marginal part in predicting the colour stability of muscles, as muscles of similar colour stabilities had significantly different *L** values. Furthermore, the grouping of the muscles according to anatomical location was not observed for *L** which may also intimate to its lack of use in predicting colour stability in muscles.

To the authors knowledge no other studies have observed gender differences for %OMb (Fig. 4a,b). Furthermore, the GxM and GxT interactions for the %OMb were not significant (Table 1). Only the male and female IS muscles differed in %OMb for the GxM interaction (Fig. 4a) and no differences were observed between the days for the male and female muscles for the GxT interaction (Fig. 4b); the significant interaction observed is related to the differences in the temporal trend between the male and female muscles rather than differences between days. Thus, the biological significance of the gender effect is questionable and warrants further investigation.

The *a** and %OMb values give an indication of the redness of muscle, whereas the hue and %MMb values give an indication of discolouration or browning (Mancini & Hunt, 2005). Higher *a** and %OMb, and higher hue and %MMb values are indicative of redder and browner muscle, respectively. The *a** (Fig. 1b) and hue (Fig. 1d) results suggest that the colour stability of the muscles are in descending order of BF>SM>IS=SS>ST. Furthermore, Wiklund *et al.* (2001) established a cut-off point of 12 for *a** values; values of 12 and above are considered acceptable by consumers for venison colour and values below 12

unacceptable. If this cut-off point is applied (Fig. 1b), the BF and SM would be considered to have an acceptable surface colour for the duration of the trial, whereas the ST, and the IS and SS would be considered unacceptable in colour after D2 and D4, respectively. The cut-off point thus echoes the colour stability sequence mentioned previously. These results are comparable to those of McKenna *et al.* (2005) who analysed the colour stability of 19 bovine muscles over eight days. McKenna *et al.* (2005) found that the SM had high, and the IS and SS low *a** values. Conversely, McKenna *et al.* (2005) noted that the ST also had high *a** values and that the BF had mid-range *a** values. In addition, McKenna *et al.* (2005) concluded that the ST had a high colour stability and IS and SS very low colour stabilities. King *et al.* (2011) noted similar trends in *a** values to those of McKenna *et al.* (2005) for the IS, BF, SM and ST (SS was not analysed) of bovine muscles. Other authors have also reported similar findings for bovine muscles (Seyfert *et al.,* 2006). The differences noted between the current study and those of other authors may be attributed to species differences. Furthermore, the temporal decrease and increase in the *a** (Fig 1b) and hue values (Fig 1d), respectively, is expected under aerobic conditions due to the decrease in OMb and the subsequent formation of MMb (Faustman & Cassens, 1990; Mancini & Hunt, 2005). This trend has also been noted by others (Stevenson *et al.,* 1989; Gatellier *et al.,* 2001; Jacob *et al.,* 2007; Sawyer *et al.,* 2007; Luciano *et al.,* 2009; Kannan *et al.,* 2001; Kim *et al.,* 2011; King *et al.,* 2011; Rosenvold & Wiklund, 2011; Holmgaard Bak *et al.,* 2012; Liu *et al.,* 2015). However, the results from the *a** and hue values did not correspond with what was visually observed for the colour stability of the muscles and are somewhat contradictory to the result observed for the %MMb (Fig. 3b) and %OMb (Fig. 4c). Although contrary to other literature regarding the colour stability of various bovine muscles (McKenna *et al.,* 2005; Seyfert *et al.,* 2006; King *et al.,* 2011), the temporal trends observed for the %MMb and %OMb corresponded better to visual observations. The results for the %MMb and %OMb indicate that the colour stability of the muscles is in descending order of IS>SS>BF=SM=ST. The contradictions observed between the current study and those of other authors can most likely be attributed to the differences in species. The reason(s) for the poor correlation between these attributes is not readily explained and warrants further investigation. McKenna *et al.* (2005) also noted discrepancies between *a** and the K/S ratios (indication of %OMb and %MMb) values for various bovine muscles. These authors attributed the discrepancies to the fact that OMb is characterised by a bright red colour and that *a** only measures the redness of muscles. Other authors have also noted that, although commonly employed to measure the colour of meat, the CIELab ordinates do not indicate the Mb form present at the surface of the meat and that the use of reflectance spectra gives a better indication of the temporal colour changes which occur on the surface of meat (Holmgaard Bak *et al.,* 2012).

The higher colour stability of the IS in relation to the other muscles in this study could be attributed to its significantly higher pH in relation to the other muscles (Fig. 5). Higher pH values delay the oxidation of OMb and vice versa (Gotoh & Shikama, 1974; Ledward, 1985). This does, however, not explain the colour stability differences between all the muscles as the pH of the SS does not differ significantly from the other muscles but does differ in colour stability. This suggests that pH alone does not influence colour stability but rather that it is influenced by numerous factors (Madhavi & Carpenter, 1993).

It has been noted that lipid oxidation is a major contributor to the decrease in colour stability of meat (Faustman *et al.,* 2010). A correlation between lipid oxidation and Mb oxidation in meat has been observed, with an increase in one leading to an increase in the other (Faustman & Cassens, 1990; Renerre, 1990; Renerre & Labadie, 1993; Yin *et al.,* 1993; O'Grady *et al.,* 1998; Gatellier *et al.,* 2001; Kim *et al.,* 2011). Despite a temporal increase in TBARS values being observed in this study (Fig. 6), no differences were observed for the various muscles (Table 1). This may be due to the limited number of sampling periods used, and it would be suggested that, for future research, the same number of sample periods be used as for the various surface colour attributes and Mb redox forms, to determine the temporal trend in TBARS values and any possible interactions. In addition, other researchers have also noted a temporal increase in TBARS for venison (Wiklund *et al.,* 2006; Daszkiewicz *et al.,* 2011).

CONCLUSION

The results indicate that the colour stability of the blesbok muscles is in descending order of IS>SS>BF=SM=ST. These findings are in contradiction to those noted for other species and highlight the need for further, more in-depth research on the colour stability of game meat and the factors which influence it. The results also indicated that the colour stabilities of certain muscles were the same or similar and/or that the muscles followed similar temporal trends. The similarities between these muscles would allow for the muscles in each group to be processed, packaged and marketed in the same or a similar manner. Additionally, the muscles in each group could be processed together. For example, the IS and SS would be ideal for the production of minced meat due to their high colour stability and low value of these muscles, while the BF and SM would be ideal for the production of steaks due to their low colour stability and higher value by comparison. Furthermore, the similarities between the muscles would allow for the reduction of the number of samples in future studies, as the changes in colour stability of one muscle can be used to predict the changes in other muscles. Significant effects were observed for gender, muscle and time and thus all these variables need to be included in future studies regarding the colour stability of game meat.

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CHAPTER 4

MUSCLE-SPECIFIC COLOUR STABILITY OF BLESBOK (*DAMALISCUS PYGARGUS PHILLIPSI*) MEAT

ABSTRACT

The increased demand for meat from alternative species, such as blesbok, gives rise to the need for the quantification of all meat quality factors from these species. Since colour is an important determinant of consumer purchasing decision, it is one of the meat quality factors which require investigation. The objective of this study was to determine the meat colour stability of three blesbok muscles, *infraspinatus* (IS)*, longissimus thoracis et lumborum* (LTL) and *biceps femoris* (BF) stored under aerobic conditions at 2±0.60°C for eight days. Surface colour attributes (*L**, *a**, *b**, hue, chroma and R (630/580)), surface myoglobin redox forms (deoxymyoglobin, oxymyoglobin and metmyoglobin) and various biochemical factors which influence colour stability (pH, metmyoglobin reducing activity, oxygen consumption, lipid oxidation, heme, non-heme and total iron, and total myoglobin) were measured at various time intervals. The colour stability of the three blesbok muscles was determined as, in descending order, IS, LTL, BF. The shelf-life colour stability was determined to be more than eight days for the IS and one day for the LTL and BF. The results suggest that species and muscle specific strategies should be employed to improve the shelf-life colour stability of blesbok meat to increase consumer acceptability and sales.

Keywords Venison, Game Meat, Ungulate, Myoglobin, Lipid oxidation

INTRODUCTION

Blesbok (*Damaliscus pygargus phillipsi*) is a popular and economically significant South African game species as it is hunted for both its meat and hide, and is an attraction at many game parks. The increasing global demand for alternative (Boland *et al.,* 2013) and healthier red meat sources (Hoffman & Wiklund, 2006; Dahlan & Norfarizan Hanoon, 2008) means that the demand for blesbok meat, other game meat species and venison will increase in the future (Hoffman & Cawthorn, 2012). The need to evaluate all factors influencing game meat quality is thus becoming increasingly important.

With consumer purchase intent based largely on meat colour (Faustman & Cassens, 1990; Risvik, 1994; Mancini & Hunt, 2005; Suman *et al.,* 2014), one of the most important quality factors to quantify in game meat is colour stability. Surface discolouration of meat is inevitable and the period for which fresh meat colour is acceptable is brief (Faustman &

Cassens, 1990). Meat which is discoloured is often reworked into mince or other processed products and sold at reduced prices leading to a loss in revenue (Faustman & Cassens, 1990; Mancini & Hunt, 2005; McKenna *et al.,* 2005; Suman *et al.,* 2014). Meat colour stability is not only species (O'Keeffe & Hood, 1982; Faustman & Cassens, 1990) but also muscle specific (O'Keeffe & Hood, 1982; McKenna *et al.,* 2005) and different strategies may be required to increase the colour stability of different species and muscles.

The objective of the present study was to investigate the colour stability of blesbok meat during refrigerated storage by evaluating three muscles, one from the forequarter (*infraspinatus*; IS), one from the trunk/mid-section (*longissimus thoracis et lumborum*; LTL) and one from the hindquarter (*biceps femoris*; BF), by evaluating surface colour attributes, surface Mb redox forms and various biochemical aspects which influence colour stability of meat. The larger muscles, LTL and BF, are commonly sold as fresh muscles, whereas the IS usually forms part of composite cuts or processed products.

MATERIALS AND METHODS

Animal harvesting and muscle sample collection

Twelve (six male and six female) mature blesbok (live weight, bled 54.0 ± 5.82 kg) were harvested on the farm Brakkekuil (34°18'24.0"S and 20°49'3.9"E), situated near Witsand in the Western Cape province of South Africa. The animals were harvested according to the standard operating procedure number SU-ACUM14-001SOP (Stellenbosch University Animal Care and Use Committee) in June 2014 which is winter in South Africa. The animals were harvested at night to reduce stress and were shot in the head or the high neck area with a .308 calibre rifle. Once shot, the animals were exsanguinated in the field (two to three minutes after being shot). As far as possible all steps were taken to prevent or reduce any unnecessary ante-mortem stress experienced by the animals. The exsanguinated, undressed carcasses were transported to abattoir facilities at Brakkekuil where the legs, head and skin were removed and the animals eviscerated. After dressing, the carcasses were hung in a cold room (± 2°C) for 24 h by both hind legs. Subsequently, the entire *infraspinatus* (IS) *longissimus thoracis et lumborum* (LTL) and *biceps femoris* (BF) muscles were removed from the left and right side of each carcass. All visible intermuscular and subcutaneous fat was removed and the muscles were individually vacuum packed in a composite plastic (70 µm polyethylene and nylon; moisture vapour transfer rate of 2.2 gm^{-2} 24 h^{-1} 1 atm^{-1}, O_2 permeability of 30 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1} and a CO_2 permeability of 105 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1}) bag with a residual pressure of 5 mb (as per the machine gauge pressure reading) (Multivac, Model C200; Sepp Haggenmuller, Wolfertschwenden, Germany). The samples were transported to the Department of Animal Sciences at Stellenbosch University and placed into a cold room (2 ±

0.60°C) upon arrival. The samples were laid out on racks in a single layer to ensure even cooling of the samples.

Muscle fabrication

The muscles were stored for 48 hours at 2±0.60°C prior to the commencement of the colour stability trial. The muscles from the left and right sides of each carcass were considered an experimental unit (variation between the left and right muscles was considered negligible). The muscles were removed from the vacuum packaging and blotted dry with paper towel to remove any surface moisture. The anterior and posterior ends the muscles were removed and not used for analysis. Each muscle was cut through the centre perpendicular to the long axis of each muscle and three 2.5 cm steaks were cut perpendicular to the long axis of each muscle from each half, either side the centre of the muscle (six steaks per muscle; twelve steaks per experimental unit). Steaks were packed in a single layer by muscle type (IS, LTL, BF) and carcass in polystyrene trays and overwrapped with low-density polyethylene wrap (LDPE) (moisture vapour transfer rate of 585 gm^{-2} 24 h^{-1} 1 atm^{-1}, O_2 permeability of 25 000 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1} and a CO_2 permeability of 180 000 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1}). The overwrapped trays were packed in a single layer in the cold room to reduce variation between samples in terms of light exposure, temperature variation and gas permeation through the LDPE film.

Colour stability trial

The colour stability trial was conducted over an eight day period with samples being taken at six time periods: day zero (D0); day one (D1); day two (D2); day four (D4); day six (D6); and day eight (D8). The samples were stored in a cold room at 2±0.60°C under fluorescent lights (OSRAM L58W/640, Energy saver, Cool White, 4600 Lumen, 4000°K, 65 CRI) for the duration of the trial.

Surface colour attributes

1. *Instrumental colour measurement*

The surface colour of the steaks was measured on D0, D1, D2, D4, D6 and D8 using the same methodology as detailed in *Chapter 3*.

2. *R (630/580)*

Concurrent to the surface colour measurements, the colorimeter also measures surface reflectance. The reflectance is measured from 400-700 nm at 10 nm increments. This reflectance data can be used to calculate R (630/580) using equation 3 (AMSA, 2012).

Equation 3: R (630/580) = 630 nm/580 nm

Surface myoglobin redox forms

The surface Mb redox forms were measured on D0, D1, D2, D4, D6 and D8 using the same methodology as detailed in *Chapter 3.*

Biochemical attributes

1. *pH*

The pH of the meat samples were determined at each time point using the iodoacetate method as described by Jeacocke (1977). The meat sample (0.5 g) was homogenised in 5 mL of a 5 mM Na-iodoacetate and 150 mM KCl (adjusted to pH 7 with KOH) solution. The pH of the homogenate was measured in duplicate with a calibrated Jenway 3510 bench top pH meter with ATC probe (IJEN351201, Lasec SA, Cape Town, South Africa).

2. *Oxygen consumption (OC)*

The OC was measured at three time points: D0; D4; and D8. A 3 cm x 3 cm x 2 cm cube was removed from the centre of each steak. If the sample surface was not freshly cut (D1, D2, D4, D6 and D8) a thin section of the surface layer was removed to expose a fresh surface layer. The samples were allowed to bloom for 2 h at 2±0.60°C to ensure uniform oxygenation and covered in overwrap (LDPE) (moisture vapour transfer rate of 585 gm^{-2} 24 h^{-1} 1 atm^{-1}, O_2 permeability of 25 000 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1} and a CO_2 permeability of 180 000 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1}) to prevent desiccation. The samples were vacuum packed in a composite plastic (70 µm polyethylene and nylon; moisture vapour transfer rate of 2.2 gm^{-2} 24 h^{-1} 1 atm^{-1}, O_2 permeability of 30 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1} and a CO_2 permeability of 105 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1}) with a residual pressure of 5 mb (as per the machine gauge pressure reading) (Multivac, Model C200; Sepp Haggenmuller, Wolfertschwenden, Germany). The bloomed surface reflectance was scanned (400-700 nm) immediately after packaging. These measurements were used to calculate the initial %OMb, using the relevant spectral values (473, 525, 572 and 700 nm), from equations 1-4 (*see Surface reflectance measurements*). The scanned samples were subsequently placed in a water bath and incubated at 25°C for 20

min. After incubation the surface reflectance was scanned again. These measurements were used to calculate the final %OMb as indicated for the initial %OMb above. OC was calculated using equation 5 (AMSA, 2012).

Equation 5: OC = [(Initial %OMb − Final % OMb) ÷ Initial %OMb] × 100

3. Metmyoglobin reducing activity (MRA)

The MRA was measured at three time points: D0; D4; and D8. A 3 cm x 3 cm x 2 cm cube was removed from the centre of each steak. The samples were submerged in 0.3% $NaNO_2$ (w/w) for 20 min to induce MMb formation. After 20 min the samples were removed, blotted dry and vacuum packed in a composite plastic (70 µm polyethylene and nylon; moisture vapour transfer rate of 2.2 gm^{-2} 24 h^{-1} 1 atm^{-1}, O_2 permeability of 30 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1} and a CO_2 permeability of 105 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1}) with a residual pressure of 5 mb (as per the machine gauge pressure reading) (Multivac, Model C200; Sepp Haggenmuller, Wolfertschwenden, Germany). The surface reflectance was scanned (400-700 nm) immediately subsequent to packaging. These measurements were used to calculate the initial %MMb, using the relevant spectral values (525, 572 and 700 nm), from equations 1 and 2 (see *Surface reflectance measurements)*. The samples were placed in a water bath at 30°C for 2 h after which the surface reflectance was scanned once more. These measurements were used to calculate the final %MMb as indicated for the initial %MMb above. The MRA was calculated using equation 6 (AMSA, 2012).

Equation 6: MRA = [(Initial %MMb − Final %MMb) ÷ Initial %MMb] × 100

4. Lipid oxidation

Lipid oxidation was measured for all time periods using the same methodology as detailed in *Chapter 3.*

5. Iron quantification

a. Heme iron

Heme iron was determined in duplicate on D0. The heme-iron concentration was determined using an acidified acetone extraction method (Hornsey, 1956). The sample (1 g) was homogenised (P-8; Kinematica, Littau, Switzerland) with 0.2 ml distilled water (70% water content in samples), 0.1 ml concentrated hydrochloric acid and 4 ml of acetone and allowed to extract in the dark for 60 min at room temperature. The samples were centrifuged (Sigma 2-16 K, Wirsam scientific, Cape Town SA), filtered (Whatman #1 filter paper) and the

absorbance of the resulting filtrate was measured at 640 nm (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). The heme iron concentration was expressed as mg heme iron g^{-1} of meat.

b. Total iron

Total iron was determined on D0 using HNO_3 digested samples analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Briefly, a 0.5 g samples was pre-digested (allowed to digest at room temperature) in concentrated HNO_3 for 20 min. The samples were then digested fully in a MARS microwave digester (MARS 240/50; CEM Corporation, USA) at 1600 W, 800 psi and 200°C for 10 minutes (ramp time of 25 min and cool down time of 25 min). The samples were diluted to 50 ml (1:100 dilution) with deionised water. The diluted, digested samples were then analysed using a Thermo ICap 6200 ICP-AES. The ICP-AES was calibrated using NIST traceable standards and verified with a control standard. The results were corrected for the dilution factor resulting from the digestion procedure.

c. Non-heme iron

Non-heme iron was calculated from the difference between the total iron and the heme iron values (Chen *et al.,* 1984).

6. Total myoglobin concentration

The total Mb concentration was quantified from samples taken on D0. Myoglobin was extracted from the samples according to the method of Tang *et al.* (2004). Briefly, a 10 g sample was homogenised (P-8; Kinematica, Littau, Switzerland) in 100 ml cold 40 mM potassium buffer (adjusted to pH 6.8) and allowed to extract for 60 min at 4°C. The extract was then centrifuged (Sigma 2-16 K, Wirsam scientific, Cape Town SA) for 30 min at 4000 rpm at 4°C. A small quantity of sodium dithionite (3-5 µg) was added to the filtrate to convert all the Mb to DMb. The absorbance of the filtrate was measured at 433 nm (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). The Mb concentration was calculated using the absorbance value at 433 nm as follows (AMSA, 2012):

Equation 7: $[Mb]\ (mg/g\ meat) = A_{433} \times (1\ M\ Mb/114\ 000) \times [(1\ mol/L)/M]$

$\times (17\ 000\ g\ Mb/mol\ Mb) \times (1000\ mg/g) \times$ dilution factor of 0.10 L/10 g meat

Statistical analysis

Mixed model repeated measures ANOVA was used to investigate the differences of measurements between muscles taking into account gender and time effects. Thus gender, muscle and time were treated as fixed effects, and animal nested in gender as random effect. For post hoc testing, the Fisher least significant difference (LSD) test was used. Correlation analyses were done using Pearson correlation. A 5% significance level ($P \leq 0.05$) was used as guideline for significant effects and correlations.

RESULTS

The significant interactions between the main effects for the various analyses are shown in Table 1. No significant interactions were observed between gender and time (GxT), and gender and muscle (GxM). Gender as an individual main effect also had no significant effect on the colour stability of blesbok meat. Where applicable, only the significant interactions and significant individual main effects will be reported and discussed further.

An interaction between muscle and time (MxT) was observed for all the surface colour attributes (L^*, a^*, b^*, hue, chroma and R (630/580)) and surface Mb redox forms (%OMb, %DMb and %MMb) (Table 1) indicating that the muscle type and the time for which the muscles were stored had the most significant effect in the colour of the blesbok meat.

Surface colour attributes

Initially (D0), the LTL had lower ($P \leq 0.05$) L^* values compared to the IS and BF, which did not differ ($P > 0.05$) from one another (Fig. 1a). A similar temporal trend was observed for all the muscles except that the L^* values of the LTL and BF decreased from D0 to D1, while those of the IS increased. A steep increase in L^* was observed for all the muscles from D1 to D2, followed by a decrease (D2 to D4) and subsequent plateau (D4 to D8). An overall increase (D0 to D8) ($P \leq 0.05$) in L^* was observed for the IS, whereas no change was observed for the LTL and BF. On D8, all the muscles differed significantly, with the IS having the highest L^* values and the LTL the lowest.

Table 1 The *P*-values[1] indicating the impact of gender, muscle and time on the various colour stability attributes measured for blesbok meat

Attributes	GxMxT[2]	MxT[3]	GxT[4]	GxM[5]	Gender	Muscle	Time
*L**	0.322	**0.000**	0.372	0.528	0.999	**0.000**	**0.000**
*a**	0.460	**0.000**	0.939	0.228	0.179	**0.000**	**0.000**
*b**	0.845	**0.000**	0.784	0.920	0.206	**0.000**	**0.000**
Hue	0.268	**0.000**	0.733	0.093	0.662	**0.000**	**0.000**
Chroma	0.751	**0.000**	0.952	0.590	0.144	**0.000**	**0.000**
%OMb	0.172	**0.000**	0.487	0.653	0.757	**0.000**	**0.000**
%DMb	0.345	**0.036**	0.396	0.148	0.483	**0.015**	**0.000**
%MMb	0.245	**0.000**	0.805	0.737	0.817	**0.000**	**0.000**
R (630/580)	0.680	**0.000**	0.942	0.591	0.711	**0.000**	**0.000**
pH	0.480	**0.001**	0.854	0.587	0.964	**0.000**	**0.000**
MRA	**0.003**	0.304	0.814	0.341	0.272	**0.000**	**0.002**
OC	0.122	**0.007**	0.993	0.417	0.698	0.129	0.535
Lipid oxidation	0.892	**0.000**	0.626	0.363	0.875	**0.000**	**0.000**
Heme iron	-	-	-	0.156	0.678	**0.000**	-
Non-heme iron	-	-	-	0.914	0.951	**0.000**	-
Total iron	-	-	-	0.689	0.937	**0.000**	-
Total Mb	-	-	-	0.619	0.665	**0.009**	-

[1]*P*-values in bold indicate a significant interaction/difference at $P \leq 0.05$.
[2]Interaction between gender, muscle and time.
[3]Interaction between muscle and time.
[4]Interaction between gender and time.
[5]Interaction between gender and muscle.

On D0, the *a** values for all three muscles did not differ significantly (Fig. 1b). A subsequent temporal decrease ($P \leq 0.05$) was observed for all three muscles. The LTL and BF had similar, but steeper declines than the IS, which demonstrated only a gradual decline. As a result the IS had the highest ($P \leq 0.05$) *a** value on D8, whereas the LTL and BF had the lowest albeit similar to each other ($P > 0.05$).

The *b** values of the LTL differed significantly from the IS and BF on D0, whereas those for the IS and BF did not differ ($P > 0.05$) (Fig. 1c). The *b** values for the LTL and BF followed a similar, erratic temporal trend; an initial decrease (D0 to D1) ($P \leq 0.05$), followed by an increase (D1 to D2) ($P \leq 0.05$), a decrease (D2 to D6) ($P \leq 0.05$) and ending in a plateau (D6 to D8) ($P > 0.05$). On the other hand, the IS increased initially (D0 to D2) ($P \leq 0.05$) and then decreased (D2 to D8) ($P \leq 0.05$). On D8, all the muscles differed significantly, with the IS having

the highest *b** values and the LTL the lowest. An overall increase ($P \leq 0.05$) in the *b** values was observed for the IS, whereas a decrease ($P \leq 0.05$) was observed for the LTL and BF.

