Physicochemical and Microbiological Attributes of Black Wildebeest (*Connochaetes gnou*) Muscles

By

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Declaration

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Abstract

The study characterised the physicochemical and microbiological-related meat quality attributes of six muscles [*Longissimus thoracis et lumborum* (LTL), *Biceps femoris* (BF), *Infraspinatus* (IS), *Supraspinatus* (SS), *Semimembranosus* (SM) and *Semitendinosus* (ST)] harvested from culled mature male and female black wildebeest (*Connochaetes gnou*). Descriptive information regarding the respective muscles included carcass characteristics, physical attributes (pH, surface colour, drip loss percentage, weep loss percentage, cooking loss percentage and Warner-Bratzler shear force), chemical attributes (moisture, protein, fat, ash, lipid oxidation and fatty acids profile) and microbiological attributes (total viable count and *Enterobacteriaceae*). Sex and harvesting year were considered as main effects when analysing carcass characteristics, while sex and muscle type were the main effects regarding determination of the physicochemical quality attributes of all muscles. Muscle type and ageing time were the main effects considered in determining the influence of ageing of the LTL and BF muscles.

The average live weight of animals harvested in 2016, did not differ from that harvested in 2017 (149.5 ± 4.23 kg vs. 163.4 ± 5.92kg). Male black wildebeest had a heavier live weight (141.4 ± 5.92kg), and heavier warm and cold carcass weight (89.8 ± 1.91 kg and 85.8 ± 1.99 kg), when compared to the live weight (117.4 ± 4.23kg) and warm and cold carcass weight (71.0 ± 2.67kg and 68.6 ± 2.79 kg) recorded for female animals. The dressing percentage for male black wildebeest (50.2 ± 0.62%) did not differ from that of female animals (48.6 ± 0.87%). Weights of the trachea and lungs were heavier in the animals harvested in 2017. Heavier hide, head, tongue, trotters, heart and spleen weights were recorded for male black wildebeest. Consumable offal (excluding the gastrointestinal tract) contributed 12.7% to male live weights, and 11.2% to female live weight, respectively.

A significant sex effect was observed on the % composition of total polyunsaturated fatty acids (PUFA), total saturated fatty acids (SFA) and polyunsaturated fatty acids to saturated fatty acids ratio (PUFA:SFA). Male black wildebeest meat samples had higher levels of PUFA (35.0%) when compared to the female meat samples (28.9%). This differences was also reflected in a higher PUFA:SFA ratio in males when compared to the females (0.80 vs. 0.60).

The pH_u values of the muscles ranged from 6.50 to 6.59, which is indicative of dark, firm and dry (DFD) meat. Mean CIE L*, CIE b*, Chroma and hue angle values of the muscles ranged from 27.0-33.4, 8.0-10.2, 14.0-15.9 and 34.3-39.7, respectively. Mean cooking loss percentages and Warner-Bratzler shear force (WBSF) values of the muscles ranged from 25.9-33.5% and 3.9-6.5 kg/cm ø, respectively. The LTL and SM muscles had overall lighter appearance than other muscles. The IS muscle had the lowest cooking loss percentage while

the ST muscle had the highest value. The IS and SS muscles had the lowest mean WBSF values, and can thus considered to be the most tender. The LTL muscle was further classified according to pH values; pH>6 being DFD and pH<6 being classified as normal. The DFD LTL was significantly darker in colour, and had a lower cooking loss percentage and WBSF values than normal meat. The moisture, protein, fat and ash content of the respective muscles ranged between 75.6-78.1%, 19.4-22.6%, 1.3-1.8%, and 1.1-1.3%, respectively. The SS muscle had the highest moisture content, while the LTL muscle had the lowest moisture content. The LTL muscle had the highest protein and fat content, compared to the IS muscle that had the lowest fat content.