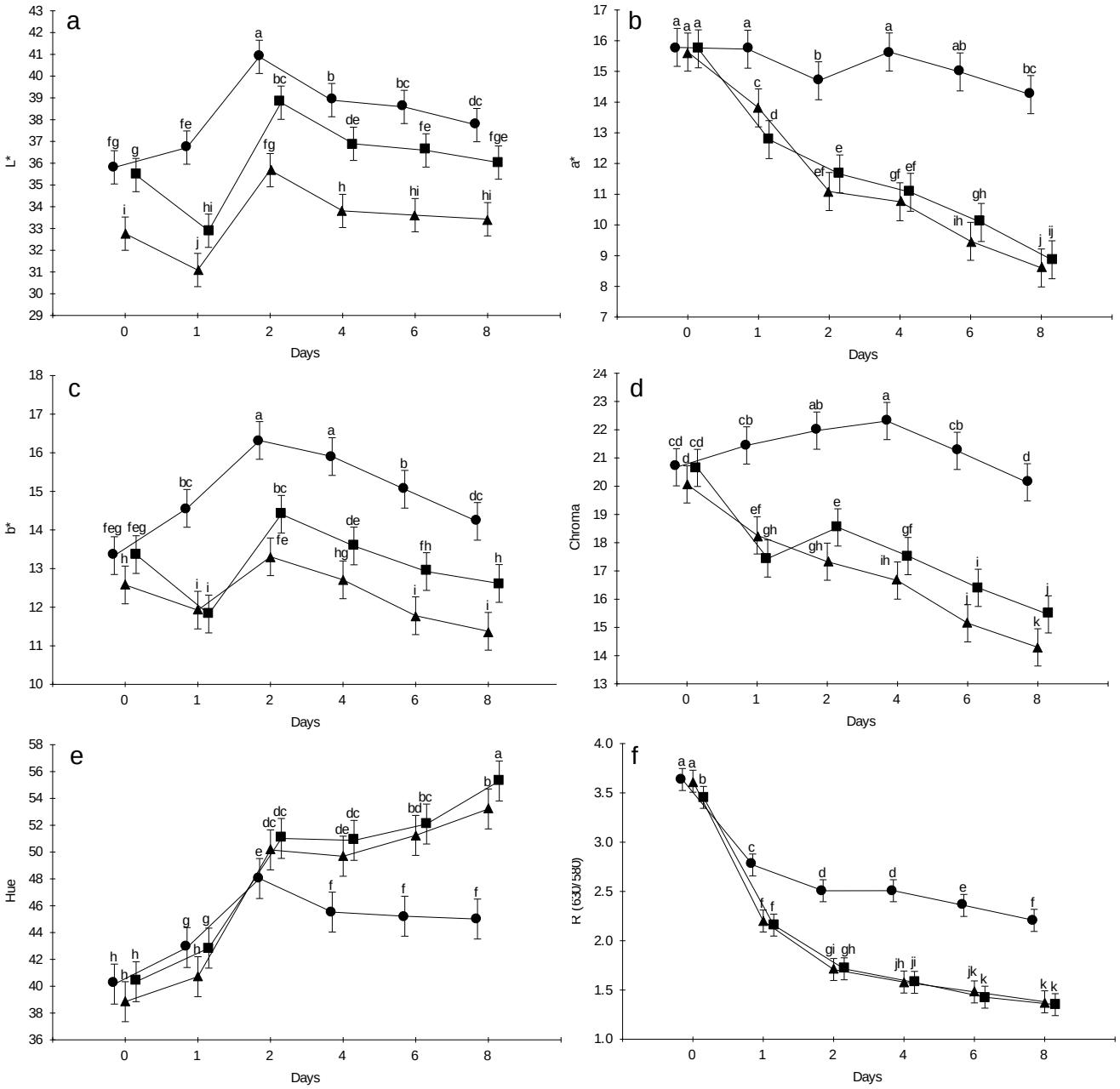

Figure 1 The temporal changes in (a) *L**, (b) *a**, (c) *b**, (d) chroma, (e) hue (f) R (630/580), (with standard error bars) for three blesbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

The chroma values did not initially differ (D0) ($P > 0.05$) for the three muscles (Fig. 1d). The LTL and BF followed a similar temporal decrease (D0 to D8) ($P \leq 0.05$), resulting in an overall decrease ($P \leq 0.05$) in chroma values. The chroma values for the IS, however, initially increased (D0 to D4) ($P \leq 0.05$) and subsequently decreased (D4 to D8) ($P \leq 0.05$), resulting in

no overall change ($P>0.05$) in chroma values. On D8, the IS had the highest ($P\leq0.05$) chroma value, followed by the BF, with the LTL having the lowest ($P\leq0.05$) chroma values.

The initial (D0) hue values for all three muscles did not differ significantly (Fig. 1e). A similar temporal increase ($P\leq0.05$) was observed for the LTL and BF. The IS initially followed a similar temporal increase in hue values as the LTL and BF (D0 to D2) after which the IS values decreased (D2 to D4) ($P\leq0.05$) and subsequently plateaued (D4 to D8) ($P>0.05$). On D8, all the muscles differed significantly, with the BF having the highest hue values and the IS the lowest.

The initial R (630/580) values for the BF differed ($P\leq0.05$) from the IS and LTL (Fig.1f). A temporal decrease ($P\leq0.05$) in R (630/580) was observed for all three muscles, with the IS demonstrating a more gradual decrease than the LTL and BF. The LTL and BF had almost identical slopes. Consequently, the IS had the highest ($P\leq0.05$) R (630/580) values on D8, with those of the LTL and BF not differing ($P>0.05$).

Surface myoglobin redox forms

A similar temporal decrease ($P\leq0.05$) in the %DMb values was observed for all three muscles (Fig. 2a). The %DMb values of the muscles only differed on D2, where the BF had lower ($P\leq0.05$) values than the LTL and BF, and on D8, where the IS differs ($P\leq0.05$) from the BF and the LTL differs from neither ($P>0.05$).

The %OMb values for the IS were higher ($P\leq0.05$) than those of the BF and LD over the entire colour stability trial (D0 to D8) (Fig. 2b). The %OMb values for the IS remained the same from D0 to D1 ($P>0.05$) after which the values decreased ($P\leq0.05$) (D1 to D2) and then plateaued ($P>0.05$). The LTL and BF have a similar temporal decrease in %OMb, with values only differing ($P\leq0.05$) on D2, where the BF values were higher. On D2 for the LTL and D4 for the BF, the %OMb plateaued.

A temporal increase ($P\leq0.05$) in %MMb was observed for all three muscles, which all followed a similar curved trend. The LTL and BF had the steepest increases (Fig. 1i) and only differed ($P\leq0.05$) on D6 and D8, with the BF having the highest values. The %MMb values for the IS differed ($P\leq0.05$) from the LTL and BF on all days, with the IS values being the lowest. The values for all three muscles plateaued after D6.

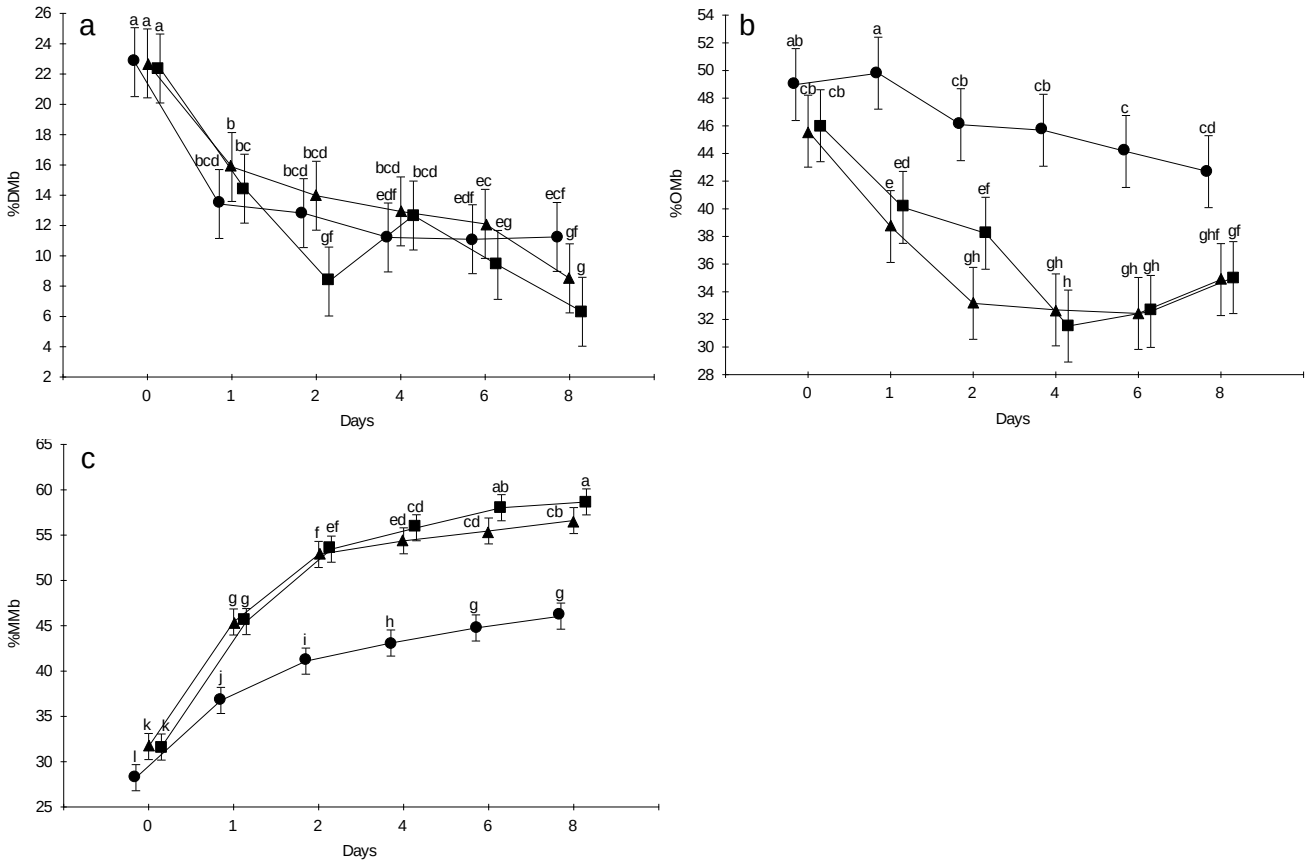

Figure 2 The temporal changes in (a) deoxymyoglobin (%DMb), (b) oxymyoglobin (%OMb), (c) metmyoglobin (%MMb) (with standard error bars) for three blesbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

Biochemical attributes

An interaction between MxT was observed for pH (Table 1). The IS had higher ($P \leq 0.05$) pH values in comparison to the LTL and BF for all days (Fig. 3). The LTL and BF did not differ ($P > 0.05$) for any of the days. An increase ($P \leq 0.05$) in pH was observed for the IS and LTL over time.

An interaction between gender, muscle and time (GxMxT) was observed for the MRA (Table 1). The MRA values for the male IS differed significantly from the LTL and BF on D0 and D8, with the IS values being higher ($P \leq 0.05$) (Fig. 4). The MRA values for the male IS values decreased from D0 to D4 ($P \leq 0.05$) and increased from D4 to D8. The male LTL and BF values only differed on D4, with the BF having higher values. An increase ($P \leq 0.05$) in MRA was observed for both the LTL and BF from D0 to D8. The female IS initially (D0) differed ($P \leq 0.05$) from the LTL but not the BF. The MRA of the female IS gradually increased ($P \leq 0.05$) over time (D0 to D8). The MRA for the female IS differed ($P \leq 0.05$) from the MRA on D4 and

D8. The MRA for the female LTL and BF did not differ (P>0.05) on any day. No change (P>0.05) in MRA was observed for the female BF over time but the female LTL did demonstrate an increase (P≤0.05) in MRA over time (D0 to D8). The MRA values for the various muscles were within the same range between the male and female muscles.

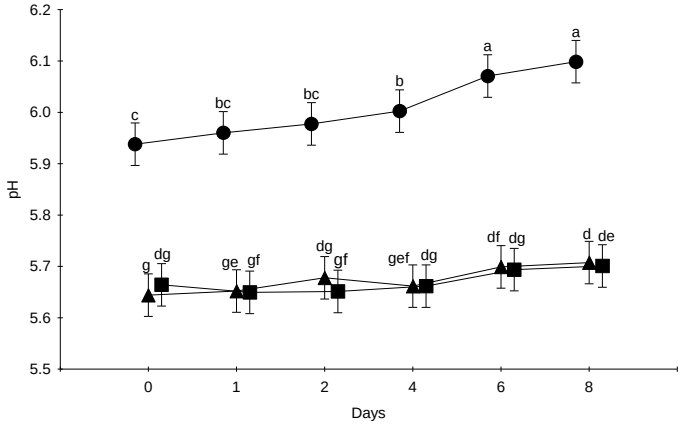

Figure 3 The temporal change in ultimate pH (with standard error bars) for three blesbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means with different letters differ significantly (P≤0.05).

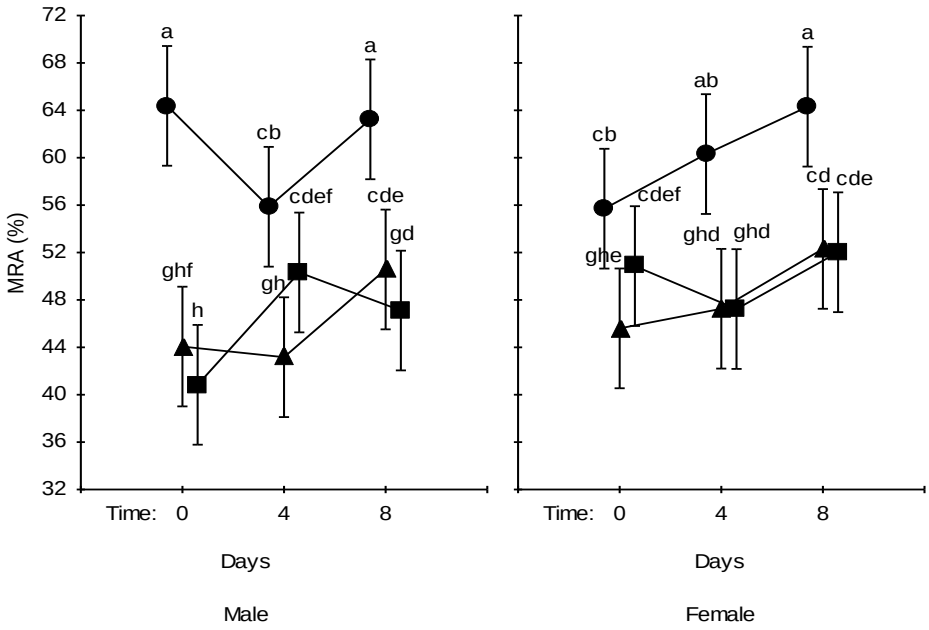

Figure 4 Temporal change in metmyoglobin reducing activity (MRA) (with standard error bars) for three male and female blesbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means with different letters differ significantly (P≤0.05).

An interaction between muscle and time was observed for the OC (Table 1). No differences (P>0.05) between the muscles was observed on any of the days, except for the IS on D8, where it had lower (P≤0.05) values than those of the LTL and BF (Fig. 5). The OC

remained constant (P>0.05) over time for the LTL and BF but decreased for the IS (D4 to D8) (P≤0.05).

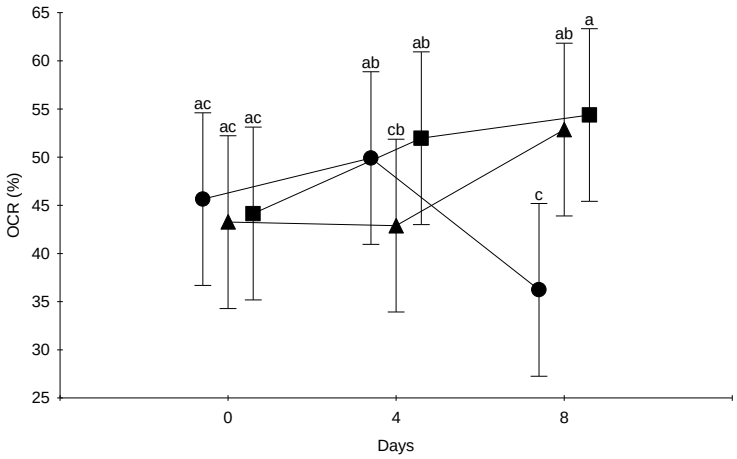

Fig. 5. Temporal change in oxygen consumption (OC) (with standard error bars) for three blesbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means with different letters differ significantly (P≤0.05).

An interaction between muscle and time was observed for the OC (Table 1). A temporal increase in TBARS was observed for all the muscles (Fig. 6). Initial TBARS values for the IS differed from the LTL and the BF, with the IS having the lowest values. On D8 the muscles all differed significantly in TBARS concentration with the LTL having the highest values and the IS the lowest.

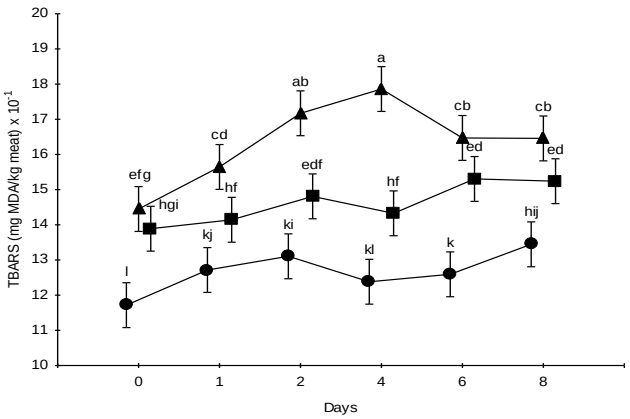

Figure 6 Temporal change in TBARS concentration (with standard error bars) for three blesbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means with different letters differ significantly (P≤0.05).

A significant muscle effect was observed for the total iron, heme iron, non-heme iron and total Mb (Table 2). The IS had the lowest (P≤0.05) total iron and non-heme iron and highest (P≤0.05) heme iron values. The total Mb content of the IS differed significantly from

the LTL but not from the BF (P>0.05). The LTL had the highest (P≤0.05) total iron value and did not differ (P>0.05) from the BF for the heme iron, hon-heme iron and total Mb.

Table 2 The means and standard error of the mean (SEM) of the total iron, heme iron, non-heme iron and total Mb measured on D0 of storage for the IS, LTL and BF of blesbok meat

Attribute	IS[1]	LTL[2]	BF[3]	SEM
Total iron (µg/g meat)	28.12[c]	36.85[a]	33.97[b]	0.922
Heme iron (µg/g meat)	20.06[a]	18.98[ab]	18.24[cb]	0.478
Non-heme iron (µg/g meat)	8.06[b]	17.87[a]	15.73[a]	0.921
Total Mb (mg/g meat)	6.86[b]	7.53[a]	7.17[ab]	0.259

[a-c]Means in rows with different superscripts differ significantly (P≤0.05).
[1]IS - *Infraspinatus.*
[2]LTL - *Longissimus thoracis et lumborum.*
[3]BF - *Biceps femoris*

DISCUSSION

Surface colour stability

Previous studies either observed an increase (Farouk *et al.,* 2007; Luciano *et al.,* 2009; Jacob & Thomson, 2012) or a decrease (Kim *et al.,* 2011; King *et al.,* 2011) in *L** values during storage. McKenna *et al.* (2005) observed that the LTL of beef had the lowest mean *L** values over time. These observations concur with the findings in this study (Fig. 1a). Furthermore, McKenna *et al.* (2005) noted that *L** appears to play only a nominal role in measuring colour stability as different muscles of the same colour stability had significantly different *L** values. This argument was further substantiated by the poor correlation observed between colour stability determinants (K/S ratio). Similarly, the colour stability determinants from this study, R (630/580) (r=-0.01, P>0.05), also correlated poorly to *L**. It has been noted that high pH values lead to darker meat (lower *L** values) and *vice versa* (Lawrie & Ledward, 2006). However, this link between pH and *L** contradicts the results from this study where the IS was found to have the highest *L** values (Fig.1a) (lightest) despite also having the highest pH (Fig. 3).

The %OMb had moderate to strong correlations with all the surface colour attributes commonly used to indicate redness, *a** (r=0.72, P≤0.05), chroma (r=0.68; P≤0.05) and R (630/580) (r=0.74; P≤0.05), which reiterates the use of these surface colour attributes to represent the presence of OMb at the surface of meat. A strong correlation was also observed between *a** and R (630/580) (r=0.84, P≤0.05). The chroma (saturation) results indicate that the IS had a more saturated red colour and was more colour stable (retains high chroma

values over time) than the LTL and BF. The relationship between chroma and meat redness is reiterated by the moderate to strong correlations observed between *a** and chroma (r=0.93, $P \leq 0.05$), and %OMb (r=0.68, $P \leq 0.05$). The red colour saturation of meat (high chroma values) is determined by the amount of OMb formed at the surface and the depth to which the OMb layer penetrates into the muscle (O'Keeffe & Hood, 1982; Mancini & Hunt, 2005). Thus, factors affecting the formation of OMb affect the chroma values (saturation). These factors include pH, MRA and OC as they can affect the blooming of the muscle (AMSA, 2012). The contradiction noted above, between the *L** values and pH (higher pH did not lead to lower *L** values) in this study for the IS, may be explained by the higher chroma values observed for the IS. Higher *L** values have been correlated to higher chroma values at the surface of meat (Onyango *et al.,* 1998) and thus the higher *L** values noted for the IS may be explained by the higher chroma values, which counteract the darkening (lower *L** values) effect of higher pH values.

As with the chroma, the results of the *a** (Fig. 1b), R (630/580) (Fig. 1f) and %OMb (Fig. 2b), indicate that the IS was the most colour stable of the three muscles over eight days of storage. In comparison the LTL and BF were less colour stable and displayed similar colour stabilities over the storage period. In contrast to these findings, McKenna *et al.* (2005) found that *a** values of the LTL of beef decreased the least over five days of storage at 2°C, followed by the BF and then the IS. Despite the initial *a** values being similar for the three muscles, they differed significantly after five days. The differences in observations between the current study and those of McKenna *et al.* (2005) could be attributed to species differences, which may have major implications on the way blesbok meat is processed in comparison to beef. Other authors have also noted the high colour stability of beef LTL in comparison to other muscles (Madhavi & Carpenter, 1993; Seyfert *et al.,* 2006, 2007; Kim *et al.,* 2009; Joseph *et al.,* 2012). Furthermore, the *a** values (Fig. 1 a) for both the LTL and BF fell below the *a** value of 12 after D1, which has been established as the cut-off point for consumer acceptability of venison colour (Wiklund *et al.,* 2001). Although no such value has specifically been established for game meat, the similarities between venison and game meat allows for the assumption that it would be similar. Thus, only the surface colour of the blesbok IS would be considered acceptable by consumers after D1. However, it has also been noted that R (630/580) values below 3 results in a meat surface colour which is considered undesirable by consumers (Purchas *et al.,* 2010). When R (630/580) was used to assess the colour stability of lamb, it was observed that it took 2.5 days for the LTL to reach a R (630/580) value of 3. For the same study, venison LTL reached a value of 3 at approximately 150 min. This illustrates the well-known tendency of venison/game meat colour to deteriorate faster than popular domestic red meat species (e.g. beef and lamb) (Stevenson-Barry *et al.,* 1999). Furthermore, Trout and Gutzke (1995) observed that colour stability at 5°C for lamb was 3.1

days, beef was 4.7 days and venison was 1.6 days. In this study, R (630/580) (Fig.1 f) for all the muscles had fallen below 3 by D1. This contradicts the *a** value cut-off point as the *a** values of the IS remained well above the cut-off point for the entire trial. This contradiction between the cut-off points warrants further research. The temporal decrease in the *a** (Stevenson *et al.,* 1989; Jakobsen & Bertelsen, 2000; Gatellier *et al.,* 2001; Wiklund *et al.,* 2006; Farouk *et al.,* 2007; Jacob *et al.,* 2007; Sawyer *et al.,* 2007; Luciano *et al.,* 2009; Kim *et al.,* 2011; King *et al.,* 2011; Holmgaard Bak *et al.,* 2012), R (630/580) values (Gatellier *et al.,* 2001; Purchas *et al.,* 2010; Jacob & Thomson, 2012; Mancini & Ramanathan, 2014; Canto *et al.,* 2015) and %OMb (Hood, 1980; Feldhusen *et al.,* 1995), under aerobic conditions has been noted by numerous other authors. Decreases in these values are expected as the concentration of OMb will reduce, and the concentration of MMb increase over time, under aerobic conditions (Faustman & Cassens, 1990; Mancini & Hunt, 2005) i.e. as the redness decreases, the browning of the meat surface increases over time. The increase in browning over time is evident from the increase in % MMb (Fig. 2c) and hue (Fig. 1e) values over time for all the muscles. Similar temporal increases in hue (Farouk *et al.,* 2007; Jacob *et al.,* 2007; Sawyer *et al.,* 2007; Luciano *et al.,* 2009; Kim *et al.,* 2011; Rosenvold & Wiklund, 2011; Holmgaard Bak *et al.,* 2012; Jacob & Thomson, 2012) and %MMb (Ledward, 1971; Luciano *et al.,* 2009; Mancini & Ramanathan, 2014) have been noted by other researchers. In addition, the hue (Fig. 1e) and %MMb (Fig. 2c) results further reiterate that the IS was the most colour stable (lower hue and %MMb values) of the three muscles. The hue (Fig. 1e) and %MMb (Fig. 2c) results would seem to imply that the BF was less colour stable over time (higher values) than the LTL, as higher values were noted after D6 and on D8 for the %MMb and hue, respectively. However, these findings were not reiterated by the *a** (Fig. 1a), chroma (Fig. 1d), R (630/580) (Fig. 1f) and %OMb (Fig. 2b), values. Temporal variation in hue values (Jacob *et al.,* 2007) and MMb accumulation (Ledward, 1971, 1985; McKenna *et al.,* 2005) at the surface of meat has previously been noted for different muscles. Ledward (1971) observed higher %MMb at the surface of the BF of beef compared to the SM and LTL, which echoes some of the observations made in this study. The results from the surface colour attributes and surface Mb redox forms indicate that the blesbok IS was the most colour stable of the three muscles and that the LTL and BF had similar colour stabilities.

Biochemical attributes influencing surface colour stability

The high pH values (Fig. 3) observed for the IS and concurrent low values observed for the LTL and BF could contribute to the varying colour stabilities of these muscles (Faustman & Cassens, 1990; Mancini & Hunt, 2005; McKenna *et al.,* 2005; Jeong *et al.,* 2009; Jacob *et al.,* 2013; Calnan *et al.,* 2014). High pH values retard the oxidation of OMb (remains red for

longer) whereas lower pH values accelerate it (turns brown quicker) (Gotoh & Shikama, 1974; Ledward, 1985). This relationship was reiterated in this study, where the IS, which had the highest pH values of the three muscles, had correspondingly higher %OMb (Fig. 2b), *a** (Fig. 1b), chroma (Fig. 1d) and R (630/580) (Fig. 1f) values in comparison to the LTL and BF for the majority of the trial (slower oxidation of OMb). By comparison, the LTL and BF, which had significantly lower pH values to the IS, demonstrated a more rapid oxidation of OMb. A moderate correlation was observed in this study between the %OMb and pH (r=0.50, $P \leq 0.05$), which further reiterates this relationship. McKenna *et al.* (2005) also observed significantly higher pH values for the IS (pH=5.93) of beef in comparison to that of the LTL (pH=5.77-5.78) and BF (pH=5.69). However, these results again contradicted the results from this study as the IS, LTL and BF from beef were classified as having "very low", "high" and "low" colour stabilities, respectively. As previously mentioned, these variations may be attributed to species differences.

The differences between the colour stability of beef and blesbok meat may also be ascribed to variations in the relative proportions of the different muscle fibre types. Studies done on beef have noted that the LTL (colour stable) and *psoas major* (colour labile) contains higher proportions of type IIA and type I muscle fibres, respectively (Seyfert *et al.,* 2006). These differences explain the variations in colour stabilities between these muscles, with type IIA muscle fibres being less oxidative than type I muscle fibres (Pette, 1985). Although the relationship between muscle fibre type and colour stability has not been investigated in venison or game meat, previous studies have examined the muscle fibre type in muscles from these species. It has been noted that in both venison (Kiessling & Kiessling, 1984; Essén-Gustavsson & Rehbinder, 1985) and game meat (Kohn *et al.,* 2011; Curry *et al.,* 2012; North, 2014), muscle fibre types occur in descending order of type IIX, IIA and I. However, the dark colour and rapid colour deterioration, indicative of both venison and game meat, would suggest high proportions of type I muscle fibres (Curry *et al.,* 2012) and is thus contradictory to what is expected. These unexpected results and differences between species warrant further research.

Metmyoglobin reducing activity (MRA) has been linked to the colour stability of muscles (Faustman & Cassens, 1990; Bekhit & Faustman, 2005; Mancini & Hunt, 2005) with higher MRA within a muscle leading to more colour stable muscles (AMSA, 2012). MRA allows for the reduction of MMb back to DMb. Subsequently, this DMb formed can then be re-oxygenated to form OMb thereby maintaining the colour stability of the meat (Madhavi & Carpenter, 1993). To the authors' knowledge no other studies have found gender differences in MRA (Fig. 4). Thus, whether the gender effect observed in this study is biologically significant is debatable as the MRA was the only oxidative stability indicator which significantly differed for gender. If the gender differences in the MRA were strongly significant, other

variables would also have differed significantly for gender. The results will thus be discussed as a whole and not per gender. The MRA for the IS, LTL and BF in this study, are in agreement with the findings of McKenna *et al.* (2005) who observed similar results in beef, with the IS having the highest ($P \leq 0.05$) MRA compared to the LTL and the BF which had similar ($P > 0.05$) values. However, McKenna *et al.* (2005) concluded that MRA did not influence colour stability as the least colour stable muscles had the highest MRA values and *vice versa*. The results from this study contradict those of McKenna *et al.* (2005) as the muscles with the highest colour stability (IS) were also observed as having the highest MRA values and *vice versa*. Furthermore, a positive correlation was observed between MRA and *a** (r=0.22, $P \leq 0.05$), chroma (r=0.23; $P \leq 0.05$) and %OMb (r=0.26, $P \leq 0.05$) in this study. MRA thus seemed to have an effect on the colour stability of blesbok meat. Contradictions regarding the influence of MRA on colour stability have previously been noted (Ledward, 1971; Atkinson & Follett, 1973; O'Keeffe & Hood, 1982; Renerre & Labas, 1987; Madhavi & Carpenter, 1993). The variation in MRA (Fig. 4) between the muscles may be explained by the differences in their pH (Fig. 3). Higher pH values have been correlated to higher MRA (Ledward, 1970; Stewart *et al.,* 1965), which would explain the higher ($P \leq 0.05$) MRA values observed for the IS compared to those of the LTL and BF. In this study a moderate correlation was found between MRA and pH (r=0.50, $P \leq 0.05$). In agreement with the results from this study, temporal increases in the MRA have been observed by other researchers (Feldhusen *et al.,* 1995; Bekhit *et al.,* 2001). Bekhit *et al.* (2001) observed a 20% increase in MRA over a 6 week period in ovine LTL stored at 2°C. Feldhusen *et al.* (1995) observed an increase in the MRA in beef LTL over 13 days of storage at 5°C whereas Echevarne *et al.* (1990) observed either no change or an increase in the MRA, which was muscle dependant. In contrast, other researchers have also seen a decrease in MRA over time (Madhavi & Carpenter, 1993; Sammel *et al.,* 2002; McKenna *et al.,* 2005; Seyfert *et al.,* 2006; King *et al.,* 2011; Mancini & Ramanathan, 2014). An increase in MRA could be attributed to an increase in NAD concentration post-mortem which has been suggested to be muscle dependant (Jerez *et al.,* 2003). Enzymatic reduction of MMb occurs via the microsomal electron transfer system driven by NADH and involves NADH-cytochrome b5 reductase and cytochrome b5 (Hagler *et al.,* 1979; Arihara *et al.,* 1995). Although numerous studies suggest low and/or a decrease in levels of NAD post mortem (Atkinson *et al.,* 1969; Atkinson & Follett, 1973; Faustman & Cassens, 1990; Sammel *et al.,* 2002), others have proposed mechanisms for its regeneration post-mortem (Bodwell *et al.,* 1965; Watts *et al.,* 1966). Bekhit *et al.* (2007) suggested that NAD regeneration post-mortem could be the reason for MMb reduction occurring in the LTL of red deer up to six weeks post-mortem. Thus the increase seen in some muscles and not others in this study could be attributed to a muscle dependant NAD regeneration mechanism.

The oxygen consumption (OC) of meat has also been linked to meat colour stability. It refers to the residual mitochondrial respiration activity in meat post-mortem that will compete with Mb for oxygen, reducing the amount available for OMb production and the depth to which the oxygen can penetrate into the muscle (Bendall & Taylor, 1972). A high OC in meat leads to a decrease in colour stability because less oxygen is available to bind to the Mb, resulting in conditions ideal for MMb formation (Bendall & Taylor, 1972; O'Keeffe & Hood, 1982; Ledward, 1985; McKenna *et al.,* 2005). Although some studies have suggested a relationship between OC and MRA, arguing that OC is required to replenish the NADH required for MRA (Sammel *et al.,* 2002), this was not the case in this study as a weak, non-significant correlation was observed between MRA and OC (r=-0.11, $P>0.05$). Furthermore, negative correlations were observed for the relationship between OC and *a** (r=-0.26, $P\leq0.05$), chroma (r=-0.21, $P\leq0.05$), R (630/580) (r=-0.20, $P\leq0.05$) and %OMb (r=-0.13; $P>0.05$). The significant correlations between OC and the *a**, chroma and R (630/580) values, suggest a relationship between OC and colour stability, with lower OC values leading to increased redness/colour stability in meat. This relationship has previously been documented (Madhavi & Carpenter, 1993; Seyfert *et al.,* 2006). Although the OC (Fig. 5) results are not clear, they may suggest a higher colour stability for the IS, in comparison to the LTL and BF, which was the case in this study. Thus, this study suggests that lower OC values lead to an increase in colour stability. In contrast to these results, McKenna *et al.* (2005) found that the BF had higher OC values than the IS and the LTL, with the LTL having the lowest OC of the three. Bendall and Taylor (1972), found that the BF had the highest OC compared to the LTL, *tensor fasciae latae*, *vastus lateralis* and *rectus femoris.* Again, these differences may be attributed to specie differences. Various other researchers have also noted that the relationship between OC and colour stability was muscle dependant (Bendall & Taylor, 1972; MacDougall & Taylor, 1975; O'Keeffe & Hood, 1982; Renerre & Labas, 1987; Lanari & Cassens, 1991; Madhavi & Carpenter, 1993). As with the IS in this study, King *et al.* (2011) found that muscles with higher colour stabilities, had lower OC and higher MRA values. In addition, a decreases in OC during storage, as noted for the IS, has been observed by other authors and is due to a decrease in mitochondrial function in meat post-mortem (O'Keeffe & Hood, 1982; Madhavi & Carpenter, 1993; McKenna *et al.,* 2005; King *et al.,* 2011). Despite some trends being evident from the results for both MRA (Fig. 4) and OC (Fig. 5) and the correlations suggesting a relationship between MRA, OC and colour stability, the data are very erratic and inconclusive. Due to these findings and the disputes within the literature regarding the relative contribution of each to colour stability, further research is warranted. It would also be suggested that smaller time intervals be used in the future to elucidate whether the erratic nature of the data are due to a phenomenon in the muscles over time or biological variation.

The variations in colour stabilities between the muscles can further be explained by the differences in TBARS (Fig. 5), iron (heme, non-heme and total) (Table 2) and Mb concentrations (Table 2) observed between the muscles. Previous literature has also documented variations in these attributes between different muscles (TBARS: Jakobsen & Bertelsen, 2000; Gatellier *et al.,* 2001; iron: Field *et al.,* 1980; Purchas *et al.,* 2003; Mb: O'Keeffe & Hood, 1982; McKenna *et al.,* 2005; Jeong *et al.,* 2009; King *et al.,* 2011). It has previously been noted that higher TBARS (Faustman & Cassens, 1990; Renerre, 1990; Renerre & Labadie, 1993; Yin *et al.,* 1993; O'Grady *et al.,* 1998), iron (Lefaucheur, 2010), and Mb (O'Keeffe & Hood, 1982; McKenna *et al.,* 2005; Jeong *et al.,* 2009; King *et al.,* 2011) concentrations result in reduced colour stability in meat. The data from this study reiterates this, as the most colour stable muscle, the IS, was also found to have the lowest TBARS (Fig. 5), total and non-heme iron (Table 2), and Mb concentrations (Table 2) of the three muscles. In this study, a moderate correlation was observed between the TBARS and hue (r=0.41, $P \leq 0.05$), and %MMb (r=0.59, $P \leq 0.05$). It has also previously been noted that *a** was negatively correlated with lipid oxidation (Anton *et al.,* 1993; Renerre & Labadie, 1993), which was also the case in this study (r=-0.61, $P \leq 0.05$). However, although the low TBARS values for the IS reiterate the relationship between TBARS and colour stability, the TBARS values for the LTL and BF (Fig. 6) do not reiterate the similar colour stabilities seen for these muscles. Thus, although a relationship between lipid oxidation (TBARS) and Mb oxidation does appear to exist to some extent, it cannot, in this study, be used as a definitive indicator of which muscles would be more colour stable than others. Other researchers have also observed a temporal increase in TBARS concentration in meat with a concurrent decrease in colour stability (Greene, 1969; Greene *et al.,* 1971; Faustman *et al.,* 1992; Mitsumoto *et al.,* 1993; Mercier *et al.,* 1995; Lee *et al.,* 1998; Kannan *et al.,* 2001; O'Grady *et al.,* 2001; McKenna *et al.,* 2005; Luciano *et al.,* 2009). In contrast to the findings from this study (Fig. 6), McKenna *et al.* (2005) found that the BF of beef had the highest TBARS values after six days of retail display, followed by the IS and then the LTL. These contradicting results could be due to species differences.

It has also previously been demonstrated that non-heme iron serves as a catalyst for lipid oxidation (Igene *et al.,* 1979; Chen *et al.,* 1984), lipid oxidation in turn is implicated in Mb oxidation (Liu & Watts, 1970; Igene *et al.,* 1979; Kanner & Harel, 1985; Rhee & Ziprin, 1987; Rhee *et al.,* 1987) and thus high non-heme iron concentrations reduces the colour stability of meat. However, Liu and Watts (1970) noted that both the heme iron and the non-heme iron accelerate lipid oxidation. Thus, total iron concentration can be used to determine the oxidative stability of muscles. Both the total iron (r=0.40, $P \leq 0.05$) and non-heme iron (r=0.55, $P \leq 0.05$) demonstrated significant positive correlations to TBARS. However, only the non-

heme iron values (Table 2) concur with the findings for the colour stability of the three muscles, reiterating its use as an indicator of muscle colour stability.

The total Mb concentration (Table 2) was not as indicative of colour stability as previously suggested. As with the TBARS values, the total Mb values for the IS and LTL were in agreement with the relationship between colour stability and total Mb; lower total Mb leads to increased colour stability and *vice versa*. However, the BF did not differ in Mb concentration from either the IS or LTL but had a similar colour stability to the LTL. Thus, the correlation between total Mb content and colour stability is questionable. Canto *et al.* (2015) and Sammel *et al.* (2002) found no differences in Mb content for colour stable and colour labile muscles. This contradiction in the results reiterates that various intrinsic and extrinsic factors influence the colour stability of meat (Madhavi & Carpenter, 1993) and that these factors are not mutually exclusive but act together to influence the colour stability of meat.

CONCLUSION

The IS was observed to be the most colour stable among the three blesbok muscles (IS, LTL, and BF) with regards to surface colour attributes, Mb redox forms and biochemical attributes. Furthermore, based exclusively on the surface colour data, on which consumers base purchasing intent, blesbok LTL was found to be more colour stable than the BF. The observed muscle-specific colour stability emphasizes the need for employing muscle-specific processing strategies to retail fresh blesbok meat.

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CHAPTER 5

MUSCLE-SPECIFIC COLOUR STABILITY OF SPRINGBOK (*ANTIDORCAS MARSUPIALIS*) MEAT

ABSTRACT

Springbok, one of the most popular game meat species in South Africa has great potential in terms of meat production. Despite this potential very little research regarding its meat quality has been conducted. To produce a stable and sustainable market for springbok meat, more research should be done on its meat quality to ensure a consistent product of high quality. Considering the importance of colour in influencing the purchasing intent of consumers, colour stability of springbok meat is an important quality factor which should be investigated. The objective of this study was to evaluate the colour stability of the three springbok muscles, the *infraspinatus* (IS)*, longissimus thoracis et lumborum* (LTL) and *biceps femoris* (BF) during storage at 2±0.60°C under aerobic conditions for eight days. Surface colour attributes (*L**, *a**, *b**, hue, chroma and R (630/580)), surface myoglobin redox forms (deoxymyoglobin, oxymyoglobin and metmyoglobin) and biochemical factors which influence colour stability (pH, metmyoglobin reducing activity, oxygen consumption, lipid oxidation, heme, non-heme and total iron, and total myoglobin) were evaluated at various time intervals to determine the colour stability of the three muscles. It was determined that the IS was the most colour stable. The LTL and BF were the least colour stable and did not differ in colour stability. The results indicated that muscle-specific processing methods should be utilised to ensure optimum colour stability for springbok meat.

Keywords Game meat, Ungulate, Myoglobin, Lipid oxidation

INTRODUCTION

Springbok (*Antidorcas marsupialis*) are one the most common game species found on farms within South Africa. In addition, it is the most extensively harvested and exported game species from South Africa, with springbok comprising roughly 70% of the game animals harvested in 2008 (Eloff, 2002). Springbok are also synonyms with South Africa as it is the South African national animal and lends it name to the national rugby team, an international sport for which South Africa is well known. Game viewing of springbok and consumption of its meat is thus often perceived by tourists to form part of the Safari (South African) experience (Hoffman & Wiklund, 2006). Thus, not only do springbok have great potential in terms of tourism but also meat production. However, a sustainable and stable game meat market will

only be achieved through the delivery of products of consistently high quality (Hutchison *et al.,* 2010). Since the purchasing intent of fresh meat by consumers is based largely on meat colour (Faustman & Cassens, 1990; Risvik, 1994; Mancini & Hunt, 2005; Suman *et al.,* 2014), the colour and colour stability of game meat is an important quality attribute which needs to be kept consistent.

Despite the large potential for the development of a sustainable game meat market within South Africa, very little research has been done on the meat quality of game meat and none on its colour stability. Since it has been noted that colour stability of meat is both species (O'Keeffe & Hood, 1982; Faustman & Cassens, 1990) and muscle specific (O'Keeffe & Hood, 1982; McKenna *et al.,* 2005), it is pertinent to investigate the colour stability of various species and muscles as different approaches may be required to increase their colour stability.

The objective of the current study was to examine the colour stability of springbok meat during refrigerated storage (2 ± 0.60°C) by evaluating three muscles, the *infraspinatus* (IS) from the forequarter; the *longissimus thoracis et lumborum* (LTL) from the trunk/mid-section; and the *biceps femoris* (BF) from the hindquarter. The colour stability was determined by evaluating the surface colour attributes, surface myoglobin (Mb) redox forms and various biochemical aspects which influence the colour stability of meat. In commercial practice, the IS is a low value muscle which is commonly sold as a composite cut, or as mince or processed into various products. The LTL and BF, are high and intermediate value muscles, respectively, and are commonly sold as fresh whole muscles.

MATERIALS AND METHODS

Animal harvesting and muscle sample collection

Twelve (six male and six female) mature springbok (live weight, bled 28.6 ± 3.18 kg) were harvested on the farm Brakkekuil (34°18'24.0"S and 20°49'3.9"E). The farm is situated near Witsand in the Western Cape province of South Africa. The animals were harvested according to standard operating procedures (SU-ACUM14-001SOP - Stellenbosch University Animal Care and Use Committee) in May 2014 (winter in South Africa). The animals were harvested using the same procedure as detailed in *Chapter 4*.

Muscle fabrication

The muscles were fabricated using the same methodology as detailed in *Chapter 4*.

Colour stability trial

The colour stability trial was conducted over an eight day period with samples being taken at six time periods: day zero (D0); day one (D1); day two (D2); day four (D4); day six (D6); and day eight (D8). The samples were stored in a cold room at 2±0.60°C under fluorescent lights (OSRAM L58W/640, Energy saver, Cool White, 4600 Lumen, 4000°K, 65 CRI) for the duration of the trial.

Surface colour attributes

1. *Instrumental colour measurement*

The surface colour of the steaks was measured on D0, D1, D4, D6 and D8 using the same methodology as detailed in *Chapter 3*.

2. *R (630/580)*

R (630/580) of the steaks was measured on D0, D1, D4, D6 and D8 using the same methodology as detailed in *Chapter 4*.

Surface myoglobin redox forms

The surface Mb redox forms were measured on D0, D1, D4, D6 and D8 using the same methodology as detailed in *Chapter 3.*

Biochemical attributes

1. *pH*

The pH of the meat samples were determined at each time point using the same methodology as detailed in *Chapter 4.*

2. *Oxygen consumption (OC)*

The OC was measured at three time points: D0; D4; and D8, using the same methodology as detailed in *Chapter 4.*

3. *Metmyoglobin reducing activity (MRA)*

The MRA was measured at three time points: D0; D4; and D8, using the same methodology as detailed in *Chapter 4.*

4. *Lipid oxidation*

Lipid oxidation was measured for all time periods (D0, D1, D4, D6 and D8) using the same methodology as detailed in *Chapter 3.*

5. *Iron quantification*

a. *Heme iron*

Heme iron was determined in duplicate on D0 using the same methodology as detailed in *Chapter 4.*

b. *Total iron*

Total iron was determined on D0 using the same methodology as detailed in *Chapter 4.*

c. *Non-heme iron*

Non-heme iron was calculated using the same methodology as detailed in *Chapter 4.*

6. *Total myoglobin concentration*

The total Mb concentration was quantified from samples taken on D0 using the same methodology as detailed in *Chapter 4.*

Statistical analysis

Mixed model repeated measures ANOVA was used to investigate the differences of measurements between muscles taking into account gender and time effects. Thus gender, muscle and time were treated as fixed effects, and animal nested in gender as random effect. For post hoc testing, the Fisher least significant difference (LSD) test was used. Correlation analyses were done using Pearson correlation. A 5% significance level ($P \leq 0.05$) was used as guideline for significant effects and correlations.

RESULTS

All the interactions between the main effects for the various analyses are shown in Table 1. Where applicable, only the significant interactions and significant individual main effects will be reported and discussed further.

Table 1 The *P*-values[1] indicating the impact of gender, muscle and time on the various colour stability attributes measured for springbok meat

Attributes	GxMxT[2]	MxT[3]	GxT[4]	GxM[5]	Gender	Muscle	Time
*L**	0.695	**0.004**	0.116	0.077	0.139	**0.000**	**0.000**
*a**	**0.023**	**0.000**	**0.036**	**0.001**	0.850	**0.000**	**0.000**
*b**	0.454	**0.000**	0.567	0.069	0.202	**0.000**	**0.000**
Chroma	0.073	**0.000**	0.154	**0.010**	0.338	**0.000**	**0.000**
Hue	**0.011**	**0.000**	**0.008**	0.845	0.521	**0.000**	**0.000**
R (630/580)	0.399	**0.000**	**0.022**	0.912	0.114	**0.000**	**0.000**
Deoxymyoglobin (%)	0.090	**0.000**	0.296	0.727	0.084	**0.000**	**0.000**
Oxymyoglobin (%)	0.324	**0.000**	0.431	0.681	**0.045**	**0.000**	**0.000**
Metmyoglobin (%)	0.740	**0.000**	0.322	0.657	0.915	**0.000**	**0.000**
Ultimate pH	0.442	**0.000**	0.555	0.902	0.637	**0.000**	**0.000**
Metmyoglobin reducing activity	0.079	0.336	**0.039**	0.501	0.250	**0.000**	**0.000**
Oxygen consumption	0.343	0.858	0.076	0.612	0.443	0.121	0.164
Lipid oxidation	0.940	**0.000**	0.914	0.334	**0.027**	**0.000**	**0.000**
Total iron				0.370	0.704	**0.000**	
Heme iron				0.694	**0.042**	0.187	
Non-heme iron				0.325	0.223	**0.003**	
Total myoglobin				0.187	0.551	**0.024**	

[1]*P*-values in bold indicate a significant interaction/difference at $P \leq 0.05$.
[2]Interaction between gender, muscle and time.
[3]Interaction between muscle and time.
[4]Interaction between gender and time.
[5]Interaction between gender and muscle.

Surface colour attributes

A significant interaction between muscle and time (MxT) was observed for the *L** values whilst gender had no effect (Table 1). A similar trend was observed for all the muscles; an initial (D0 to D1) increase ($P \leq 0.05$) in *L** values, followed by a temporal decrease ($P \leq 0.05$) (Fig. 1). The *L** values for the LTL and BF plateaued on D4 and D6, respectively. The IS had the highest values ($P \leq 0.05$) for the duration of the trial and the LTL the lowest ($P \leq 0.05$).

A significant interaction between gender, muscle and time (GxMxT) was observed for the *a** values (Table 1). A similar trend was observed between the male and female IS, and the male and female LTL and BF; the IS values remained relatively constant over time, whereas those of the LTL and BF decreased ($P \leq 0.05$) (Fig. 2). The figures for the male and female animals have been split into two separate graphs for ease of reading. On D0, the

female LTL and IS did not differ from each other (P>0.05), whereas the BF differed (P≤0.05) from both. The same trend was observed for the male muscles. Furthermore, the female BF values did not differ from any of the male muscles on D0 whilst the female IS and LTL values did not differ from their male counterparts for any of the days. The female BF had lower values (P≤0.05) than the male BF on D6 and D8. On D8, the male and female IS have the highest values (P≤0.05) and the male LTL, BF and female LTL have the lowest values (P≤0.05).

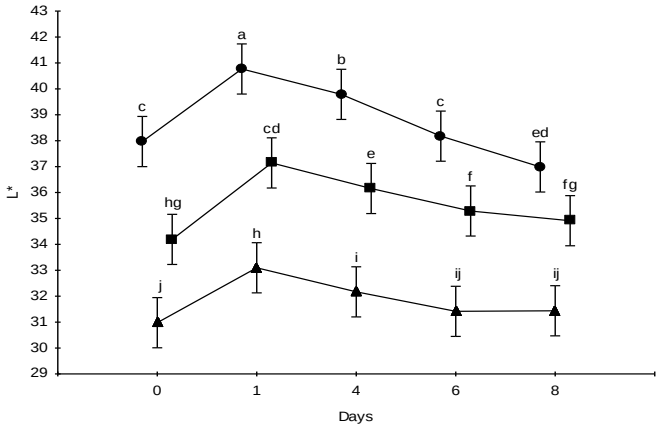

Fig. 1. The temporal changes in *L** (with standard error bars) for three springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0.60°C. Means in figures with different letters differ significantly (P≤0.05).

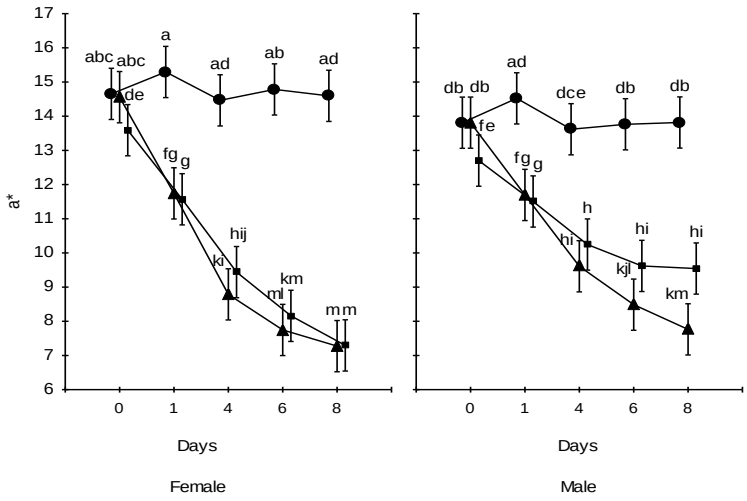

Figure 2 The temporal changes in a* (with standard error bars) for three female and male springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0.60°C. Means in figures with different letters differ significantly (P≤0.05).

A significant MxT interaction was observed for the *b** values whilst gender had no effect (Table 1). Initially (D0), the IS had the highest values (P≤0.05), whilst the LTL and BF did not differ (P>0.05) (ure3). An increase (P≤0.05) was observed for the IS and BF from D0 to D1,

whereas no such increase (P>0.05) was observed for the LTL. A decrease (P≤0.05) in b^* values was observed for the LTL and BF from D1 to D4, where after the values plateaued (P>0.05). A decrease (P≤0.05) was also observed for the IS from D4 but the values only plateaued (P>0.05) after D6. All the muscles differed significantly on D8, with the IS having the highest b^* value (P≤0.05) and the LTL the lowest (P≤0.05).