The fatty acid profile of black wildebeest muscles contained the highest level of SFA, followed by PUFA and lastly the MUFA. The IS muscle contained the highest composition (%) of SFA (68.8 ± 3.71%), whilst the ST had the lowest composition of 47.0 ± 3.70%. The SS muscle had the highest MUFA content of 20.9 ± 1.57%, compared to the IS muscle which had the lowest MUFA content of 8.1 ± 1.57%. The LTL and ST muscles had the highest PUFA content (40.0 ± 3.30% and 40.0 ± 3.29%, respectively), compared to the IS that had the lowest PUFA composition (23.0 ± 3.30%). The LTL and ST muscles had the highest PUFA:SFA ratio (1.0 ± 0.1 and 0.9 ± 0.11, respectively). The BF muscle had the highest *ω-6:ω-3* of 4.5 ± 0.72, whilst the SM muscle contained the lowest ratio (1.20 ± 0.77).

The effect of *post-mortem* ageing on the physicochemical and microbiological attributes of the LTL and BF muscles was investigated. The LTL muscle had a higher weep loss percentage (0.56%), when compared to the BF (0.38%). The TBARS value of the BF muscle was higher (1.32 mgMDA/kg meat) than that of the LTL muscle (1.11 mgMDA/kg meat). The mean pH, cooking loss percentage and Warner-Bratzer shear forces values decreased with an increase in ageing time. Mean TBARS values, total viable counts and *Enterobacteriaceae* counts increased with a longer ageing period. After considering the changes in the aforementioned attributes, it was concluded that black wildebeest meat should be aged for at least 12 days under chilled vacuum packaging. Black wildebeest meat microbial counts remained within the suggested limits for human consumption in this study. The meat is also characterized by a low fat content and high protein content, which make it suitable for consumption by consumers looking for healthy red meat.

Opsomming

Die studie het die fisiochemies- en mikrobiologies-verwante eienskappe betreffende vleisgehalte van ses spiere [*Longissimus thoracis et lumborum* (LTL), *Biceps femoris* (BF), *Infraspinatus* (IS), *Supraspinatus* (SS), *Semimembranosus* (SM) en *Semitendinosus* (ST)] verkry van volwasse manlike en vroulike swartwildebees (*Connochaetes gnou*) wat uitgedun is, vasgestel. Beskrywende inligting oor die onderskeie spiere sluit in karkaseienskappe, fisiese eienskappe (pH, oppervlakkleur, drupverliespersentasie, weegverliespersentasie, kookverliespersentasie en Warner-Bratzler skeurkrag), chemiese eienskappe (vog, proteïen, vet, as, lipied oksidasie en vetsuurprofiel) en mikrobiologiese eienskappe (totale mikrobe telling en *Enterobacteriaceae*). Geslag- en oesjaar is as hoof-effekte in die analise van karkaseienskappe beskou, terwyl geslag en spiertipe die belangrikste effekte was in die analise van die fisio-chemiese eienskappe van al die spiere. Spiertipe en verouderingstyd was die belangrikste effekte wat oorweeg was om die invloed van veroudering op die LTL- en BF-spiere te bepaal.

Die gemiddelde lewendige gewig van diere wat in 2016 geoes is, het nie verskil van dié diere wat in 2017 geoes is nie (149.5 ± 4.23 kg vs 163.4 ± 5.92kg). Manlike swartwildebeeste het 'n swaarder lewende gewig (141.4 ± 5.92 kg) en swaarder koue karkasgewig (89.8 ± 1.91 kg en 85.8 ± 1.99 kg) in vergelyking met die lewendige gewig (117.4 ± 4.23 kg) en warm en koud karkasgewig (71.0 ± 2.67kg en 68.6 ± 2.79 kg) van die vroulike swartwildebeeste gehad. Die uitslagpersentasie vir manlike swartwildebeeste (50.2 ± 0.62%) het nie verskil van die -persentasie van die vroulike diere nie (48.6 ± 0.87%). Gewigte van die tragea en longe was swaarder in die diere wat in 2017 geoes is. Swaarder vel-, kop-, tong-, poot-, hart- en miltgewigte is aangeteken vir manlike swartwildebeeste. Afval (uitgesonderd die spysverteringskanaal) het onderskeidelik 12.7% en 11.2% tot die lewende gewig van die manlike en vroulike swartwildebeeste bygedra.