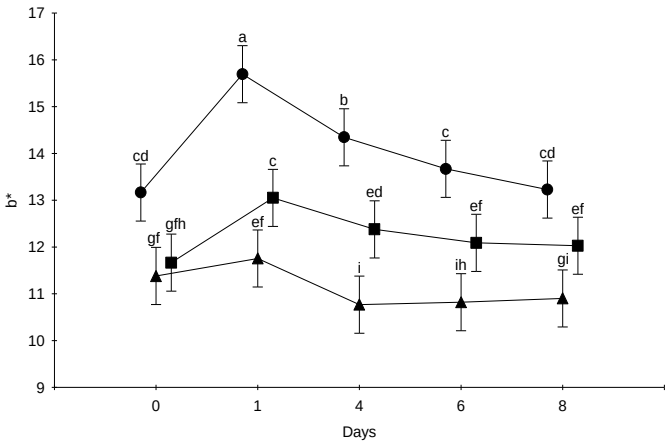

Figure 3 The temporal changes in b^* (with standard error bars) for three springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0. 60°C. Means in figures with different letters differ significantly (P≤0.05).

A significant gender and muscle (GxM) and MxT interaction was observed for the chroma values (Table 1). The GxM interaction showed that the chroma values of the male and female IS, and the male and female LTL did not differ significantly, whereas the female BF had significantly lower chroma values in comparison to the male BF (Table 2). The female and male LTL and female BF did not differ in chroma values. The IS of both genders had the highest values (P≤0.05), and the female and male LTL and female BF the lowest (P>0.05). Initially (D0), for the GxT interaction, the IS had the highest values (P≤0.05) and the LTL and BF the lowest values (P≤0.05) (Fig. 4). A similar trend was observed for the a^* values and chroma values. The IS had the highest values (P≤0.05) for the duration of the trial. The IS values remained relatively constant (P>0.05) for the duration of the trial except for a spike (P≤0.05) on D1. A temporal decrease (P≤0.05) in chroma values was observed for the LTL and BF, with the values plateauing after D6. On D8, the IS had the highest values (P≤0.05) and the LTL the lowest (P≤0.05).

A significant GxMxT interaction was observed for the hue values (Table 1). The figures for the male and female animals have been split into two separate graphs for ease of reading. Similar temporal trends were observed for the male and female hue values with slight differences being observed between the female and male LTL and BF (Fig. 5). The male and female IS, and the male and female LTL muscles did not differ (P>0.05) for any of the days.

The female BF differed ($P \leq 0.05$) from the male BF on D0 and D1, with the values being higher for the male muscles. For both the female and male IS, an increase ($P \leq 0.05$) was observed from D0 to D1, followed by a temporal decrease ($P \leq 0.05$). For the LTL and BF of both genders, a temporal increase ($P \leq 0.05$) was observed. Initially (D0), the female IS and male IS and BF had the highest ($P \leq 0.05$) hue values and the female LTL and BF and male LTL the lowest ($P \leq 0.05$). On D8, the LTL and BF of both genders had the highest ($P \leq 0.05$) values and the IS the lowest ($P \leq 0.05$).

Table 2 The average chroma values (with standard error bars) for the male and female springbok muscles, *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL) and *biceps femoris* (BF), stored at 2±0.60°C. Means in rows and columns with different superscripts differ significantly ($P \leq 0.05$)

Muscle	Gender		SEM
	Female	Male	
IS	20.53^{a}	19.61^{a}	0.356
LD	14.95^{c}	15.36^{c}	0.356
BF	15.40^{c}	16.83^{b}	0.356

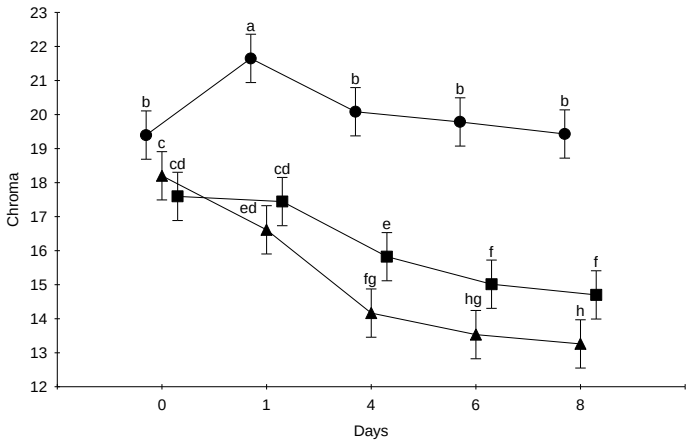

Figure 4 The temporal changes in chroma (with standard error bars) for three springbok muscles, (●) *infraspinatus* (IS), (▲) *longissimus thoracis et lumborum* (LTL) and (■) *biceps femoris* (BF), stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant gender and time (GxT) and MxT interaction was observed for the R (630/580) values (Table 1). For the GxT interaction a similar temporal trend was observed for the male and female muscles (Fig. 6a). The female and male muscles had the same values for all time points except D0, where the female muscles had significantly higher values. For the MxT, a temporal decrease in R (630/580) values for all the muscles was observed (Fig.

6b). Initially (D0), the IS and LTL had the highest values ($P \leq 0.05$) and the BF the lowest ($P \leq 0.05$). The IS maintained the highest values ($P \leq 0.05$) from D1 to D8. The LTL and BF values do not differ ($P > 0.05$) from D1 to D8, with the values plateauing on D6 ($P > 0.05$).

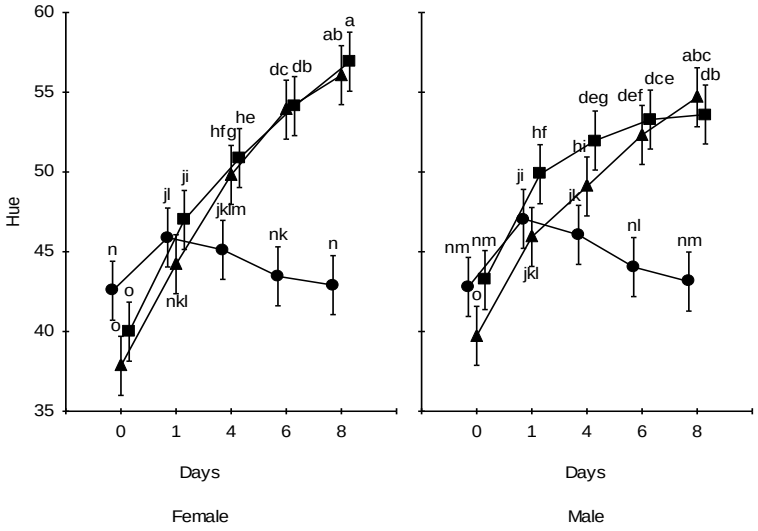

Fig. 5. The temporal changes in hue (with standard error bars) for three female and male springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

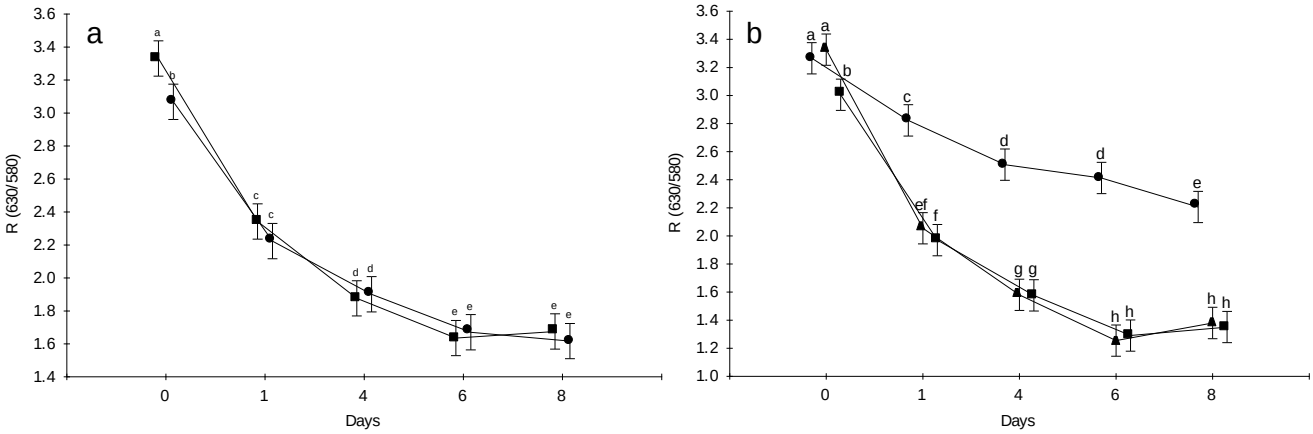

Figure 6 The temporal changes for the (a) (■) female and (●) male (with standard error bars), and (b) R (630/580) (with standard error bars) for three springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

Surface myoglobin redox forms

A significant MxT interaction was observed for the percentage deoxymyoglobin (%DMb) values whilst gender had no effect (Table 1). A temporal decrease in %DMb values was observed for all the muscles (Fig. 7a). No significant differences ($P > 0.05$) were observed

between the muscles from D0 to D4. From D6 to D8, the IS had the highest %DMb values ($P \leq 0.05$). The LTL and BF had similar values ($P > 0.05$) on D6, with the BF having the lowest ($P \leq 0.05$) %DMb values on D8.

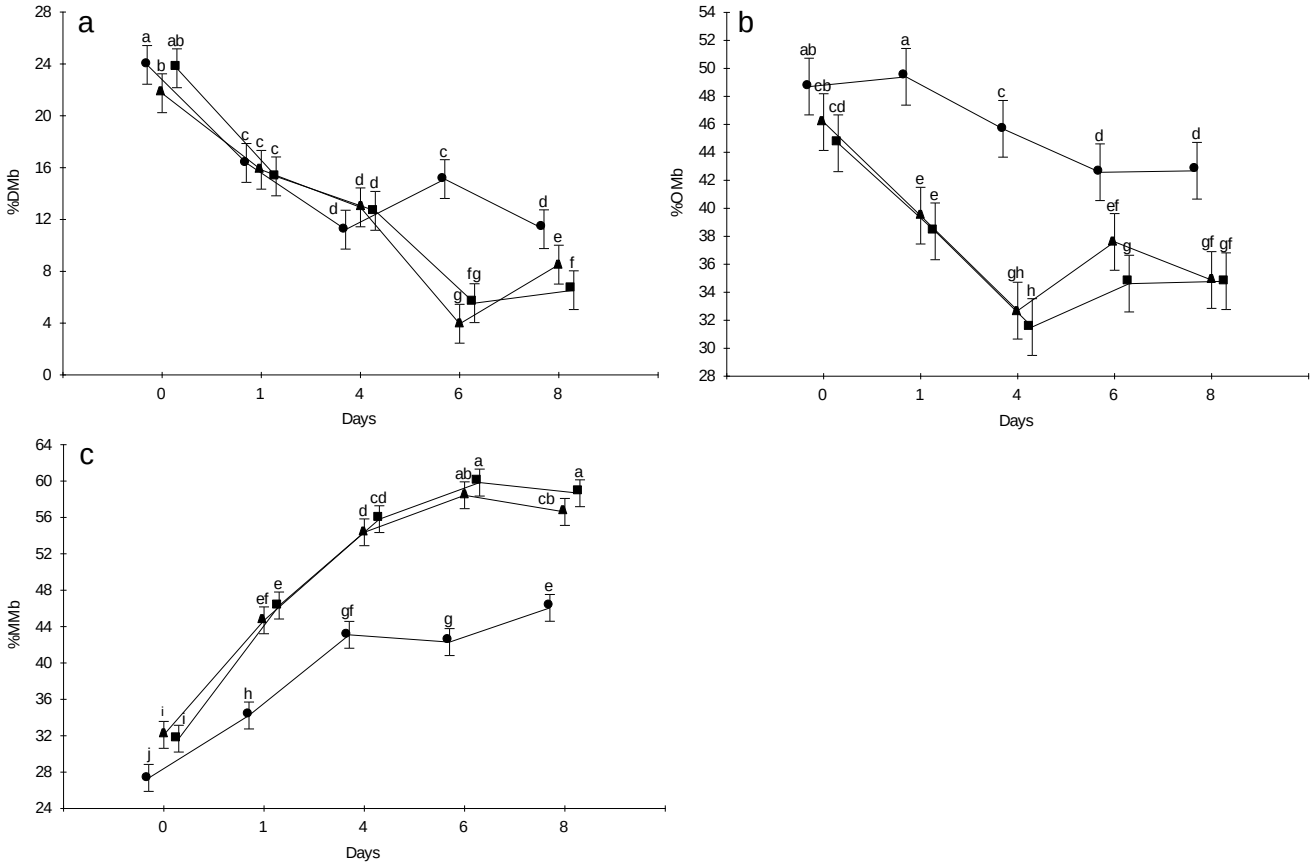

Figure 7 The temporal changes for the (a) %DMb, (b) %OMb, and (c) %MMb, (with standard error bars) for three springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant MxT interaction and a gender effect were observed for the percentage oxymyoglobin (%OMb) values (Table 1). A temporal decrease in %OMb values was observed for all the muscles (Fig. 7b). Initially, the IS had the highest values and did not differ significantly from the LTL, and the BF had the lowest values and also did not differ significantly from the LTL. The IS had the highest values ($P \leq 0.05$) from D1 to D8. The LTL and BF had similar values ($P > 0.05$) for most of the trial, except on D6, where the LTL had higher values

(*P*≤0.05). The values for the muscles plateaued (*P*>0.05) from D6. The gender effect indicated that the female muscles (40.85±0.372) had higher values in comparison to the male muscles (39.65±0.372).

A significant MxT interaction was observed for the %MMb values whilst gender had no effect (Table 1). A temporal increase in %MMb values was observed for all the muscles (Fig. 7c). The IS had the lowest %MMb values (*P*≤0.05) for the duration of the trial. A similar temporal trend was observed for the LTL and BF, with their values only differing on D8, where the BF had higher values (*P*≤0.05) than the LTL.

Biochemical attributes

A significant MxT interaction was observed for the pH values whilst gender had no effect (Table 1). A temporal increase (*P*≤0.05) in pH values was observed for all the muscles (Fig. 8). The IS had the highest pH values (*P*≤0.05) for the duration of the trial. A similar temporal trend was observed for the LTL and BF and their values did not differ (*P*>0.05) on any of the days.

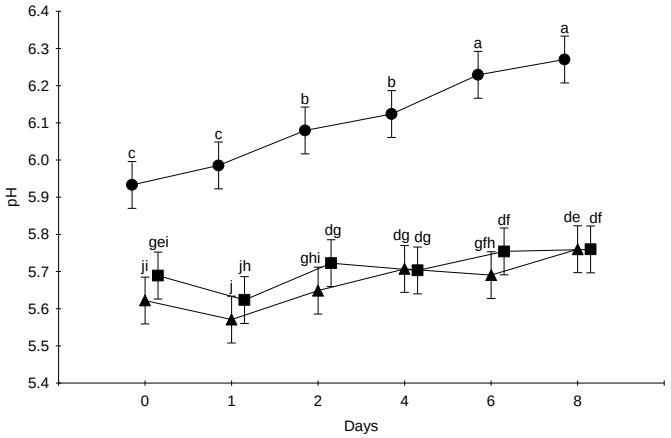

Figure 8 The temporal changes in ultimate pH (with standard error bars) for three springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0.60°C. Means in figures with different letters differ significantly (*P*≤0.05).

A significant GxT interaction and muscle effect was observed for the MRA values (Table 1). An increase (D0 to D4) (*P*≤0.05), followed by a decrease (D4 to D8) (*P*≤0.05) was observed for the male MRA values (*P*>0.05) (Fig. 9). No significant difference was observed between D0 and D8. No initial increase (*P*>0.05) was observed for the female muscles but a significant decrease was observed from D4 to D8. The female and male muscles only differed on D8. The muscle effect indicated that the IS had the highest (*P*≤0.05) MRA values and the LTL and BF the lowest (*P*≤0.05) (Table 3).

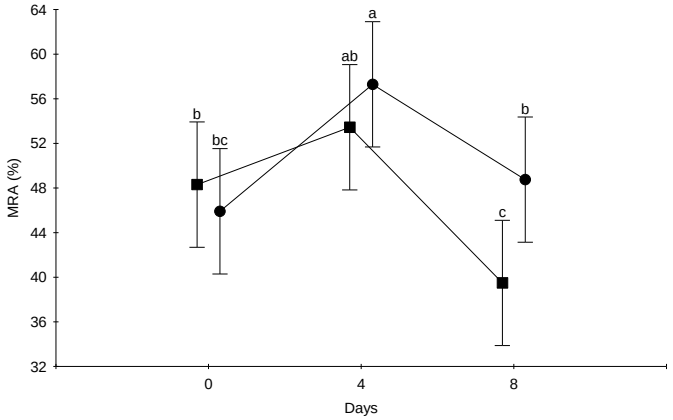

Figure 9 The temporal changes in metmyoglobin reducing activity (MRA) (with standard error bars) for (■) female and (●) male springbok muscles stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

No significant interactions or differences were observed for the OC values (44.70±1.756) (Table 1).

A significant MxT interaction was observed for the TBARS values whilst gender had no effect (Table 1). The IS has the lowest TBARS values ($P \leq 0.05$) for the duration of the trial (Fig. 10). The LTL and BF had similar temporal trends. The LTL had higher TBARS values ($P \leq 0.05$) on D0 and lower values ($P \leq 0.05$) on D4 and D8 in comparison to the BF.

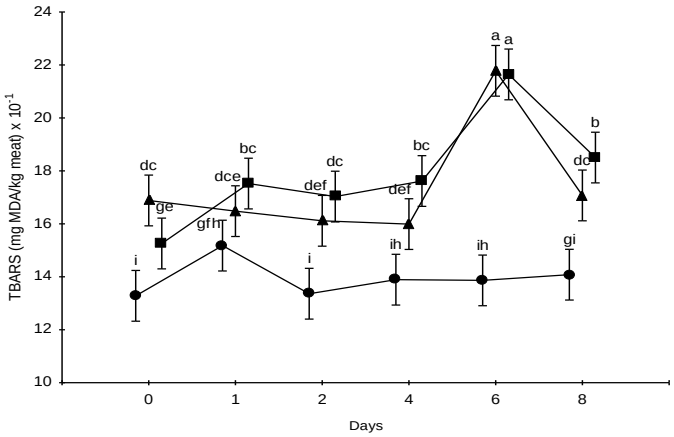

Figure 10 The temporal changes in TBARS (with standard error bars) for three springbok muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2±0.60°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant muscle effect was observed for the total and non-heme iron concentrations whilst no gender was observed (Table 1). The LTL and BF had the highest ($P \leq 0.05$) and the IS the lowest total and non-heme iron concentrations (Table 3). A significant gender effect was observed for the heme iron concentrations but no muscle effect (Table 1),

with the female muscles having significantly higher heme iron concentrations than the male muscles.

A significant muscle effect was observed for the total Mb concentration but no gender effect was observed (Table 1).The LTL and IS differed ($P \leq 0.05$) in total Mb, with the LTL having the highest and the IS the lowest values (Table 3). The BF differed from neither ($P > 0.05$).

Table 3 The means and standard error of the mean (SEM) of the metmyoglobin reducing activity (MRA), total iron, heme iron, non-heme iron and total myoglobin (Mb) for the IS, LTL and BF of springbok meat

Attribute	IS[1]	LTL[2]	BF[3]	SEM
MRA (%)	59.65[a]	43.16[b]	43.80[b]	2.184
Total iron (µg/g meat)	28.04[b]	33.83[a]	31.96[a]	0.854
Non-heme iron (µg/g meat)	10.10[b]	15.23[a]	14.89[a]	1.089
Total Mb (mg/g meat)	8.01[b]	8.59[a]	8.21[ab]	0.202

[a-c]Means in rows with different superscripts differ significantly ($P \leq 0.05$).
[1]IS - *infraspinatus.*
[2]LTL - *longissimus thoracis et lumborum.*
[3]BF - *biceps femoris*

DISCUSSION

Surface colour stability

The *L** values indicate that the IS was the lightest/brightest followed by the BF, with the LTL being the least light/bright (Fig. 1). These results are consistent with those in *Chapter 4* where a similar sequence (IS>BF>LTL) was noted for the corresponding blesbok (*Damaliscus pygargus phillipsi*) muscles. Other researchers have also observed a similar sequence for the corresponding beef muscles (King *et al.,* 2011). Although comparisons of the *L** values of the 19 bovine muscles measured by McKenna *et al.* (2005) (which include the BF, IS and LTL) were not specifically given, the *L** values for the LTL was noted as being the lowest, which is in agreement with the results from this study. Furthermore, the *L** values for the blesbok muscles (*Chapter 4*) fell in similar ranges as those for the corresponding muscles measured in this study. In contrast, the *L** values for the IS (46.3-47.2), LTL (39.8-43.0) and BF (42.6-45.4) of beef noted by King *et al.* (2011) where considerably higher than those for the muscles in this study, indicating that beef is lighter and brighter in colour than springbok. Similarly, beef was found to be lighter in comparison to eland (*Taurotragus oryx*) (Bartoň *et al.,* 2014), bison (*Bison bison*) (Koch *et al.,* 1995), reindeer (*Rangifer tarandus*) and caribou (*Rangifer tarandus*) (Rincker *et al.,* 2006), and red deer (*Cervus elaphus*) (Farouk *et al.,* 2007). These

can be attributed to species differences and is most likely caused by the higher Mb content (Vestergaard *et al.,* 2000; Díaz *et al.,* 2002; Kritzinger *et al.,* 2003; Daszkiewicz *et al.,* 2011), lower quantities of intramuscular fat (IMF) (Hoffman *et al.,* 2005) and differences in muscle fibre types (Curry *et al.,* 2012; North & Hoffman, 2015) found in game meat and venison in comparison to domestic red meat species. Rincker *et al.* (2006) noted that the Mb concentrations of beef (7.29 $mg.g^{-1}$) was significantly lower than the reindeer (9.79 $mg.g^{-1}$) and the caribou (8.59 $mg.g^{-1}$). In addition, impala (*Aepyceros melampus)* was found to have a higher Mb content (7.25-7.50 $mg.g^{-1}$) than that of beef (5.80 $mg.g^{-1}$) and was stated as the reason for the darker colour of the impala meat (Hoffman *et al.,* 2005). The total Mb concentration of the muscles in this study are higher than those reported by Hoffman *et al.* (2005) for beef and could thus be the reason for the lower *L** values observed for the muscles in this study compared to those noted by King *et al.* (2011). Interestingly, an initial decrease in *L** values for LTL and BF, and no change for the IS of blesbok was noted (D0 to D1) (*Chapter 4*), whereas an initial increase was noted for the muscles from this study. After D4, the muscles from both species exhibited similar temporal trends. The differences observed can most likely be attributed to species differences. Despite the *L** values being lower for springbok in comparison to beef, *L** values seems to be a poor colour stability indicator. Muscles in this study with similar colour stabilities (LTL and BF) had significantly different temporal *L** values. Other researchers have also noted the poor correlation between *L** values and muscle colour stability (McKenna *et al.,* 2005). This poor correlation is substantiated by poor correlations between *L** and colour stability determinants such as the K/S ratio (McKenna *et al.,* 2005) and R (630/580) values (*Chapter 4*). Similarly, a poor correlation between the *L** values and R (630/580) values (r=0.25; $P \leq 0.05$) were noted for the muscles in this study. In addition, muscles with high and low pH values are generally darker (lower *L** values) and lighter (higher *L** values), respectively (Lawrie & Ledward, 2006). This relationship between the lightness of the muscle and pH was not observed in this study as the muscle with the highest pH (Fig.8), the IS, was also the lightest. This relationship was also not noted for the blesbok muscles (*Chapter 4*).

The surface colour attributes, *a** (r=0.75; $P \leq 0.05$), chroma (r=0.68; $P \leq 0.05$), and R (630/580) (r=0.82; $P \leq 0.05$), commonly used to represent the redness/concentration of OMb at the surface of meat, all had moderate to strong correlations with %OMb. The *a** values also correlated strongly to both chroma (r=0.94; $P \leq 0.05$) and R (630/580) (r=0.85; $P \leq 0.05$). In addition, chroma and R (630/580) (r=0.71; $P \leq 0.05$) also correlated strongly. These correlations enforce the relationship between these surface colour attributes and their aptness to indicate the redness/concentration of OMb at the surface of meat. Similarly, the strong correlation noted between the hue and %MMb values (r=0.83; $P \leq 0.05$) enforces the use of

hue to indicate browning or MMb accumulation at the surface of meat. Almost identical correlation values where noted for the blesbok muscles (*Chapter 4*). In contrast, gender was found to play a more significant role in the surface colour attributes and surface Mb redox forms in springbok than in blesbok, where no gender interactions were observed for the surface colour stability attributes (*Chapter 4*). Despite the gender interactions observed, similar temporal trends for all the muscles were observed for the blesbok (*Chapter 4*) and springbok for all the surface colour attributes (Figs. 2, 4b, 5 & 6b) and surface Mb redox forms (Figs.7b & 7c).

The results for the *a** values (Fig. 2) indicated that the IS was the most colour stable of the three muscles for both genders as it retained the highest *a** values for the duration of the trial in comparison to the LTL and BF. The higher colour stability of the IS was reiterated by the results observed for the chroma (Fig. 4), hue (Fig. 5), R (630/580) (Fig. 6), %OMb (Fig. 7b) and %MMb (Fig. 7c). The colour stabilities of the LTL and BF were not as distinct as that of the IS. At first glance it would seem that the LTL and BF have similar colour stabilities. However, gender variation for these muscles, particularly for the BF, makes it more difficult to determine the colour stabilities of these two muscles. Several anomalies also appear to exist for the results of the various surface colour stability attributes. The colour stabilities of the springbok muscles are similar to those for blesbok (*Chapter 4*). Conversely, McKenna *et al.* (2005) noted that the IS, BF and LTL had “very low”, “low” and “high” colour stabilities, respectively. In agreement, King *et al.* (2011) noted similar colour stabilities for the IS, LTL and BF of beef. These observational differences may be attributed to processing and species differences, which may have significant consequences for the processing of springbok meat in comparison to beef.

With regard to the gender differences observed for the *a** (Fig. 2), chroma (Table 2) and hue values (Fig. 5), it appears that the only minor temporal variations occur for the IS and LTL, whereas the BF seems to be the most affected by gender. The fact that gender variation was more prevalent in the BF in comparison to other muscles may suggest that colour stability is not only muscle specific but muscle and gender specific. However, the R (630/580) (Fig. 6) and %OMb results indicate that all muscle were influenced by gender differences. Thus discrepancies exist regarding the influence of gender on surface colour stability attributes for springbok meat. The *a** results suggests that the male BF may be more colour stable than the female BF (higher *a** values for D6 and D8), with the male LTL and BF and female LTL having similar colour stabilities. It would appear that the chroma values (Fig. 4) also suggest that the male BF is more colour stable than the female BF and LTL from both genders (higher chroma values). However, the higher chroma values cannot, in this case, be interpreted alone. When higher chroma values are accompanied by higher hue angle values, as is the case with the BF (Fig. 7c), it indicates that the muscle is more saturated (higher chroma values) brown

in colour (higher hue values) i.e. the muscle is browner in colour in comparison to the LTL, which has lower chroma values in comparison to the BF and therefore is less saturated brown in colour. The LTL thus appears to be more colour stable than the BF. In particular, the male BF may be less colour stable than the male LTL and female LTL and BF as it has higher overall chroma values (Table 2). These results directly contradict those of the *a** values, which indicated the higher colour stability of the male BF. The %OMb results indicate that all the female muscles had higher values in comparison to the male muscles indicating that the female muscles may be more colour stable than their male counterparts. The %OMb results also indicate that, regardless of the gender, the LTL and BF had similar colour stabilities (Fig. 7b). As with the %OMb, the R (630/580) values (Fig. 6a) also indicated a gender difference for all muscle. However, the gender difference was only noted for D0 and is thus not indicative of differences in colour stability but only that the female muscles were initially redder than their male counterparts. As with the %OMb values, the R (630/580) values indicated similar colour stabilities for the BF and LTL. The %MMb values (Fig. 7c) also indicated similar colour stabilities for the BF and LTL, with the D8 values possibly indicating that the LTL was more colour stable than the BF (significantly lower hue values for the LTL in comparison to the BF). Interestingly, no gender differences were observed for the %MMb, which would be expected considering the gender differences observed for the majority of the surface colour attributes, specifically the %OMb values. However, the gender effect for the %OMb values was not strongly significant (P=0.045) (Table 1) and may explain the lack of gender differences observed for the %MMb values. In addition, it should be noted that no genders differences in colour stability was visually perceived for the duration of the trial for any of the muscles. The biological significance of the gender differences for the colour stability attributes is thus highly questionable and requires additional research. Furthermore, the colour stability differences noted for the LTL and BF for some of the attributes was also not visually perceived. The colour stability of the muscles thus seems to be in descending order of IS>LTL=BF. These findings are similar to those for the blesbok muscles (*Chapter 4*), where the IS was also observed to be the most colour stable. In contrast, the LTL of the blesbok was concluded as being more colour stable than its BF.

Meat colour differences between genders is mainly attributed to differences in Mb content, with male animals having higher concentrations resulting from higher activity levels in comparison to their female counterparts (Seideman *et al.,* 1982). These higher concentrations lead to male animals having darker meat in comparison to females. In addition, these higher Mb concentrations may also lead to increased oxidation and reduced colour stability in meat obtained from male animals (Insausti *et al.,* 1999; Farouk *et al.,* 2007; Purchas *et al.,* 2010). However, no gender differences in *L** where observed in this study and the meat from the male animals was thus not darker than that of the meat from female animals.

Furthermore, no gender differences in the Mb concentration was observed in this study (Table 3) which may explain the lack of gender differences observed for the *L** values. The literature regarding the effect of gender on the colour of venison and game meat varies widely (Daszkiewicz *et al.,* 2012). Hoffman *et al*. (2009) observed no differences in the Mb content of male and female kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus)* meat. Hoffman *et al.* (2009) did not however report their findings on *L** but found no genders differences for *a**, chorma and hue, which is in contrast to this study. Gender differences in meat colour have been observed for gemsbok (*Oryx gazella*), with females having lower *L**, hue and chroma values (Hoffman & Laubscher, 2010). These findings contradict the theory that male animals have darker meat than females and also are in contrast to the findings from this study. Gender differences have also been observed for springbok, with females having higher *a** and chroma values due to significantly higher pH values (Hoffman *et al.,* 2007). These findings are in contrast to the current study and may be attributed to seasonal and processing differences. In addition, no gender differences in pH were observed in the current study (Fig. 8). It should be noted that no colour stability studies have previously been conducted on game meat. The results from literature only give initial colour values and were frequently conducted on only one muscle, the LTL, which may also contribute to the differences in the observed results. Several studies on venison have noted no colour stabilities differences between genders (Daszkiewicz *et al.,* 2009; Purchas *et al.,* 2010; Daszkiewicz *et al.,* 2012).

Cut-off values for the consumer acceptability of surface colour have been established for the *a**, R (630/580) and %MMb values. The *a** cut-off values established for venison is 12 (Wiklund *et al.,* 2001). Meat with *a** values above 12 is considered acceptable by consumers in terms of surface colour and values below 12 unacceptable. Assuming that this value can be used for game meat, it would indicate that the surface colour of the IS would remain acceptable for the duration of the trial, whereas the LTL and BF fall below acceptable levels after D1. Similarly, Wiklund and Johansson (2011) found that reindeer venison fell below an *a** value of 12 after approximately 1 day. Conversely, it has been noted that the times after which red deer (*Cervus elaphus*) (Wiklund *et al.,* 2006) and fallow deer (*Dama dama*) (Wiklund *et al.,* 2005) fell below 12 were approximately 70 (±3 days) and 200 hours (±8 days), respectively. These variations in time are possible due to the differences in display temperatures used for the different studies. The display temperature for the reindeer meat was 4°C, whereas the red deer and fallow deer were displayed at -1.5°C; lower storage temperatures increase the colour stability of meat (Lawrie & Ledward, 2006). A cut-off value above 3 has been stablished for R (630/580) (Purchas *et al.,* 2010). Using this value, all the muscle fell below an acceptable level after D1, which is contradictory to the results for the *a** cut-off value and what was visually perceived. Similar results were observed for the blesbok

muscles (*Chapter 4*). When Purchas *et al.* (2010) used this cut-off value for the LTL of red deer venison, it was noted that the cut-off value was reached after 150 min. In comparison, Jacob *et al.* (2007) found that the LTL of lamb only reached the cut-off value after 2.5-3 days. However, different R (630/580) and *a** cut-off values have been observed in literature. Khliji *et al.* (2010) noted that lamb meat colour was considered unacceptable when either the R (630/580) value fell below 3.3 or the *a** value fell below 14.8. Since both the R (630/580) and *a** values vary from literature and an *a** cut-off value of 12 has specifically been determined for venison, it may suggest that these cut-off values are species and perhaps even breed specific. The cut-off values for the %MMb varies in literature from 40% (Filgueras *et al.,* 2010) to 60% (Brooks, 1938). Values above these percentages would be deemed unacceptable in surface colour by consumers. The LTL and BF reached the 40% value on or around D1, whereas the IS reached the 40% value on or around D3. For the 60% cut-off value, all the muscles remained acceptable in colour for the duration of the trial. For both of the suggested cut-off percentages for MMb, the results are contradictory to the results for the *a** cut-off value and what was visually observed. These cut-off values were not established for venison. The cut-off values used were established for beef (60%) and rhea (40%). Thus, as with the R (630/580) cut-off values, the %MMb cut-off values may be species specific. If we use the *a** cut-off point as a reference, as it was found to be the most accurate in determining colour stability of the muscles in relation to what was visually observed, then the cut-off value for springbok should be approximately 2.0 and 50% for the R (630/580) and %MMb, respectively. Furthermore, Morrissey *et al.* (2008) noted that *a** values better reflected consumer preference for meat colour than did the R (630/580) values, which is unexpected since the R (630/580) measures discolouration and the formation of MMb (Hunt, 1980). Thus, if only the *a** cut-off value is used, it reiterates that the IS was the most colour stable of the muscles and that the LTL and BF had similar colour stabilities in terms of consumer preference in muscle colour.

Biochemical attributes influencing surface colour stability

Higher pH values have been noted to delay the oxidation of OMb resulting in increased colour stability, with the opposite being true for low pH values (Gotoh & Shikama, 1974; Ledward, 1985; Gutzke & Trout, 2002). Thus, the significantly higher pH noted for the IS (Fig.8) may have contributed to its higher colour stability in comparison to the LTL and BF. Similarly, the low pH values observed for the LTL and BF could contribute to the low colour stabilities observed for these muscles. Furthermore, the similarities noted in the pH values for the LTL and BF suggest similarities in the colour stabilities of these muscles which reiterates the findings above. Similar results were noted for the blesbok muscles (*Chapter 4*). Although, McKenna *et al.* (2005) also noted significantly higher pH values for the IS of beef in relation to

the LTL and BF, the IS was noted to have a lower colour stability in relation to the LTL and BF, with the LTL having the highest colour stability. These differences may suggest that the influence of pH on colour stability is species specific. The relationship between pH and colour stability may be linked to the relationship between pH and MRA. Various studies have shown that the MRA increases with increasing pH (Cutaia & Ordal, 1964; Stewart *et al.,* 1965; Ledward, 1970). However, despite a moderate correlation between pH and MRA (r=0.46; $P \leq 0.05$), no such relationship was observed in this study.

It has been suggested that higher MRA in meat lead to an increased colour stability in muscles (AMSA, 2012). However, a great deal of debate regarding the influence of MRA on colour stability exists in literature. While some authors feel that it does influence colour stability, other authors argue it has no influence (Ledward, 1971; Atkinson & Follett, 1973; O'Keeffe & Hood, 1982; Renerre & Labas, 1987; Madhavi & Carpenter, 1993). In this study, MRA did appear to influence colour stability. Significantly higher MRA values were observed for the IS (most colour stable) in comparison to the LTL and BF (least colour stable) (Table 3). Furthermore, no differences in MRA were observed for the LTL and BF further echoing the conclusions drawn in the previous section regarding the similarities in colour stabilities between these two muscles. These results are in agreement with those observed for the blesbok muscles (*Chapter 4*), where the IS was also observed to have the highest colour stability and correspondingly, the highest MRA values. Similarly, King *et al.* (2011) noted that muscles with higher colour stabilities had higher MRA values. In contrast, the GxT interaction results for the MRA (Fig. 9) appears to contradict the colour stability results; significantly lower MRA values were observed for the female muscles in comparison to the male muscle on D8, however, higher %OMb values were noted for all the muscles for the duration of the trial. No explanation for this phenomenon could be found. A gender effect was also noted for the blesbok muscles (*Chapter 4*) but due to the lack of other gender effects, it was considered biologically insignificant. Furthermore, due to the contradictions found in literature and the contradictions found in this study, more comprehensive research is required to elucidate the reasons for these differences. In addition, it would be advised that more time points be used to clarify the exact temporal nature of the MRA.

Lipid oxidation accelerates the formation of MMb in meat and consequently decreases its colour stability (Faustman *et al.,* 2010). The TBARS values from this study supports this notion, with the most colour stable muscles, the IS, also having significantly lower TBARS values in comparison to the LTL and BF, which are less colour stable (Fig. 10). The TBARS values for the LTL and BF also reiterates their similarities in colour stability as they have similar temporal TBARS values. It thus seems plausible that the TBARS values for springbok meat can be used as a reliable predictor for its colour stability. In contrast, the TBARS values for the blesbok muscles were not necessarily indicative of colour stability (*Chapter 4*). Similarly,

McKenna *et al.* (2005) also noted that the TBARS values were not indicative of colour stability for beef muscles. It would thus appear that the use of TBARS values as an indicator for the susceptibility of muscle to colour stability may be species specific.

Iron is a pro-oxidant and thus serves as a catalyst of lipid oxidation (Lawrie & Ledward, 2006), which consequently results in a reduction in the colour stability of meat. Both heme and non-heme iron have been implicated in accelerating lipid oxidation (Igene *et al.,* 1979; Chen *et al.,* 1984) and as such the total iron should also be indicative of colour stability. Muscle difference were observed for the total and non-heme iron results (Table 3). These differences reiterated the link between colour stability and iron content; lower total and non-heme iron values were noted for the IS (most colour stable) and similar values for the LTL and BF (similar colour stabilities). In contrast, only the non-heme iron values were found to correlate to the colour stability in the blesbok muscles (*Chapter 4*). Furthermore, no significant positive correlations were observed between the total and non-heme iron, and TBARS as was observed for the blesbok muscles. It could thus be concluded that the use of total and non-heme iron in predicting the colour stability of muscles is species specific. Interesting, unlike the blesbok muscles, no muscle differences were noted for the heme iron. However, as with the blesbok muscles, the heme iron was not found to be indicative of muscle colour stability. Furthermore, the gender differences noted for the heme iron results (female muscle higher content than male muscles) also did not correlate to muscle colour stability. In fact, the results would suggest that heme iron enhances muscle colour stability.

The Mb content of muscles is often implicated in its colour stability, with higher concentrations leading to a decrease in colour stability (Jeong *et al.,* 2009; King *et al.,* 2011). As with the heme iron results, the Mb values were not linked to the muscle colour stability. The BF, which differed significantly from the IS in colour stability but not from the LTL, did not differ significantly from either with regard to its total Mb content. Similarly, the Mb content of the blesbok muscles was also not found to be indicative of muscle colour stability. Other researchers have also noted that the Mb content is not a good indicator of muscle colour stability (Sammel *et al.,* 2002; McKenna *et al.,* 2005; Canto *et al.,* 2015)

CONCLUSION

Based on the results of the surface colour attributes, surface Mb redox forms and biochemical attributes, the IS was determined to be the most colour stable of the three muscles investigated. The LTL and BF were observed to be the least colour stable and have similar colour stabilities. The strong gender influence on the various attributes measured warrants further research as the influence was not visually perceived. The L^* was observed to be a poor predictor of colour stability as was the heme iron and total Mb. In addition, the muscle

and species specificity of the various attributes measured highlights the requirement for employing not only muscle-specific but species specific processing strategies to game meat.

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CHAPTER 6

MUSCLE-SPECIFIC COLOUR STABILITY OF FALLOW DEER (*DAMA DAMA*) MEAT

ABSTRACT

Fallow deer meat still comprises a relatively small proportion of the game meat market in South Africa despite having great market potential. Should this market potential be exploited, the meat quality of South African fallow deer should be investigated to ensure a consistent, desirable, high quality product is delivered to consumers. The objective of this study was to establish the colour stability of the three fallow deer muscles, the *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL) and *biceps femoris* (BF) stored for eight days at 2±0.60°C under aerobic condition. Surface colour attributes surface myoglobin redox forms and biochemical factors influencing colour stability were evaluated at various time intervals. Although significant gender differences in colour stability were observed for several of the attributes measured, these differences were disregarded as no gender differences were visually perceived. It was determined that the colour stability of the muscles was in descending order of IS>LTL>BF. The IS was determined to be colour stable for the duration of the trial (eight days), whereas the LTL and BF remained colour stable for one to two days. The results highlighted the need for muscle-specific processing methods to optimise the colour stability for fallow deer meat.

Keywords Game meat, Ungulate, Myoglobin, Lipid oxidation

INTRODUCTION

Fallow deer (*Dama dama*) are not indigenous to South Africa and, although it is uncertain when they were first introduced to South Africa from Europe, the first record of their occurrence is at Newlands House, Cape Town in 1869 (Mills & Hes, 1997). The fallow deer adapted well to the South African climate and consequently their population numbers grew rapidly (Curry *et al.,* 2012). They have since been translocated to various areas and today they can be found on many game farms as well as free roaming throughout South Africa. Despite the drastic increase in their population numbers, fallow deer meat still comprises a relatively small proportion of the game meat market in South Africa in comparison to springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus pygargus phillipsi*) and kudu (*Tragelaphus strepsiceros*) (Hoffman & Wiklund, 2006). However, fallow deer are one of the most commonly farmed cervid species in Europe, Canada and the US and the popularity of deer farming as a whole is increasing globally (Chakanya *et al.,* in press; Hoffman & Wiklund, 2006). This popularity

may stem from the relatively low input system, in comparison to other species, required to rear deer and the minimal impact their husbandry has on the environment (Volpelli *et al.,* 2003). Thus, market potential exists for the increased production of fallow deer for export and local consumption within South Africa. In order to create a sustainable and reliable fallow deer meat market, products of consistent and high quality need to be delivered to the market (Hutchison *et al.,* 2010). To do this, the factors which affect the quality of fallow deer meat need to be investigated. Although research on the quality of venison originating from farmed fallow deer has been conducted, limited research has been conducted on venison originating from fallow deer in South Africa. Game meat and venison are distinguished from each other as venison commonly refers to meat obtained from cervids in countries outside South Africa, which are increasingly being replaced by domesticated, farmed animals, whereas African game meat is still obtained from wild, free-roaming animals (Hoffman & Wiklund, 2006).

One important quality attribute of fallow deer meat which must be investigated is its colour stability. The colour of meat largely dictates the willingness of a consumer to purchase meat (Faustman & Cassens, 1990; Risvik, 1994; Mancini & Hunt, 2005; Suman *et al.,* 2014). Thus, desirable and consistent meat colour, and prolonged colour stability will result in increased sales resulting in increased revenue for the game meat industry.

The objective of the this study was therefore to determine the colour stability of three fallow deer muscles, the *infraspinatus* (IS), *longissimus thoracis et lumborum* (LTL), and *biceps femoris* (BF), during refrigerated storage (2±0.60°C). The colour stability was determined by evaluating the surface colour attributes, surface Mb redox forms and various biochemical aspects which influence the colour stability of meat over an eight day period.

MATERIALS AND METHODS

Animal harvesting and muscle sample collection

Twelve (six male and six female) mature fallow deer (live weight, bled 51.8 ± 12.16 kg) were harvested according to standard operating procedure (SU-ACUM14-001SOP issued by the Stellenbosch University Animal Care and Use Committee) in June 2014 (winter in South Africa). The animals were harvested on the farm Brakkekuil (34°18’24.0”S and 20°49’3.9”E), Witsand, Western Cape Province of South Africa. The animals were harvested using the same procedure as detailed in *Chapter 4*.

Muscle fabrication

The muscles were fabricated using the same methodology as detailed in *Chapter 4*.

Colour stability trial

The colour stability trial was conducted over an eight day period with samples being taken at six time periods: day zero (D0); day one (D1); day two (D2); day four (D4); day six (D6); and day eight (D8). The samples were stored in a cold room at 2±0.60°C under fluorescent lights (OSRAM L58W/640, Energy saver, Cool White, 4600 Lumen, 4000°K, 65 CRI) for the duration of the trial.

Surface colour attributes

1. *Instrumental colour measurement*

The surface colour of the steaks was measured on D0, D1, D2, D4, D6 and D8 using the same methodology as detailed in *Chapter 3*.

2. *R (630/580)*

R (630/580) of the steaks was measured on D0, D1, D2, D4, D6 and D8 using the same methodology as detailed in *Chapter 4*.

Surface myoglobin (Mb) redox forms

The surface Mb redox forms were measured on D0, D1, D2, D4, D6 and D8 using the same methodology as detailed in *Chapter 3.*

Biochemical attributes

1. *pH*

The pH of the meat samples were determined at each time point using the same methodology as detailed in *Chapter 4.*

2. *Oxygen consumption (OC)*

The OC was measured at three time points: D0; D4; and D8, using the same methodology as detailed in *Chapter 4.*

3. *Metmyoglobin (MMb) reducing activity (MRA)*

The MRA was measured at three time points: D0; D4; and D8, using the same methodology as detailed in *Chapter 4.*

4. *Lipid oxidation*

Lipid oxidation was measured for all time periods using the same methodology as detailed in *Chapter 3.*

5. *Iron quantification*

a. *Heme iron*

Heme iron was determined in duplicate on D0 using the same methodology as detailed in *Chapter 4.*

b. *Total iron*

Total iron was determined on D0 using the same methodology as detailed in *Chapter 4.*

c. *Non-heme iron*

Non-heme iron was calculated using the same methodology as detailed in *Chapter 4.*

6. *Total Mb concentration*

The total Mb concentration was quantified from samples taken on D0 using the same methodology as detailed in *Chapter 4.*

Statistical analysis

Mixed model repeated measures ANOVA was used to investigate the differences of measurements between muscles taking into account gender and time effects. Thus gender, muscle and time were treated as fixed effects, and animal nested in gender as random effect. For post hoc testing, the Fisher least significant difference (LSD) test was used. Correlation analyses were done using Pearson correlation. A 5% significance level ($P \leq 0.05$) was used as guideline for significant effects and correlations.

RESULTS

The interactions between the main effects for all the various analyses are shown in Table 1. Only the significant interactions and significant individual main effects will be reported and discussed further.

Table 1 The *P*-values[1] indicating the impact of gender, muscle and time on the various colour stability attributes measured for fallow deer meat

Attributes	GxMxT[2]	MxT[3]	GxT[4]	GxM[5]	Gender	Muscle	Time
*L**	**0.039**	**0.000**	**0.005**	**0.009**	**0.016**	**0.000**	**0.000**
*a**	**0.004**	**0.000**	**0.000**	0.568	0.140	**0.000**	**0.000**
*b**	**0.003**	**0.000**	**0.003**	0.208	**0.014**	**0.000**	**0.000**
Chroma	**0.002**	**0.000**	**0.001**	0.502	**0.040**	**0.000**	**0.000**
Hue	**0.017**	**0.000**	**0.000**	**0.024**	0.579	**0.000**	**0.000**
R (630/580)	0.399	**0.000**	0.137	0.892	0.053	**0.000**	**0.000**
%DMb	0.141	**0.000**	**0.010**	**0.002**	**0.002**	**0.000**	**0.000**
%OMb	0.384	**0.000**	**0.032**	0.279	**0.004**	**0.000**	**0.000**
%MMb	0.918	**0.000**	**0.036**	0.452	**0.035**	**0.000**	**0.000**
pH_u	0.927	**0.000**	0.824	0.845	0.132	**0.000**	**0.000**
MRA	0.145	0.052	**0.046**	**0.005**	**0.0433**	**0.000**	**0.001**
OC	0.218	**0.014**	0.577	0.140	0.727	**0.001**	**0.000**
Lipid oxidation	0.439	0.173	0.455	0.327	**0.022**	**0.000**	**0.002**
Total iron				0.536	0.594	**0.000**	
Heme iron				0.204	**0.008**	0.180	
Non-heme iron				0.700	0.382	**0.000**	
Total Mb				0.341	0.530	**0.001**	

[1]*P*-values in bold indicate a significant interaction/difference at $P \leq 0.05$.
[2]Interaction between gender, muscle and time.
[3]Interaction between muscle and time.
[4]Interaction between gender and time.
[5]Interaction between gender and muscle.

Surface colour attributes

A significant gender, muscle and time (GxMxT) interaction was observed for the *L** values (Table 1). Initially (D0), the IS has the highest ($P \leq 0.05$) *L** values for both genders (Fig. 1) and these high values were maintained throughout the shelf-life period. However, the male IS had higher values ($P \leq 0.05$) than the female IS from D2 onwards. Also, the initial values for the female IS was similar ($P > 0.05$) to those of the male IS and BF. The initial female LTL and BF values did not differ ($P > 0.05$) from their male counterparts. The female IS followed a similar trend to the male BF, with their values not differing significantly on any of the days. The male BF and LTL differed ($P \leq 0.05$) on all the days, except D0, with the BF having higher ($P \leq 0.05$) values. The female LTL and BF followed a similar trend, with their values not differing significantly on any of the days.

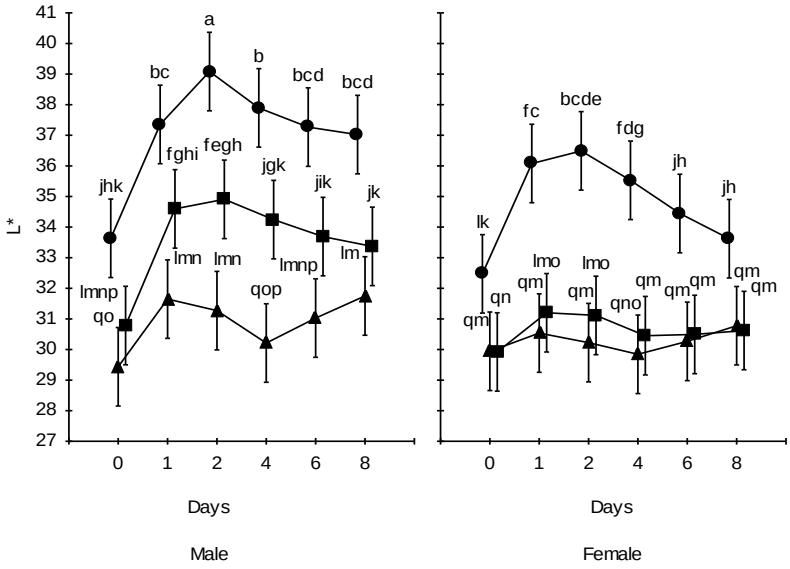

Figure 1 The temporal changes in *L** (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant GxMxT interaction was also observed for the *a** values (Table 1). Initially (D0), the male IS values did not differ from the male LTL or BF, with the LTL having the highest and the BF the lowest values (Fig. 2). The female LTL differed from the female IS and BF on D0, with the LTL having the highest ($P \leq 0.05$) *a** value and the IS and BF the lowest ($P \leq 0.05$). The male IS and LTL values did not differ from that of the female LTL, and the male IS and BF did not differ from the female IS and LTL on D0. A temporal decrease ($P \leq 0.05$) in *a** values was observed for all the muscles, with the least amount of decrease being observed for the IS of both genders. The *a** values of the male IS and female IS plateaued ($P > 0.05$) after D2 and D4, respectively. The IS *a** values of both genders were the highest for most of the trial

and were also similar (P>0.05) except on D8 where the male IS had higher values (P≤0.05) than the female IS. The male LTL and BF a^* values only differed on D0 and D1, with the LTL having higher values (P≤0.05). The female LTL and BF follow a similar temporal trend but differed on D0, D2 and D6, with the LTL having higher a^* values (P≤0.05). The male and female LTL a^* values did not differ significantly for the duration of the trial. The male BF had higher a^* values (P≤0.05) in comparison to the female BF from D4 to D8. On D8, the male IS had the highest value (P≤0.05) in comparison to the male BF and LTL, which did not differ (P>0.05). The same trend was observed for the female muscles. The male LTL and BF did not differ (P≤0.05) from the female LTL but did differ from the female BF (P>0.05), which had the lowest a^* values on D2, D4, D6 and D8.