‘n Betekenisvolle invloed van geslag is waargeneem in terme van die persentasie samestelling van totale poli-onversadigde vetsure (PUFA) en totale versadigde vetsure (SFA), asook die poli-onversadigde vetsure tot versadigde vetsuur verhouding (PUFA:SFA). Manlike swartwildebees vleismonsters het hoër vlakke van PUFA (35.0%) in vergelyking met die vroulike vleismonsters (28.9%) gehad. Dié verskille is ook weerspieël in 'n hoër PUFA: SFA verhouding in die vleismonsters van die manlike swartwildebeeste, wanneer vergelyk met die -monsters van die vroulike swartwildebeeste (0.80 vs. 0.60).

Die pH_u waardes van die onderskeie spiere het varieer tussen 6.50 en 6.59, wat aanduidend is van donker, ferm en droë (DFD) vleis. Die gemiddelde CIE L *, CIE b *, Chroma en kleurvoorkomswaardes van die spiere het onderskeidelik tussen 27.0 en 33.4, 8.0 en 10.2, 14.0 en 15.9 en 34.3 en 39.7 gevarieer. Die gemiddelde kookverliespersentasie en Warner-

Bratzler skeurkrag (WBSF) waardes van die spiere het onderskeidelik van 25.9-33.5% en 3.9-6,5 kg/cm ø gewissel. Die LTL en SM spiere het ’n algehele ligter voorkoms as die ander spiere gehad. Die IS-spier en die ST-spier het onderskeidelik die laagste en hoogste kookverliespersentasie gehad. Die IS- en SS-spiere het die laagste gemiddelde WBSF waarde gehad en kan dus as die sagste spiere beskou word. Die LTL spier is ook volgens pH-waardes geklassifiseer, met ’n pH>6 wat as DFD en ’n pH<6 wat as normaal geklassifiseer word. Die DFD LTL was aansienlik donkerder van kleur en het 'n laer kookverliespersentasie en WBSF waarde as die ander normaal geklassifiseerde vleismonsters gehad. Die vog-, proteïen-, vet- en as-inhoud van die onderskeie spiere het onderskeidelik tussen 75.6-78.1%, 19.4-22.6%, 1.3-1.8% en 1.1-1.3%, gewissel. Die SS- en LTL spier het onderskeidelik die hoogste en laagste voginhoud gehad. Die LTL spiere is gekenmerk deur die hoogste proteïen- en vetinhoud, in vergelyking met die IS spiere wat die laagste vetinhoud gehad het.

Die vetsuurprofiel van swartwildebees spiere bevat die hoogste vlak van SFA, met medium vlakke van PUFA en lae vlakke van MUFA. Die IS-spier het die hoogste SFA samestelling (68.8 ± 3.71%) gehad, terwyl die ST die laagste samestelling van 47.0 ± 3.70% gehad het. Die SS-spier het die hoogste MUFA-inhoud van 20.9 ± 1.57%, in vergelyking met die IS-spier wat die laagste MUFA-inhoud van 8.1 ± 1.57% gehad het. Die LTL- en ST-spier het die hoogste PUFA-inhoud (onderskeidelik 40.0 ± 3.30% en 40.0 ± 3.29%)gehad, wanneer dit met die IS vergelyk is, wat die laagste PUFA-samestelling gehad het (23.0 ± 3.30%). Die LTL- en ST-spier het die hoogste PUFA: SFA verhouding (1.0 ± 0.1 en 0.9 ± 0.11, onderskeidelik) gehad. Die BF spier het die hoogste ω-6: ω-3 van 4.5 ± 0.72 gehad, terwyl die SM-spier die laagste verhouding (1.20 ± 0.77) bevat.