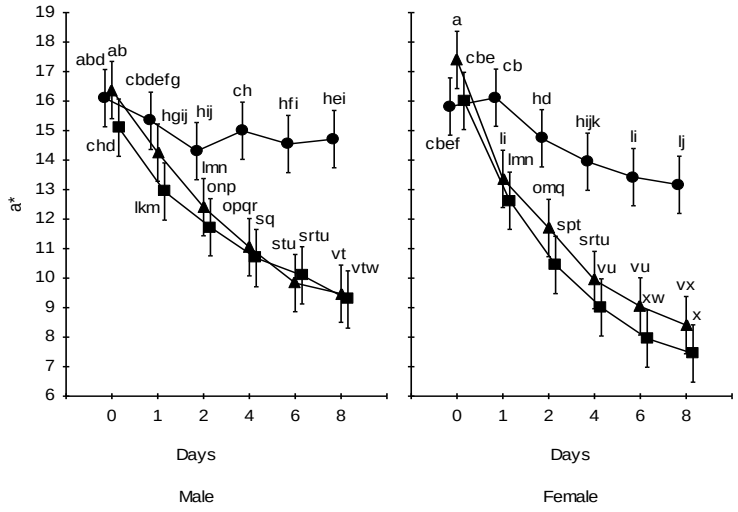

Figure 2 The temporal changes in a^* (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly (P≤0.05).

A significant GxMxT interaction was observed for the b^* values (Table 1). A temporal increase (P≤0.05) in b^* was observed for the male IS, whilst no overall change (P>0.05) was observed for the male BF and female IS, and a decrease (P≤0.05) was observed for the male LTL and female LTL and BF (Fig. 3). Initially (D0), the male BF had the lowest b^* values (P≤0.05) and differed (P≤0.05) from the male IS and LTL. The female IS did not differ (P>0.05) from the female LTL or BF on D0, with the LTL having the highest and the BF the lowest b^* values. An initial increase in b^* values, followed by a subsequent decrease was observed for the male and female IS, with the values for both being the highest from D1 to D8. The female IS b^* values were lower than those of the male from D4 onward. The male LTL and BF b^* values did not differ (P>0.05) on any of the days, except D0; a similar trend was observed for the female muscles. The male LTL and female LTL differed (P≤0.05) on D1 and D2 with the

male LTL having higher *b** values ($P \leq 0.05$). The male and female BF differ ($P \leq 0.05$) on all days, except D1 and D8, with the male BF having higher *b** values ($P \leq 0.05$).

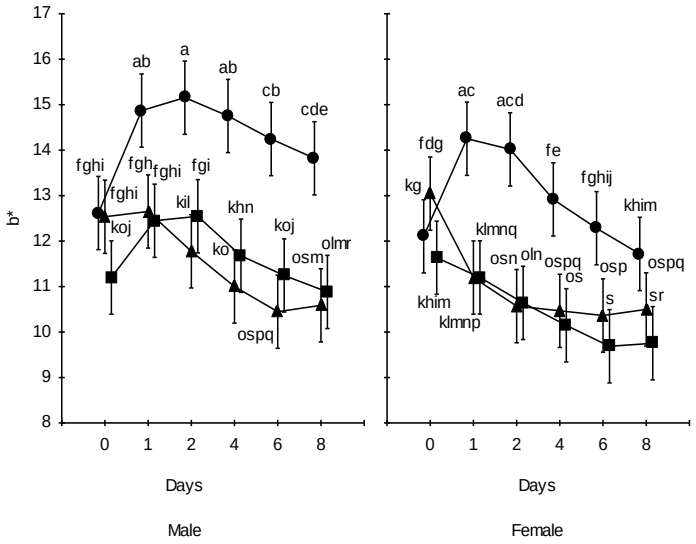

Figure 3 The temporal changes in *b** (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant GxMxT interaction was observed for the chroma values (Table 1). A temporal decrease was observed for all the muscles, except the male IS, for which no overall change was observed (Fig. 4). On D0, the male BF had the lowest chroma value ($P \leq 0.05$) and differed significantly from the male IS and LTL. For the female muscles, the LTL had the highest chroma values ($P \leq 0.05$) and differed from the IS and BF on D0. The female IS had lower chroma values ($P \leq 0.05$) than the male IS from D4 to D8. The IS of both genders had the highest chroma values from D1 to D8 for their respective gender counterparts. The male LTL and BF did not differ from D1 to D8; a similar trend was observed for the female LTL and BF. The male and female LTL differed ($P \leq 0.05$) on D2, with the male LTL having higher chroma values. The male BF had higher chroma values ($P \leq 0.05$) than the female BF from D2 onward.

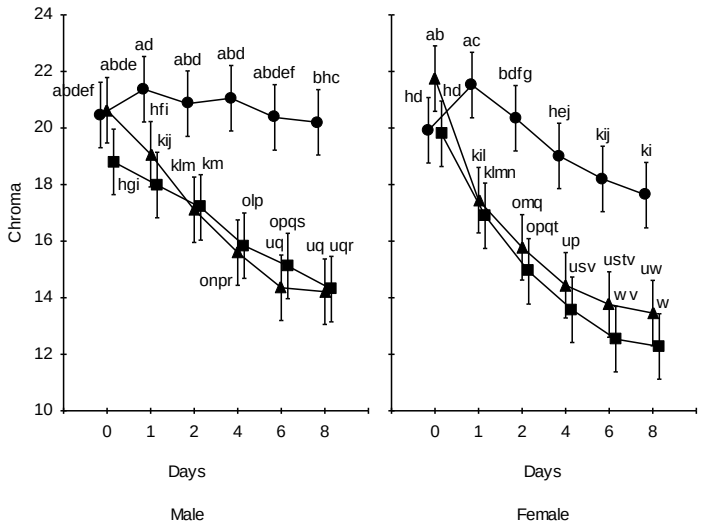

Figure 4 The temporal changes in chroma (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant GxMxT interaction was observed for the hue values (Table 1). A temporal increase in hue values was observed for all the muscles (Fig. 5). Initially (D0), all the muscles did not differ ($P > 0.05$) in hue values. On D1 and D2, the male LTL had the lowest values ($P \leq 0.05$) and differed from the male IS and BF. On D4, the male BF had the highest hue value ($P \leq 0.05$) and differed from the male IS and LTL. On D6 and D8, the male IS had the lowest hue value ($P \leq 0.05$) and differed from the male BF and LTL. On D1, none of the female muscles differed from each other ($P > 0.05$). On D2, the female IS did not differ ($P > 0.05$) from either the female LTL or female BF, with the BF having the highest value. The female IS had the lowest hue values ($P \leq 0.05$) from D4 to D8. The female LTL had lower hue values ($P \leq 0.05$) than the female BF on D4 and D6 but did not differ ($P > 0.05$) from the female BF on D8. The male and female IS differed on D1 and D2, with the male IS having higher hue values. The male LTL only differed from the female LTL on D8, where the male LTL had lower hue values ($P \leq 0.05$). The male BF differed from the female BF on D6 and D8, where the male BF had lower hue values ($P \leq 0.05$).

A significant muscle and time (MxT) interaction was observed for the R (630/580) values whilst gender had no effect (Table 1). A similar temporal decrease ($P \leq 0.05$) was observed for all the muscles, with a more gradual decrease being observed for the IS (Fig. 6). Initially (D0), the R (630/580) values differed for all the muscles, with the LTL having the highest values ($P \leq 0.05$) and the BF the lowest ($P \leq 0.05$). The IS had the highest R (630/580) values from D1 to D8. The BF had the lowest values from D0 to D2, after which the values

did not differ (P>0.05) from the LTL. The IS R (630/580) values, and the LTL and BF values plateaued (P>0.05) after D4 and D6, respectively.

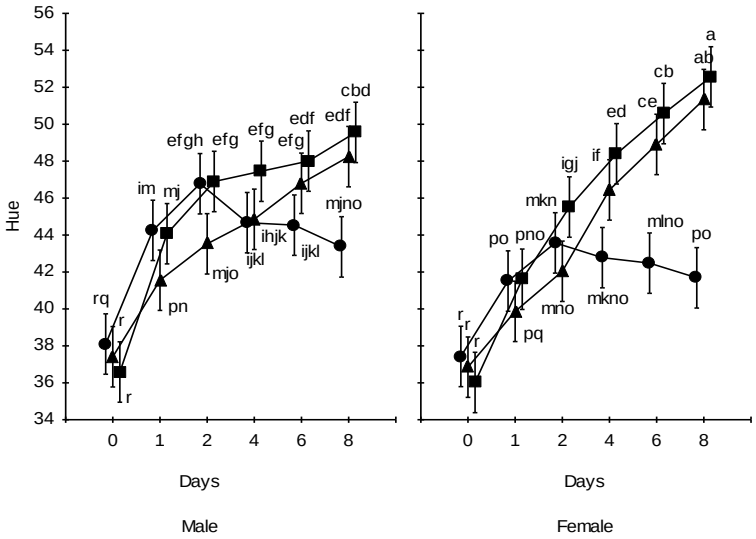

Figure 5 The temporal changes in hue (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly (P≤0.05).

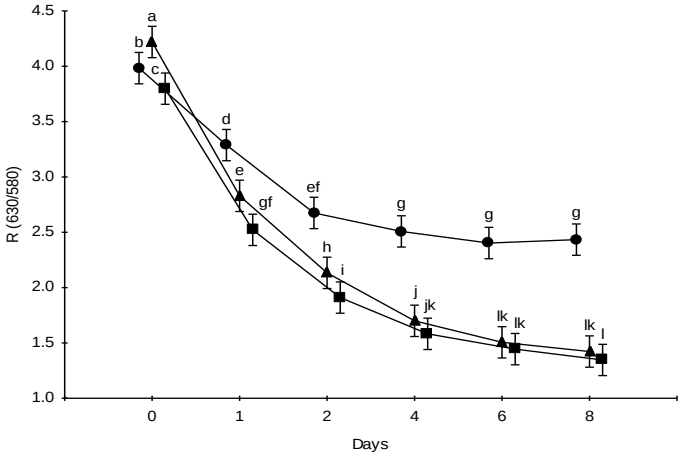

Figure 6 The temporal changes in R (630/580) (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly (P≤0.05).

Surface Mb redox forms

A significant MxT, gender and time (GxT), and gender and muscle (GxM) interaction was observed for the %DMb values (Table 1). For the MxT interaction, a temporal decrease in values was observed for all the muscles. Initially (D0), the %DMb differed (P≤0.05) for all the muscles, with the IS having the highest (P≤0.05) values and the LTL the lowest (P≤0.05) (Fig. 7a). On D1 and D2, the muscles did not differ (P>0.05) in their %DMb. On D4, the LTL did

not differ (P>0.05) from either the IS or BF, with the IS having the highest %DMb and differing (P≤0.05) from the BF with the lowest value (P≤0.05). On D6 and D8, the IS had the highest %DMb which differed (P≤0.05) from the LTL and BF. For the GxT interaction, a temporal decrease (P≤0.05) was observed for both genders (Fig. 7b). Furthermore, the male muscles had higher %DMb for the duration of the trial in comparison to the female muscles. However, the GxM interaction shows that overall the male and female IS did not differ significantly, whereas the LTL and BF for both genders did differ significantly (Fig. 7c).

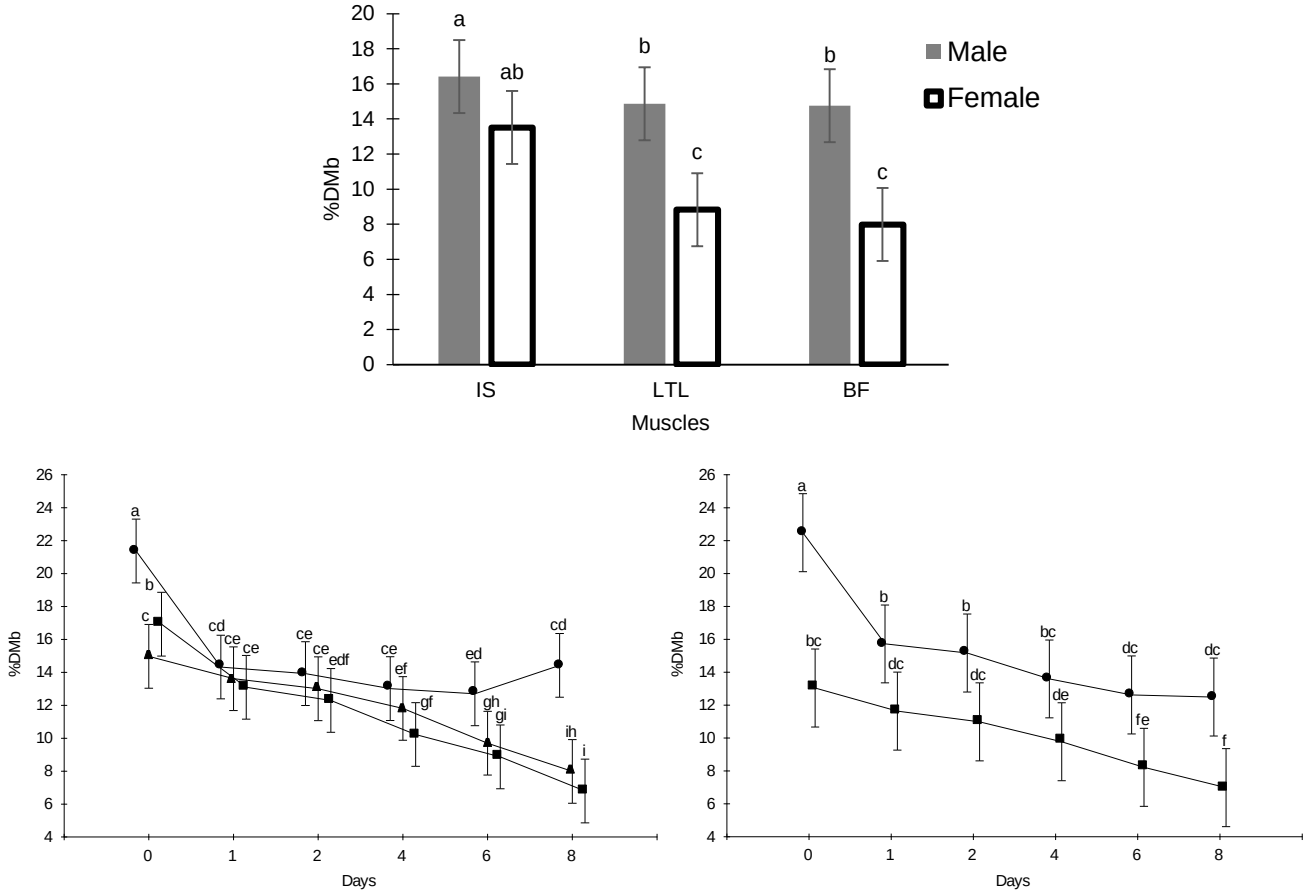

Figure 7 (a) the temporal changes in %DMb (with standard error bars) for three fallow deer muscles, (●) *infraspinatus* (IS), (▲) *longissimus thoracis et lumborum* (LTL) and (■) *biceps femoris* (BF), (b) the temporal changes for the averages of the (●) male and (■) female fallow deer muscles (with standard error bars), and (c) the average %DMb values (with standard error bars) for the male and female fallow deer muscles, and, stored at 2°C. Means in figures with different letters differ significantly (P≤0.05).

A significant MxT and GxT interaction was observed for the %OMb (Table 1). A temporal decrease in %OMb was observed for the for the MxT and GxT interactions (Fig. 8). Initially (D0), for the MxT interaction, the LTL had the highest value (P≤0.05) and the IS and BF the lowest (P≤0.05) (Fig. 8a). From D1 to D8, the IS had the highest %OMb (P≤0.05).

Except for D0 and D2, where the LTL had higher values ($P \leq 0.05$) than the BF, their values did not differ ($P > 0.05$). The %OMb for all the muscle plateaued ($P > 0.05$) after D4. The GxT interaction showed that the female muscles had higher values ($P \leq 0.05$) on D0 and D1, after which the values did not differ ($P > 0.05$) (Fig. 8b).

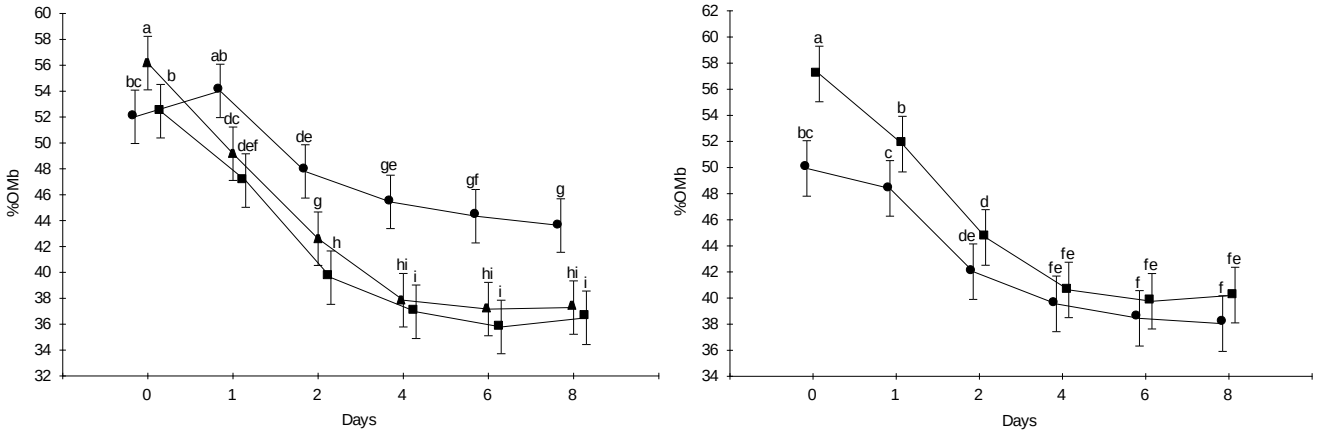

Figure 8 (a) the temporal changes in %OMb (with standard error bars) for three fallow deer muscles, (●) *infraspinatus* (IS), (▲) *longissimus thoracis et lumborum* (LTL) and (■) *biceps femoris* (BF), (b) the temporal changes for the averages of the (●) male and (■) female fallow deer muscles (with standard error bars), stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

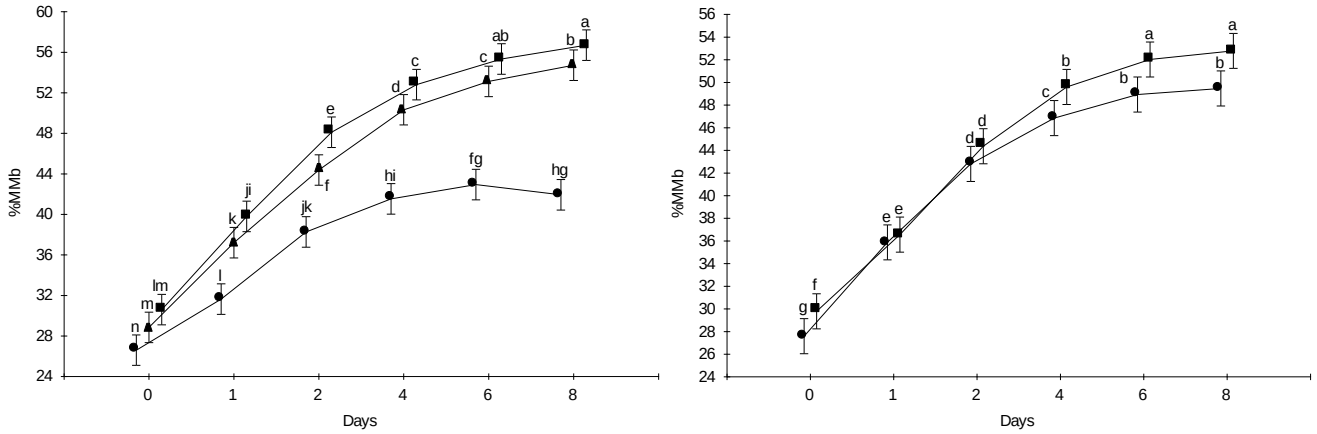

Figure 9 (a) the temporal changes in %MMb (with standard error bars) for three fallow deer muscles, (●) *infraspinatus* (IS), (▲) *longissimus thoracis et lumborum* (LTL) and (■) *biceps femoris* (BF), (b) the temporal changes for the averages of the (●) male and (■) female fallow deer muscles (with standard error bars), stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant MxT and GxT interaction was observed for the %MMb (Table 1). A temporal increase in %MMb was observed for both the MxT and GxT interactions (Fig. 9). A more gradual increase was observed for the IS in comparison to the LTL and BF (Fig. 9a). For the MxT interaction, the IS had lowest %MMb ($P \leq 0.05$) for the duration of the trial. The

BF had the highest values ($P \leq 0.05$) for the duration of the trial, except on D0, where it did not differ ($P > 0.05$) from the LTL. The GxT interaction showed that the female muscles had higher values on all the days, except D1 and D2 (Fig. 9b).

Biochemical attributes

A significant MxT interaction was observed for the pH values whilst gender had no effect (Table 1). The IS had the highest ($P \leq 0.05$) values for the duration of the trial and the LTL the lowest ($P \leq 0.05$) (Fig.10).

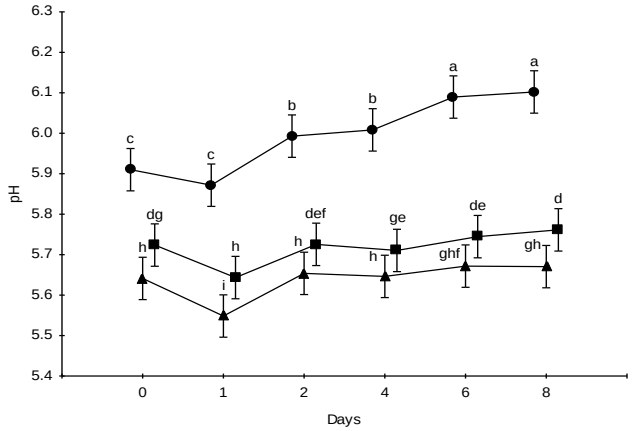

Figure 10 The temporal changes in ultimate pH (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant GxT and GxM interaction was observed for the MRA values (Table 1). The GxT interaction shows that there was an initial increase ($P \leq 0.05$) in MRA from D0 to D4 for all the muscle and both genders (Fig. 11a). Subsequently, a significant decrease was observed for the female but not the male muscles. The MRA values only differed for the genders on D8, where the males had higher values ($P \leq 0.05$). The GxM interaction showed that overall, the values for the male and female IS did not differ ($P > 0.05$) but those of the LTL and BF did differ ($P \leq 0.05$) (Fig. 11b).

A significant MxT interaction was observed for the OC values whilst gender had no effect (Table 1). A temporal decrease was observed for all the muscles (Fig. 12). On D0, no significant difference was observed for any of the muscles. On D4, the LTL differed ($P \leq 0.05$) from the IS, with the LTL having the highest values ($P \leq 0.05$); the BF differed from neither. On D8, the IS had the lowest values, which also differed ($P \leq 0.05$) from the LTL and BF.

A significant gender, muscle and time effect was observed for the TBARS values (Table 1). The gender effect showed that the female muscles had higher TBARS values (17.53 mg MDA/kg meat) overall in comparison to the male muscles (15.79 mg MDA/kg meat). The muscle effect showed that the IS had the lowest TBARS values ($P \leq 0.05$) overall in comparison to the LTL and BF, which did not differ ($P > 0.05$) from each other. For the time effect, an initial plateau ($P > 0.05$) in TBARS values was observed (D0 to D2), followed by an increase ($P \leq 0.05$) from D2 to D4, after which the values again plateaued ($P > 0.05$) (Fig. 13).

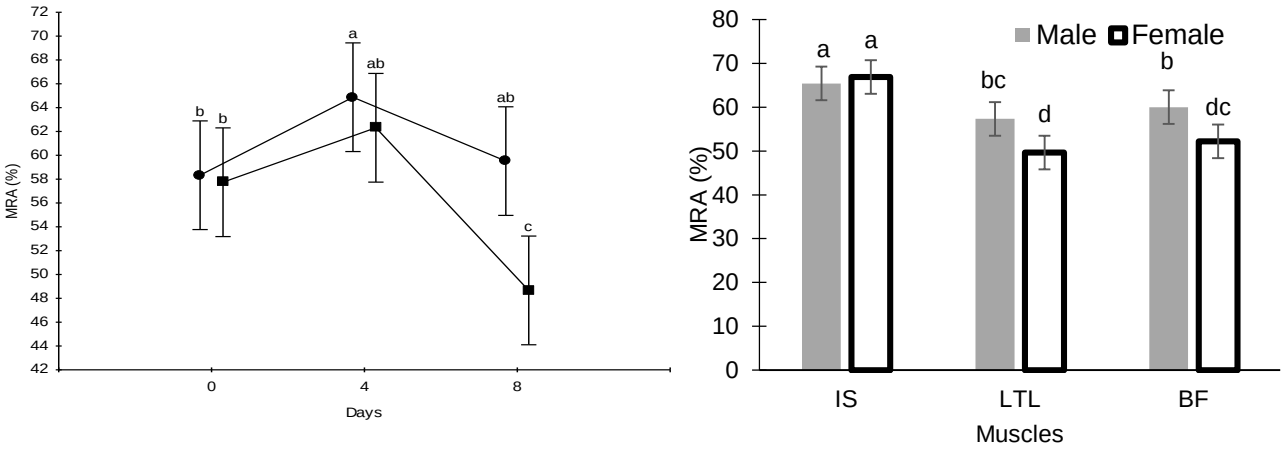

Figure 11 (a) The temporal changes for the averages of the metmyoglobin reducing activity (MRA) for the (●) male and (■) female fallow deer muscles (with standard error bars), and (b) the average MRA values (with standard error bars) for the male and female fallow deer muscles, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

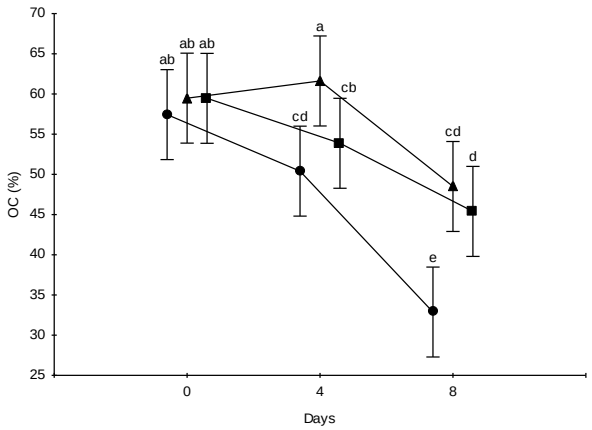

Figure 12 The temporal changes in ultimate pH (with standard error bars) for three female and male fallow deer muscles, (●) *infraspinatus*, (▲) *longissimus thoracis et lumborum* and (■) *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant muscle effect for the total and non-heme iron, and a significant gender effect for heme iron was observed. No gender effect was observed for the total and non-heme

iron, and no muscle effect was observed for the heme iron. The LTL and BF had the highest ($P \leq 0.05$) and the IS the lowest total and non-heme iron values (Table 2). The muscles of the female animals (22.28 ± 0.744 µg/g meat) had a higher heme iron concentration than the males (18.79 ± 0.744 µg/g meat).

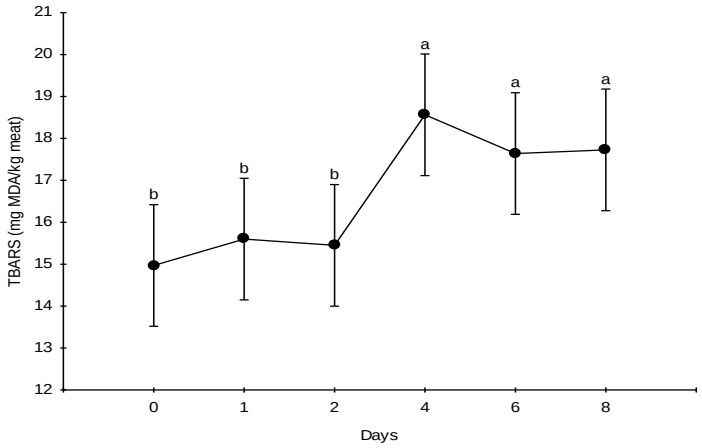

Figure 13 The average temporal changes in TBARS values (with standard error bars) for three fallow deer muscles, *infraspinatus*, *longissimus thoracis et lumborum* and *biceps femoris*, stored at 2°C. Means in figures with different letters differ significantly ($P \leq 0.05$).

A significant muscle effect was observed for the total Mb whilst gender had no effect (Table 2). The IS and LTL had the lowest values ($P \leq 0.05$) and the BF the highest ($P \leq 0.05$).

Table 2 The means and standard error of the mean (SEM) of the thiobarbituric acid reactive substances (TBARS), total iron, heme iron, non-heme iron and total Mb measured on D0 of storage for the IS, LTL and BF of fallow deer meat

Attribute	**IS**[1]	**LTL**[2]	**BF**[3]	**SEM**
TBARS (mg MDA/kg meat)	1.53[b]	1.74[a]	1.75[a]	0.434
Total iron (µg/g meat)	35.60[b]	41.40[a]	41.59[a]	1.427
Non-heme iron (µg/g meat)	14.11[b]	21.52[a]	21.35[a]	1.514
Total Mb (mg/g meat)	8.79[b]	9.16[b]	9.70[a]	0.269

[a-c]Means in rows with different superscripts differ significantly ($P \leq 0.05$).
[1]IS - *Infraspinatus.*
[2]LTL - *Longissimus thoracis et lumborum.*
[3]BF - *Biceps femoris*

DISCUSSION

Surface colour stability

Considerably more gender effects were observed in this study for the fallow deer muscles in comparison to the blesbok (*Damaliscus pygargus phillipsi*) muscles (*Chapter 4*) and the

springbok (*Antidorcas marsupialis*) muscles (*Chapter 5*). This alone indicates that there are variances in colour and colour stability between different game species.

Although the literature regarding the influence of gender on colour and colour stability is inconclusive, it is generally postulated that male animals have darker meat (lower *L** values) due to having higher concentrations of Mb in their muscles than female animals. The higher Mb concentrations result from higher physical activity levels (Seideman *et al.,* 1982). In theory, these higher Mb concentrations may also lead to a reduction in colour stability, with meat from male animals being less colour stable than meat from female animals (Insuasti *et al.,* 1999; Farouk *et al.,* 2007; Purchas et al., 2010). The higher Mb content of venison and game meat is also implicated in their darker colour (Vestergaard *et al.,* 2000; Díaz *et al.,* 2002; Kritzinger *et al.,* 2003; Daszkiewicz *et al.,* 2011) and propensity to discolour rapidly (Farouk *et al.,* 2007; Purchas *et al.,* 2010). Bureš *et al.* (2014) noted that the *L** values of red deer and fallow deer venison differed significantly from those of meat obtained from Aberdeen Angus and Holstein cattle. Similarly, other authors have also noted the darker colour of venison (Koch *et al.,* 1995; Rincker *et al.,* 2006; Farouk *et al.,* 2007; Bartoň *et al.,* 2014) and game meat (Hoffman *et al.,* 2005) in comparison to beef.

The results for *L** indicate that, for both genders, the IS is lighter in comparison to their LTL and BF counterparts (Fig. 1). Contrary to the general suggestion that the meat from male animals is darker than that of females, when the gender counterparts were lighter, the male muscles, not the female muscles were lighter. In fact, the female IS had a similar temporal trend to the male BF and not the male IS. The male BF muscles were significantly lighter in comparison to the male LTL, whereas the female LTL and female BF had similar values and did not differ from the male LTL. Interestingly, the IS and LTL did not differ in terms of total Mb concentration and the BF had the highest concentration (Table 2). In addition, no gender differences in total Mb concentration were observed in this study (Table 1). The theorised relationship between Mb concentration and *L** does thus not hold true for this study (Vestergaard *et al.,* 2000; Díaz *et al.,* 2002; Kritzinger *et al.,* 2003; Daszkiewicz et al., 2011) and propensity to discolour rapidly (Farouk *et al.,* 2007; Purchas *et al.,* 2010). Other explanations for variations in muscle colour are differences in the intramuscular fat content (IMF), with higher values resulting in lighter muscles (Lawrie & Ledward, 2006). However, this theory also does not hold true as the female muscles were found to have significantly higher (data not shown) IMF concentrations than the male muscles. Muscle fibre type is also linked to differences in colour and colour stability. Muscles containing higher proportions of oxidative muscle fibres (type I) are darker in colour in comparison to muscle containing high proportions of glycolytic fibres (type IIX) due to higher Mb concentrations. Oxidative muscles are also more prone to oxidation and thus discolour more readily (O'Keeffe & Hood 1982; Renerre & Labas, 1987). Despite the darker colour associated with venison and game meat, studies

conducted on both venison (deer and reindeer) (Kiessling & Kiessling, 1984; Essén-Gustavsson & Rehbinder, 1985) and game meat (blesbok, kudu, springbok, black and blue wildebeest) (Kohn *et al.,* 2011; Curry *et al.,* 2012; North, 2014) have noted that type IIX are present in the highest concentration in their muscles and not type I as expected. Studies have shown that increased physical activity results in an increase in either type I or type IIX fibres (Aalhus & Price, 1991; Van Vooren *et al.,* 1992; Petersen *et al.,* 1998). An increase in either, will result in an increase in the proportion of Mb, resulting in a darker muscle (lower *L** values). Although the opposite has been postulated, it may thus be that the darker colour of the female muscles results from higher activity in the female animals in comparison to the male animals. In contrast, no gender effect was observed for the *L** values of either the blesbok (*Chapter 4*) or springbok (*Chapter 5*). Despite the gender differences, a similar trend for the *L** values was observed for all the species, with the IS being the lightest and the LTL the darkest. Only the female muscles of the fallow deer indicated similarities between the *L** values of the LTL and BF. As with the blesbok (*Chapter 4*) and the springbok (*Chapter 5*), the *L** values of the male fallow deer proved to be a poor indicator of colour stability. However, the female *L** results are consistent with the colour stability results and would thus be indicative of colour stability, muscles with highest colour stabilities had the highest *L** values and vice versa. Nonetheless, overall the *L** values appear to be a poor indicator of colour stability for game meat. Similarly, McKenna *et al.* (2005) also noted that *L** values were a poor indicator of colour stability for beef muscles. As with both the blesbok (*Chapter 4*) and the springbok (*Chapter 5*), the lack of association between *L** and colour stability is reiterated by the poor correlation between *L** and the colour stability determinant R (630/580) (r=0.11 $P>0.05$). Furthermore, the *L** values for muscles of the blesbok (*Chapter 4*), springbok (*Chapter 5*) and fallow deer all fell within the same ranges (D0: 29-38; D8: 30-38). For the most part, these values are in agreement with the parameters set by Volpelli, Valusso, and Piasentier (2003), who noted that venison is characterised by *L** values below 40. Several other authors have also noted *L** values below 40 for fallow deer LTL which fall within similar ranges as those observed for the fallow deer from this study (Volpelli *et al.,* 2003; Bureš *et al.,* 2014; Cifuni *et al.,* 2014; Daszkiewicz *et al.* 2015).

As with the blesbok (*Chapter 4*) and springbok (*Chapter 5*), strong correlations were noted between the %OMb and *a** (r=0.82; $P\leq0.05$), chroma (r=0.75; $P\leq0.05$), and R (630/580) (r=0.85; $P\leq0.05$) values. In addition, strong correlations were noted between *a** and chroma (r=0.97; $P\leq0.05$), and R (630/580) (r=0.92; $P\leq0.05$) as well as chroma and R (630/580) (r=0.83; $P\leq0.05$). These strong correlations substantiate the relationships between these attributes and their proficiency in measuring surface colour redness. Similarly, strong correlations were noted between the %MMb and hue (r=0.83; $P\leq0.05$) substantiating the

relationship between these attributes and their proficiency in measuring surface discolouration (browning) of meat. Strong correlations between %MMb and hue were also noted for the blesbok (*Chapter 4*) and springbok (*Chapter 5*).

As with the springbok (*Chapter 5*), gender played a more significant role in the surface colour attributes of fallow deer in comparison to those of the blesbok (*Chapter 4*). In fact, it appears that gender had an even greater effect on the surface colour attributes of fallow deer than it did with the blesbok. Furthermore, similar trends were observed for the various surface colour attributes for the blesbok (*Chapter 4*), springbok (*Chapter 5*) and fallow deer.

Despite the gender differences observed, the results for the surface colour attributes and surface Mb redox forms indicate that the IS was the most colour stable of the three fallow deer muscles. It maintained the highest *a** (Fig. 2), chroma (Fig. 4), R (630/580) (Fig.6) and %OMb (Fig. 8) values, and had the lowest hue (for the most part) and %MMb values for the duration of the trial. Similarly, the IS was also the most colour stable of the three blesbok (*Chapter 5*) and springbok (*Chapter 6*) muscles. The differences in colour stabilities between the LTL and BF, and the male and female muscles were not as evident from the results as several anomalies exist between the attributes. It does however appear from a cursory glance at the results that the LTL and BF have similar colour stabilities but a more detailed analysis of the results is required to elucidate their actual colour stabilities. In agreement with the initial results from this study, similar colour stabilities have been noted for beef muscle (King *et al.,* 2011). On the other hand, it has previously been noted that distinct differences in colour stabilities exist between these three muscles for beef, which are in contrast to the results from this; the IS, BF and LTL were noted as having “very low”, “low” and “high” colour stabilities, respectively (McKenna *et al.,* 2005).

With regard to the effect of gender, the *a** results appear to indicate that the female IS and BF are less colour stable than the corresponding male muscles. The *a** values for the female IS and BF are lower than corresponding male muscles on D8 and from D4 to D8, respectively (Fig. 2). No gender differences were noted between the LTL muscles. In contrast, no gender differences for the *a** values were noted for the blesbok (*Chapter 4*), whereas gender differences were noted for the *a** values of the springbok (*Chapter 5*). However, no temporal variation in *a** values for the IS of the springbok (*Chapter 5*) was noted for either gender, whereas a temporal decrease was noted for the IS of the blesbok (*Chapter 4*) and the fallow deer. Despite the gender variation, the muscles for the blesbok (*Chapter 4*), springbok (*Chapter 5*) and fallow deer all fell within the same ranges (D0: 13-18; D8: IS - 13-15, LTL and BF - 7-10) for their *a** values. Other authors have also noted similar initial *a** values for fallow deer (Volpelli *et al.* 2003; Hutchison *et al.,* 2012; Bureš *et al.,* 2014; Daszkiewicz *et al.,* 2015). Interestingly, Daszkiewicz *et al.* (2015) noted significant differences between the *a** values of farm-raised (intensive) and wild (extensive) fallow deer, probably

owing to higher Mb concentrations resulting from higher activity levels in the latter (Lawrie & Ledward, 2006). This difference between intensive and extensively reared animals may have a significant effect on the overall colour stability of the meat; Mb is a pro-oxidant, and higher concentrations could lead to a decrease in the colour stability of meat from extensively reared animals. Similarly, other meat quality factors may also be influenced by differences in rearing systems. The differences noted by Daszkiewicz *et al.* (2015) endorses the suggestion made by Hoffman and Wiklund (2006) to differentiate between venison and game meat based on the differences in their rearing systems. Wiklund *et al.* 2001 established an *a** cut-off value of 12 for venison. Values above or below this cut-off value are considered acceptable and unacceptable by consumers, respectively. If this cut-off value is applied to the *a** results from the fallow deer in this study, the IS values, for both the male and female muscles, remain above the cut-off value for the duration of the trial, reiterating the high colour stability of the IS. The female LTL and BF, and male BF fall below 12 after D1, whereas the male LTL falls below 12 after D2, suggesting that it is marginally more colour stable. The significance of the higher colour stability of the male LTL is questionable and warrants further investigation. The *a** cut-off value is consistent with the suggested colour stabilities of the three muscles, with the IS having the highest colour stability and the LTL and BF lower colour stabilities. Similarly, the *a** cut-off values for the blesbok (*Chapter 4*) and the springbok (*Chapter 5*) also indicated that the IS had the highest colour stability in comparison to the LTL and BF, with the values remaining above the cut-off value for the duration of the trial. In addition, the LTL and BF of both the blesbok and springbok fell below the cut-off value after D1, regardless of gender. Thus, only the male LTL of the fallow deer appears to differ in terms of its colour stability in comparison to the LTL and BF of the other game species, which again brings into question the significance of its higher colour stability. In agreement with the results from this study, previous authors have noted that the LTL of reindeer fell below the acceptable limit after approximately one day (Wiklund & Johansson, 2011). In contrast, red deer (*Cervus elaphus*) venison was noted to fall below 12 after 70 hours (±3 days) (Wiklund *et al.,* 2006) and fallow deer after 200 hours (±8 days) (Wiklund *et al.,* 2005). These variations can be ascribed to differences in storage temperatures, with lower storage temperatures leading to an increase in colour stability (Lawrie & Ledward, 2006). The reindeer were stored at 4°C (Wiklund & Johansson, 2011) and fallow deer (current study) at 2°C and the red deer and fallow deer (Wiklund *et al.,* 2005) at -1.5°C. The increased colour stability of venison at lower temperatures should be taken into consideration when processing and storing game meat as it could be an effective method for increasing its colour stability without the need for too much additional capital.

The chroma values follow a somewhat similar trend to the *a** values (Fig. 4) with most amount of the temporal variation between genders being observed for the IS and BF, with no

variation between genders being observed for the LTL. The female IS and BF appeared to be less colour stable compared to the corresponding male muscles as they had lower chroma values towards the end of the trial. The chroma values also indicated, that regardless of gender, that the IS was the most colour stable, and that the LTL and BF had similar colour stabilities. The chroma values for all the game species fell within similar ranges (D0: 17-22; D8: IS - 18-21; LTL and BF - 12-16) for all the muscles. As with the *a** values, gender differences for the chroma values were noted for the blesbok (*Chapter 4*) and fallow deer but not for the springbok (*Chapter 5)*. Furthermore, more variation between the chroma results for the BF and LTL of the springbok (*Chapter 5*) and blesbok was noted in comparison to the fallow deer. The female IS of the fallow deer was also the only IS muscle from all the game species to show a temporal decrease in chroma values.

The hue value results further substantiate the suggested colour stabilities of the muscles already observed for the *a** and chroma results; the IS is the most colour stable, whilst the LTL and BF have similar colour stabilities (Fig. 5). In contrast to the results for the *a** and chroma, the hue results indicate that the male LTL and BF have a higher colour stability than their female counterpart, whereas the IS, of both genders, have similar colour stabilities. The hue values for all the game species, blesbok (*Chapter 4*), springbok (*Chapter 5*) and the fallow deer, all fell within similar ranges (D0: 36-44; D8 IS - 42-44, LTL and BF - 48-56). Interestingly, only the IS showed a similar temporal trend between the three game species. The temporal trend for the LTL and BF of the springbok (*Chapter 5*) and fallow deer were similar for their respective genders but differed from the corresponding blesbok muscles.

The R (630/580) results also indicate that the IS is the most colour stable and that the LTL and BF are the least colour stable and have similar colour stabilities (Fig. 6). Similar value ranges (D0: 3-4.2; D8: IS - 2.2-2.4; LTL and BF - 1.3-1.4) and temporal trends were observed for the R (630/580) values for the blesbok (*Chapter 4*), springbok (*Chapter 5*) and fallow deer, with all the results indicating the higher colour stability of the IS and the lower colour stabilities of the LTL and BF. As with the *a** values, a cut-off value of 3 has been established for the R (630/580) values (Purchas *et al.,* 2010). Consumers perceive values above 3 to be acceptable in terms of surface colour and below 3 as unacceptable. If this cut-off value is applied to the R (630/580) results from this study, none of the muscles remain acceptable in colour after D1. This is contradictory to the suggested results and visually perceived colour stabilities of the muscles. In addition it is also contrary to the results for the *a** cut-off value, which were consistent with the suggested and perceived colour stabilities of the muscles. Similarly, the cut-off value of 3 did not reflect the colour stabilities of the blesbok (*Chapter 4*) and springbok (*Chapter 5*) muscles. In both cases, a cut-off value of 2 better reflected the colour stabilities of the muscles; this was also the case for the fallow deer muscles from this study. Different *a** (14.8) and R (630/580) (3.3) cut-off values have been noted for lamb (Jacob *et al.,* 2007).

The differences in cut-off values between species may explain why the *a** value cut-off point translates so well for the game meat but the R (6380/580) cut-off value does not; the *a** cut-off value of 12 was specifically established for venison (Wiklund *et al.,* 2001), whereas no R (6380/580) cut-off value has specifically been established for game meat or venison. Although authors have used the same R (630/580) cut-off value (3) to compare the colour stabilities to lamb and venison (Jacob *et al.,* 2007), the research done on game meat suggests that cut-off values may be species specific. As with the blesbok (*Chapter 4*) and the springbok (*Chapter 5*) a cut-off value of 2 better reflected the perceived colour stabilities of the muscles. This cut-off value indicated that the IS remained acceptable in colour for the duration of the trial, whereas the LTL and BF would be deemed unacceptable after D2. More research is warranted to establish whether a specific R (630/580) cut-off value can be established for game species as a whole and/or whether a cut-off value is required for each individual game species.

The %OMb results indicated that the IS was the most colour stable and that the LTL and BF has similar colour stabilities (Fig. 8a). However, the results also indicate that the female muscles were initially (D0 and D1) redder in colour (higher %OMb values) in comparison to the male muscles (Fig. 8b) but had similar colour stabilities from D2 to D8. The initial higher values of the %OMb are not indicative of higher colour stability but only that that the female muscle were more red in comparison to the male muscles. The %MMb results also indicate that the IS is the most colour stable, but contrary to the %OMb results indicate that the BF is less colour stable than the LTL (Fig. 9). In addition, the %MMb results indicate that the male and female muscles initially had similar colour stabilities and that the female muscles were less stable than the male muscles overall (higher % MMb values towards the end of the trial), which is also in contradiction to the %OMb results. Although the %OMb and %MMb results did reflect those of the *a** and hue values to some extent, various anomalies were noted between these results. Since the *a** value and hue values in effect give an indication of the %OMb (redness) and %MMb (brownness), respectively, the anomalies between these results indicate that either the surface colour attributes or surface Mb redox forms are inaccurate to some extent. This can most likely be attributed to the surface colour attributes describing a point in the colour space, whereas the nature of Mb redox form is more accurately described by the surface Mb redox forms. The latter is regarded as more accurate in revealing information about surface colour (Holmgaard Bak *et al.,* 2012). Thus, although the CIE *L**, *a**, and *b** parameters are often used to measure the colour and colour stability of meat and strong correlations were observed between the %OMb and *a** values, and %MMb and hue values, the surface Mb redox forms may be more sensitive in measuring temporal changes in colour. Similar anomalies were noted for the blesbok (*Chapter 4*) and springbok (*Chapter 5*). Despite the gender differences noted for the springbok (*Chapter 5*) and fallow

deer, similar trends and value ranges were observed for the %OMb (D0: 44-56; D8: IS - 44; LTL and BF - 34-38) and %MMb (D0: 26-32; D8: IS - 42-46; LTL and BF - 51-59) results for all the game species (blesbok (*Chapter 4*), springbok (*Chapter 5*) and fallow deer). However, no gender differences were observed for the %OMb results for the blesbok (*Chapter 4*) and those observed for the springbok (*Chapter 5*) and the fallow deer differ; the %OMb values for the springbok (*Chapter 5*) indicated that the all the female muscles had higher %OMb for the duration of the trial, whereas the female muscles of the fallow deer only had higher values initially. Furthermore, only gender differences were noted for the %MMb results of the fallow deer and not for the blesbok (*Chapter 4*) and springbok (*Chapter 5*). These variances are most likely due to species differences. The exact mechanisms behind these species differences warrant further investigation. Comparing the results of this investigation to other studies, Cifuni *et al.* (2014) noted similar %OMb (51.79±1.44%) and %MMb values (20.24±0.959) for the LTL muscle of fallow deer. Sammel *et al.* (2002) noted higher %OMb and lower %MMb values for beef *semimembranosus* (SM) over a five day colour stability trial. Similarly, Jeong *et al.* (2009) also noted higher %OMb and lower %MMb values for beef LTL, *psoas major* and SM over a seven day colour stability trial. These differences can be attributed to species differences. Venison and game meat tend to discolour more readily due to the oxidative nature of their meat (Stevenson-Barry *et al.,* 1999). This oxidative nature is attributed to higher Mb concentrations, which result in higher iron (a pro-oxidant) levels (Farouk *et al.,* 2007; Purchas *et al.,* 2010). As with the *a** and R (630/580) values, cut-off values for the %MMb has also been noted in literature. Above and below this value the surface colour of the meat is regarded as unacceptable and acceptable by consumers, respectively. As with the *a** and R (630/580) values, the cut-off value for the %MMb appears to be species specific. A value of 60% and 40% have been established for beef (Brooks, 1938) and rhea (*Rhea americana*) (Filgueras *et al.,* 2010), respectively. If these cut-off values are applied to the %MMb results from this study, either all the muscles remain acceptable for the duration of the trial (60%) or the LTL and BF, and IS are deemed unacceptable after D1 and D3, respectively. Neither of these cut-off values reflect what was visually observed. Similarly, these cut-off values did not agree with the visual observations for the colour stability of the blesbok (*Chapter 4*) and springbok (*Chapter 5*) muscles. For the blesbok and springbok a cut-off value of 50 was suggested as being more indicative of what was visually observed during the trial but would need to be verified by proper visual meat colour analysis using a consumer panel (AMSA, 2012). In the case of the fallow deer, a cut-off value of 44 gave the best results and gave similar colour stability results as the *a** and R (630/580) cut-off values; the IS remained acceptable for the duration of the trial, whereas the LTL and BF were unacceptable after D1.

The *a** (Fig. 2), chroma (Fig. 4), hue (Fig. 5), %OMb (Fig. 8) and %MMb (Fig. 9) results indicated that all or most of the female muscles oxidised more readily than their male counterparts. Interestingly, no gender differences were noted for the total Mb (Table 1) but gender differences were observed for the heme iron (Table 1), with the females having higher concentrations than the males. Thus, the differences observed for the muscles may, at least in part, be ascribed to the differences in heme iron concentration. The effect of gender on the colour and colour stability of venison and game species is inconclusive. However, several studies on the colour stability of venison have noted no gender differences in colour stability (Daszkiewicz *et al.,* 2009; Purchas *et al.,* 2010). In agreement with these studies, no gender difference in colour stability were visually perceived for any of the muscles in this study, bringing into question the biological significance of the gender differences. Similarly, despite gender differences being observed for several of the surface colour attributes and surface Mb redox forms, no colour stability differences were visually perceived for the springbok (*Chapter 4*). Further research into the effect of gender differences on the colour stability of game meat is definitely warranted. The overall colour stability of the fallow deer muscles thus seems to be in descending order of IS>LTL>BF. As with the fallow deer, the IS of the blesbok (*Chapter 4*) and springbok (*Chapter 5*) was also noted to be the most colour stable of the three muscles. For the blesbok (*Chapter 4*) muscles, the LTL was also noted as being more colour stable in comparison to the BF, whereas the springbok (*Chapter 5*) LTL and BF were noted to have similar colour stabilities.

Biochemical attributes influencing surface colour stability

The oxidation of OMb is delayed and increased at higher and lower muscle pH values, respectively (Gotoh & Shikama, 1974; Ledward, 1985; Gutzke & Trout, 2002). Thus, in theory, muscles with higher pH values will be more colour stable than muscles with lower pH values. In this study the higher colour stability of the IS in relation to the LTL and BF is explained by its higher pH value. However, contrary to this supposition, the LTL, which was observed to be more colour stable than the BF, had significantly lower pH values in comparisons to the BF. This indicates that factors, other than pH play a role in the colour stability of muscles. Furthermore, these factors may not be mutually exclusive but act together to influence the colour stability of muscles. Contrary to these results, the blesbok (*Chapter 4*) and springbok (*Chapter 5*) muscles showed a strong correlation between pH and %OMb, with the more colour stable muscles also having correspondingly higher pH values and vice versa. This may indicate that the effect of pH on the colour stability of muscles is species specific. In addition, the literature regarding the effect of muscle pH on colour stability varies. While some researchers have noted that higher pH values decrease the oxidation of OMb (Brown &

Mebine, 1969; O'Grady *et al.,* 2001) others have noted no link between pH and rate of discolouration (Hood, 1980; McKenna *et al.,* 2005).

MRA refers to the residual enzymatic and non-enzymatic systems in the muscles post-mortem which possess the ability to reduce MMb back to DMb (Bekhit & Faustman, 2005). The DMb can subsequently be converted to OMb. Thus, in theory, higher MRA values lead to an increased colour stability of muscles. However, this theory has also been disputed by several authors. While some authors firmly believe in the influence of MRA on colour stability (Ledward, 1971), others dispute the role it plays in colour stability (Atkinson & Follett, 1973; O'Keeffe & Hood, 1982; Renerre & Labas, 1987), while others still feel it is a combination of MRA and OC which influence muscle colour stability (Madhavi & Carpenter, 1993). From the results it would appear that the MRA did play a role in the colour stability of the muscles (Fig. 11). The higher MRA values noted for the male muscles on D8 and the temporal decrease in MRA for the female muscles (Fig. 11a) may, to some extent, explain the higher colour stability noted for the male muscles for several of the attributes (*a**, chroma, hue and %MMb). Furthermore, the colour stability differences noted for the muscles may also be explained by the higher overall MRA values noted for the IS in comparison to the LTL and BF (Fig. 11b). However, the MRA values for the LTL and BF did not appear to differ, despite the LTL being more colour stable than the BF. Similarly, higher %OMb values were initially observed for the female muscle but the MRA did not differ initially for the male and female muscles. As with the pH results, these results indicate that the MRA alone does not influence the colour stability of muscles but that it is rather a combination of factors that influences colour stability. Gender and muscle differences were also noted for MRA results of the blesbok (*Chapter 4*) and springbok (*Chapter 5*). However, the gender differences observed for the MRA results of the blesbok (*Chapter* 4) were disregarded as no other gender differences were observed for any of the attributes. On the other hand, the MRA result for the springbok exhibited almost an identical trend to the fallow deer muscles, with the female muscles also having lower values on D8 in comparison to the male muscles. Despite these similarities, the gender differences in MRA were not found to influence the colour stability of the springbok (*Chapter* 5) muscles. The reason for this gender differences observed for the MRA of springbok (*Chapter* 5) and fallow deer may be attributed to the differences in heme iron observed between genders of these species. In both species, the heme iron concentrations were observed to be higher in the female muscles. The higher heme iron content may lead to increased lipid oxidation which has been linked to a decrease in MRA (Bekhit & Faustman, 2005). This corresponds to the results for the springbok (*Chapter* 5) and fallow deer as higher TBARS values was observed for the female muscles of both species.

The OC of meat refers to the residual mitochondrial activity in meat post-mortem. The mitochondria compete with Mb for oxygen, thus leading to a reduction in the amount of oxygen

available to bind to the Mb. Consequently, less OMb is formed and conditions ideal for MMb formation are created, ultimately resulting in a reduction in colour stability of the muscle (Bendall & Taylor, 1972; Ledward, 1985; O'Keeffe & Hood, 1982; McKenna *et al.,* 2005). However, as with the MRA, there is a great deal of dispute in literature regarding the role of OC in colour stability. The results from this study do not wholly support the notion that lower OC values lead to a reduction in colour stability (Fig. 12). The initial (D0) OC values did not differ significantly, which is congruent to what is expected as the muscles all had relatively similar initial values for all the attributes. However, despite the IS having the lowest OC values on D8, it has similar values to the BF on D4, which was considerably less colour stable than the IS. Similarly, the LTL had higher and similar OC values to the BF on D4 and D8, respectively, despite being more colour stable. Conversely, McKenna *et al.* (2005) noted that the OC in the BF of beef had higher values in comparison to the LTL and IS, with the LTL having the lowest OC values. However, the colour stabilities for the IS, BF and LTL were concluded as being "very low", "low", and "high", respectively. Thus, as in this study, the OC results also did not wholly support the notion that lower OC values lead to a reduction in colour stability. The OC results for the blesbok (*Chapter 4*) also did not show a definitive link between OC and colour stably. Interestingly, no significant differences in OC were observed between the muscle of the springbok (*Chapter 5*), indicating that OC values are not only muscle specific but possibly also species specific. As with the OC results for the springbok (*Chapter 5*) and the MRA results for all the game species, it would be suggested that more time intervals be used to elucidate the exact temporal nature of these attributes, which may give a better understanding of their influence and the role they play in colour stability.

MMb formation is accelerated by lipid oxidation and thus increased lipid oxidation leads to a decrease in colour stability (Faustman *et al.,* 2010). The link between colour stability and lipid oxidation was reiterated to some degree in this study. The muscle with the highest colour stability, the IS, also had the lowest TBARS values (Fig. 13). However, no significant differences were noted for the TBARS results for the LTL and BF, despite the LTL being more colour stable than the BF. Thus, it appears that TBARS values are not a good indicator of colour stability in fallow deer muscles. Similarly, other researchers have also noted that TBARS values were not a good indicator of colour stability for various beef muscles (McKenna *et al.,* 2005). Similarly, the TBARS values of the blesbok (*Chapter 4*) muscles were also not found to be a good predictor of muscle colour stability. On the other hand, the TBARS values of the springbok (*Chapter 5*) muscles were observed to be a good indicator of colour stability. This would not only suggest that the use of TBARS values as an indicator of colour stability is species specific, but that factors other than TBARS play a role in the colour stability of muscles. Furthermore, these factors may not be mutually exclusive but may act together to influence the colour stability of muscles. Although no gender differences in surface colour were visually

perceived, as previously mentioned, the lower colour stability observed for the female muscles may be attributed to the higher TBARS values noted for the female muscles in this study.

Naturally occurring pro-oxidants in the muscle, such as iron, can also decrease the colour stability of the muscle. The iron serves as a catalyst for lipid oxidation which consequently leads to a reduction in colour stability (Lawrie & Ledward, 2006). Since both heme and non-heme iron have been associated with accelerating lipid oxidation (Igene *et al.,* 1979; Chen *et al.,* 1984), the total iron concentration of meat should also be indicative of muscle colour stability. No muscle differences were observed for the heme iron results for the fallow deer muscles (Table 1), and thus are not implicated in the observed muscle colour stability differences. Congruent to these results, the heme iron results were also found not to be indicative of colour stability for the blesbok (*Chapter 4*) and springbok (*Chapter 5*) muscles. However, the female muscles were noted as having higher heme iron concentrations in comparison to the male muscles in this study, which could explain the higher colour stability observed for the male muscles. Gender differences were also noted for the heme iron results of the springbok (*Chapter 5*), but were not found to correlate to muscle colour stability. No gender differences were noted for the heme iron results for the blesbok (*Chapter 5*) muscles. It would thus appear that the influence of heme iron on colour stability is species specific. The total and non-heme iron results were also not wholly indicative of colour stability for the fallow deer muscles (Table 2). Despite the lowest total and non-heme iron values being observed for the most colour stable muscle, the IS, similar values were observed for the LTL and BF, despite the LTL being more colour stable than the BF. Thus, the total and non-heme iron results would not be a dependable method for predicting the colour stability of different fallow deer muscles. Conversely, the total and non-heme iron results were found to be a good indicator of muscle colour stability for the springbok muscles (*Chapter 5*), whereas only the non-heme iron was found to be a good indicator for the blesbok muscles (*Chapter 4*). It would thus seem evident that the use of total and non-heme iron results in predicting colour stability is species specific.

Higher Mb concentrations in muscles are often associated with the accelerated discolouration (Jeong *et al.,* 2009; King *et al.,* 2011). However, the results from the present study did not agree with this association. In agreement with these results (Table 2), the Mb concentration was not found to be indicative of colour stability for any of the game species (*Chapter 4* and *5*). Similarly, other studies have also concluded that there was no relationship between Mb concentration and colour stability (Sammel *et al.,* 2002; McKenna *et al.,* 2005; Canto *et al.,* 2015).

CONCLUSION

The IS was determined to be the most colour stable among the three fallow deer muscles investigated in this study. Furthermore, the LTL was found to be more colour stable than the BF. Despite gender variations being noted for several of the measurements, which indicated that the male muscles were more colour stable than the female muscles, no such differences were visually perceived and were regarded as being biologically insignificant. Thus, based on the various attributes measured and what was visually perceived, the colour stability of the muscles was determined to be in descending order of IS>LTL>BF. As with the previous game meat studies (*Chapter 4* and *Chapter 5*), *L** was once again determined to be a poor indicator of colour stability. In addition, the majority of the biochemical attributes were found not to wholly explain the perceived colour stability of the muscles, indicating that these attributes do not act alone to determine the colour stability of muscle but that they act together. The muscle and species differences noted further highlights the need for muscle and species specific processing of game meat.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

The South African game meat industry is yet to be fully developed, but has demonstrated a great deal of potential for growth, both within the local and international markets (Hofmeyr, 2014; Cloete *et al.,* 2015). In fact, many believe that the future of the South African game farming industry as a whole depends on the development of a sustainable game meat industry (Cloete *et al.,* 2015). To create a sustainable industry, meat products of a consistently high quality must be supplied to consumers (Hutchison *et al.,* 2010). However, no guidelines or standard procedures have been implemented in South Africa for the processing of game meat to ensure the delivery of consistently high quality products. One of the major obstacles in the development of such guidelines and standard procedures is the limited research regarding the meat quality of South African game species; information sorely required for their development. Furthermore, the grouping of meat derived from game animals under generic terms such as venison and game meat, creates the erroneous perception that the meat quality of these species is uniform. This may lead to uniform processing procedures for these species, potentially resulting in the reduction of the meat quality and shelf-life. Thus, it is essential that all quality aspects associated with each different game species be investigated to ensure that the meat is processed and marketed in the best possible way. The limited information available on the meat quality of individual game species and the potential growth identified for the game meat industry creates the need for baseline research into the various aspects which influence the meat quality of these species. Meat colour is a very important quality aspect in terms of consumer purchasing intent as it is the only quality factor consumer can use at the time of purchase (Suman *et al.,* 2014) to select meat that they perceive to be wholesome, fresh and of high quality (Kropf, 1980; Faustman & Cassens, 1990; Mancini & Hunt, 2005). Discoloured meat is discounted and results in reduced revenue (Kropf, 1980). Maintaining the colour of meat for as long as possible is thus desirable to ensure maximum profits. However, no research is currently available on the colour stability of South African game meat, which creates great scope for research into the colour stability of different South African game species and the factors which affect their colour stability. Thus, the baseline research in this dissertation focussed on the colour stability of game meat from three different game meat species: springbok (*Antidorcas marsupialis*); blesbok (*Damaliscus pygargus phillipsi*); and fallow deer (*Dama dama*).

In the initial research chapter (*Chapter 3*) the colour stability of five blesbok muscles, *infraspinatus* (IS), *supraspinatus* (SS), *biceps femoris* (BF), *semimembranosus* (SM) and *semitendinosus* (ST) were investigated. The objective of this study was to gain some initial

insight into the colour stability of game meat prior to the commencement of the subsequent, more in-depth, research chapters. Thus, only basic analyses were conducted to elucidate the nature of game meat colour stability and give guidance as to the approach which should be used in subsequent chapters. The results indicated that the colour stability of the muscles were in descending order of IS>SS>BF=SM=ST. Interestingly, the colour stability of the blesbok muscles were found to be contradictory to the colour stabilities noted for beef (McKenna *et al.,* 2005; Seyfert *et al.,* 2006; King *et al.,* 2011) and was attributed to species differences. Furthermore, the muscles were also observed to group according to their anatomical location for many of the attributes measured, suggesting a link between muscle function and colour stability. This link was ascribed to differences in muscles fibre types resulting from functional differences (Hunt & Hedrick, 1977; Klont *et al.,* 1998; Lefaucheur, 2010). The differences in colour stabilities were attributed, in part, to the pH values, with higher pH values resulting in more colour stable muscles due to a decrease in oxymyoglobin (OMb) oxidation (Gotoh & Shikama, 1974; Ledward, 1985). However, this relationship between pH and OMb was not observed for all the muscles, indicating that pH alone does not determine the colour stability of blesbok muscles. Discrepancies were noted between the *a** and percentage oxymyoglobin (%OMb), and between the hue and percentage metmyoglobin (%MMb) values. Although these discrepancies could not be explained, other authors have also noted discrepancies between these attributes (McKenna *et al.,* 2005). It was suggested that, since the %OMb and %MMb results correlated better to what was visually observed during the trial, these attributes may be more accurate in measuring colour stability. Other authors have also suggested that the use of the reflectance spectra to measure myoglobin (Mb) redox forms may be better than the CIELab ordinates at measuring colour changes as the CIELab ordinates only measure a point in the colour space, whereas the reflectance spectra measures the actual Mb form present (Holmgaard Bak *et al.,* 2012). Although a temporal increase in lipid oxidation (increase in TBARS values) was observed, no muscle differences were noted and the latter could thus not be given as the reason for the observed colour stability difference. It was suggested that additional sampling periods be added to establish a possible link between colour stability and lipid oxidation. The data indicated that gender, muscle and time should be included as variables in further studies and that the number of muscle samples could be reduced due to the similarities noted between some of the muscles.

The following three research chapters (*Chapters 4-6*) investigated the colour stability of three muscles, the IS*, longissimus thoracis et lumborum* (LTL) and BF, from blesbok (*Chapter 4*), springbok (*Chapter 5*) and fallow deer (*Chapter 6*). The colour stability of the three muscles for each individual species was determined by measuring surface colour attributes (CIELab and R (630/580)), surface Mb redox forms (percentage deoxymyoglobin

(%DMb), %OMb and %MMb) and various biochemical attributes which influence colour stability (pH, oxygen consumption (OC), metmyoglobin reducing activity (MRA), lipid oxidation (TBARS), total, heme and non-heme iron and total Mb). The results of the surface colour attributes and surface myoglobin redox forms for all three species indicated that the IS was the most colour stable of the three muscles, which is congruent with the result from *Chapter 3*. However, differences in the colour stabilities of the LTL and BF were noted between the three game species. For both the blesbok and fallow deer, the LTL was found to be slightly more colour stable than the BF, whereas the LTL and BF for the springbok were found to have similar colour stabilities. As with the results from *Chapter 3*, anomalies were observed between the *a** and %OMb, and the hue and %MMb values. As before, the %OMb and %MMb results correlated better to what was visually observed during the trial, reiterating that these attributes may be more accurate in measuring the colour stability of meat. However, the anomalies between these attributes definitely warrant further investigation. It is suggested that appropriate visual meat colour measurement methods, such as those set out in the *Meat Color Measurement Guidelines* (AMSA, 2015), be employed in future studies to correlate the perceived colour stability with the attributes measured. The colour stabilities of the muscles from the game species, is contrary to the colour stabilities of the corresponding muscles in beef. It has been noted that the colour stabilities of beef IS, LTL and BF are categorised as "very low", "high" and "low", respectively (McKenna *et al.,* 2005). Thus, the results from this study indicate that, not only does colour stability appear to be muscle specific, but also species specific. This could have significant implications in the processing of game meat. It emphasises the need for the game meat industry to employ muscle and species specific processing strategies to improve colour stability.

Cut-off values that give an indication of the point at which consumers' perceive the meat colour to be undesirable have been established for the *a** (Wiklund *et al.,* 2001), R (630/580) (Purchas *et al.,* 2010) and %MMb (Brooks, 1938; Filgueras *et al.,* 2010) measurements. The *a** cut-off value given for red meat correlated with what was visually observed in these game species but those for the R (630/580) and %MMb did not. However, these cut-off values vary in literature and appear to be dependent on the species being evaluated. The *a** cut-off value used was established for venison (Wiklund *et al.,* 2001), which is similar to game meat in appearance and could thus explain why it correlated so well with what was visually perceived. On the other hand, the R (630/580) and %MMb values were established for lamb (Purchas *et al.,* 2010), and beef (Brooks, 1938) and rhea (Filgueras *et al.,* 2010), respectively. In this investigation, using the *a** cut-off value the colour stability of the IS was established to be eight days or more, and the LTL and BF one day for all three game species. Using these colour stabilities, cut-off values for R (630/580) and %MMb were proposed for the different game species. An R (630/580) value of 2 was suggested for all the

species and a %MMb value of 50 for the blesbok and springbok, and 44 for the fallow deer was suggested. It is important to remember that these cut-off values are only suggestions and need to be validated using appropriate visual meat colour measurement methods. The shelf-life stabilities observed were similar to reindeer (*Rangifer tarandus*) venison (Wiklund & Johansson, 2011) but differed from those observed for red deer (*Cervus elaphus*) (Wiklund *et al.,* 2006) and fallow deer (Wiklund *et al.,* 2005), with considerably longer colour stability observed for the latter two. It was postulated that the variations resulted from differences in display temperatures, with lower temperatures leading to significantly increased colour stability (Lawrie & Ledward, 2006); the reindeer venison (Wiklund & Johansson, 2011) was displayed at 4°C whereas the red deer (Wiklund *et al.,* 2006) and fallow deer venison (Wiklund *et al.,* 2005) were displayed at -1.5°C. It is thus suggested that game meat processors and retailers use low temperatures to increase the colour stability of game meat and that the cold chain be closely monitored. Furthermore, it would be suggested that research be done into the ideal temperature at which game meat should be stored to achieve maximum colour stability.

Although significant gender differences were observed for several of the attributes, with the least amount of gender variation seen for the blesbok and the most for the fallow deer, these differences were not visually observed and this were disregarded in the overall colour stability conclusions which were drawn. However, the visual observations were not formally quantified and it is suggested that appropriate visual meat colour measurement methods be employed in future studies to determine whether there are quantifiable visual differences and whether these difference can be correlated to the instrumental colour measurements.

High pH values impede the oxidation of OMb whereas the opposite is true for low pH values (Gotoh & Shikama, 1974; Ledward, 1985). Thus, meat with high pH values is more colour stable than those with lower pH values. This relationship between colour stability and pH was noted for the muscles of the blesbok and springbok and thus explained the colour stability differences between the muscles from these species. In contrast, the pH values did not completely explain the colour stability differences seen for the fallow deer muscles. Although the IS, which was the most colour stable, had the highest pH value, the pH values of the LTL and BF did nor correlate to their pH values. It was thus suggested that influence of pH on muscle colour stability is species dependant.

The data for the MRA and OC results were very erratic and, for the most part, inconclusive as to whether or not these attributes influenced the colour stability of the meat. It was suggested that the number of time points be increased in future research to elucidate whether the erratic nature of the data results from phenomena in the muscles or biological variation. Increasing the number of times point may also give more tangible evidence on the influence of the attributes on the colour stability of the muscle. It is suggested that high MRA

values (Ledward, 1971) and low OC values (Bendall & Taylor, 1972; Ledward, 1985; O'Keeffe & Hood, 1982; McKenna et al., 2005), although not necessarily in conjunction, increase the colour stability of meat. However, a great deal of debate exists within the literature regarding the degree to which each of these attributes affects colour stability, if at all. The data from this study show that a combination of high MRA values and low OC values related, to some extent, to higher muscle colour stability for the blesbok muscles. For the springbok, high MRA values also appeared to be associated with higher colour stability, whereas no significant interactions or effects were observed for the OC. Although some associations between MRA, OC and colour stability were observed for the fallow deer muscles, the colour stability was not fully explained by these attributes' data. The lack of conclusive evidence regarding the influence of MRA and OC on the colour stability of meat, the erratic nature of the data and the debate regarding the relative contribution of these attributes to muscle colour stability, highlights the need for future research.

The formation of MMb is accelerated by an increase in lipid oxidation, resulting in a decrease in colour stability (Faustman *et al.,* 2010). Despite the thiobarbituric acid reactive substances (TBARS) results being the lowest for the IS (most colour stable) of all the game species in comparison to the LTL and BF, only the TBARS results for the springbok was determined to be a reliable predictor of colour stability. The TBARS values for the LTL and BF of the blesbok and fallow deer did not correspond to their colour stabilities. The differences noted between the game species suggest that the relationship between colour stability and lipid oxidation is species-specific.

Both heme and non-heme iron (Igene *et al.,* 1979; Chen *et al.,* 1984), and thus by extension total iron, have been implicated in catalysing lipid oxidation, which leads to a reduction in colour stability (Lawrie & Ledward, 2006). Heme-iron was not observed to relate to the colour stability of the muscles for any of the game species. The total and non-heme was found to relate to the colour stability of the springbok muscles, whereas only the non-heme iron related to the colour stability of blesbok muscles. Despite both the total and non-heme iron having the lowest values for the fallow deer IS (most colour stable), the values for the LTL and BF did not related to their colour stabilities. The differences indicate that the use of total and non-heme iron as a predictor for colour stability is species specific.

Higher Mb concentrations have also been linked to increased muscle discolouration (Jeong *et al.,* 2009; King *et al.,* 2011). However, the total Mb did not correlate to the colour stability of the muscles for any of the species and was thus determined not to be a good indicator of colour stability. Other studies have also noted that total Mb is not a good indicator of muscle colour stability (Sammel *et al.,* 2002; McKenna *et al.,* 2005; Canto *et al.,* 2015).

The fact that so many of the biochemical attributes measured only partly explain the colour stability of the various muscles suggests that there are various factors affecting the

colour stability of meat and that these factors are not mutually exclusive but act together to influence the perceived colour of meat. It also highlights that fact that the factor involved in meat discolouration are not, as yet, fully understood (Faustman & Cassens, 1990).

The IS is a low value muscle which is not usually sold as a whole muscle but as a minced meat due to its toughness. Mincing increases the rate of OMb oxidation leading to a reduction in colour stability (Ledward *et al.,* 1977). It thus seems ideal that the most colour stable of the game meat muscles is also the one which is commonly sold as minced/ground meat. Its high colour stability may counter the increased oxidation caused by the mincing to some extent. However, to elucidate the exact temporal colour changes which will occur if the IS is minced, further research is required. Furthermore, game meat which is sold as whole, fresh muscles, such as the LTL and BF, are commonly vacuum packed and subjected to wet aging for various periods. It would thus be suggested that further research be done on these muscles to elucidate the influence of wet aging on their colour stability.

Fibre type has also been known to influence the colour stability of muscles. Muscles with greater proportions of oxidative fibres (type I) are darker in colour, more prone to oxidation and discolour faster in relation to muscles with greater proportions of glycolytic fibre (O'Keeffe & Hood 1982; Renerre & Labas, 1987). It would thus be expected that game meat, with its propensity to oxidise readily and its darker colour (Hoffman & Wiklund, 2006), would mainly be comprised of type I muscles fibres. However, research done on the fibre types of game meat (Kohn *et al.,* 2011; Curry *et al.,* 2012; North, 2014) and venison (Kiessling & Kiessling, 1984; Essén-Gustavsson & Rehbinder, 1985) have noted that the relative proportions of muscle fibre types occur in descending order of type IIX, IIA and I. This contraction in what is expected and what has been observed definitely warrants further investigation as to how the muscle fibre composition of game meat influences its colour stability. These differences may also explain the differences observed between the colour stability of the game meat when compared to beef.

Modified atmosphere packaging (MAP) could be the ideal solution for improving the colour stability of the LTL and BF of the game species. However, the higher Mb content of the game meat in relation to beef (Vestergaard *et al.,* 2000; Díaz *et al.,* 2002; Kritzinger *et al.,* 2003; Daszkiewicz *et al.,* 2011) should be kept in mind when packing game meat under MAP. If an incorrect gas mixture is used, the higher Mb content may result in blackening of the meat as is sometimes observed in bone marrow (bone blackening) of MAP meat due to its high haemoglobin content. Marrow discolouration is usually minimised through the use of ultra-low oxygen MAP (Grobbel *et al.,* 2006). Thus, it would be suggested that studies be conducted on the effect of MAP on the colour stability of game meat, specifically focussing on the use of ultra-low oxygen MAP.

In its entirety, this study provided important baseline data regarding the colour stability of game meat and the extent to which it is influenced by species, muscle and gender. In addition, this study has highlighted areas for future research and the importance of further research into the colour stability of game meat.

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