Die effek van nadoodse veroudering op die fisio-chemiese en mikrobiologiese eienskappe van die LTL- en BF-spier is ondersoek. Die LTL spier het 'n hoër uitloogverlies persentasie (0.56%), in vergelyking met die BF (0.38%) gehad. Die TBARS-waarde van die BF-spier was hoër (1.32 mgMDA/kg vleis) as dié van die LTL spier (1.11 mgMDA/kg vleis). Die gemiddelde pH, kookverliespersentasie en Warner-Bratzer skeurwaarde het afgeneem met 'n toename in verouderingstyd. Die gemiddelde TBARS waarde, totale mikrobe - en *Enterobacteriaceae* tellings het met 'n toename in verouderingstydperk toegeneem. Na oorweging van die veranderinge in bogenoemde eienskappe, is die gevolgtrekking dat swart wildebees vleis vir ten minste 12 dae onder verkoelde omstandighede en vakuumverpak verouder moet word. Swartwildebees vleis mikrobe tellings in die studie was binne die voorgestelde grense vir menslike verbruik gebly. Die lae vetinhoud en hoë proteïeninhoud maak dit ook aanvaarbaar vir gebruikers wat verkies om lae-vet rooivleis te geniet.

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List of abbreviations

AOAC	Association of Official Chemists
ANOVA	Analysis of Variance
Anon.	Anonymous
BF	*Biceps femoris* muscle
DFD	Dark, firm and dry
FA	Fatty acid
FAME	Fatty acids methyl esters
g	Gram
h	Hour
ha	Hectare
IS	*Infraspinatus* muscle
kg	Kilogram
L	Litre
LSD	Least significant difference
LTL	*Longissimus thoracis et lumborum* muscle
mg	Milligram
min	Minute
mL	Millilitre
MUFA	Monounsaturated fatty acid
pH_u	Ultimate pH
PUFA	Polyunsaturated fatty acid
PUFA/SFA	Polyunsaturated fatty acid to saturated fatty acid ratio
s	Second
SFA	Saturated Fatty acid
SM	*Semimembranosus* muscle
SS	*Supraspinatus* muscle
ST	*Semitendinosus* muscle
WHC	Water holding capacity

Notes

Results from this study have been presented at the following symposium:

50th *South African Society for Animal Science* (SASAS) congress, 18-21 September 2017, Port Elizabeth, Eastern Cape, South Africa

Student oral presentation: Physicochemical meat quality attributes of black wildebeest (*Connochaetes gnou*) muscles

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Chapter 1: General introduction

Farming of domestic livestock has proven to be a challenge due to the prevailing drought conditions in South Africa; as a result farmers are resorting to farming either a mixture of game-livestock or game exclusively. Farming of game species is growing into a valuable utilisation of game, this practice is also important owing to the current drought in South Africa. South Africa is reported as one of the most water stressed African countries (Otieno & Muchapondwa, 2016). Game species have less nutritional requirements compared to domestic livestock, the animals are also farmed in natural habitats with minimum human interventions and thus game meat has been reported to have potential of being marketed as an organic product. Game meat also contains a higher protein content and lower fat content than domestic red meat, thus it is a healthier red meat alternative which suits the modern health conscious consumer (Hoffman & Wiklund, 2006). The consumption of game meat has increased over the years however; there is still limited information about its quality attributes. The investigation of the meat quality attributes of game meat will make it competitive to other meat types as well as improve consumer perception of its quality (Kohn *et al.*, 2005).

Black wildebeest (*Connochaetes gnou*) is one of two African wildebeest species and is also commonly known as the white-tailed gnu (Smithers, 1983; Booyse & Dehority, 2012; Oberem & Oberem, 2016). Black wildebeest is known for its running outbursts during harvesting, and it thus prone to high levels of ante-mortem stress which result in the production of dark, firm and dry (DFD) meat (Shange *et al.*, 2018). Glycogen stores are harshly depleted at the time of death, thus less lactic acid accumulates in the muscle resulting in a high ultimate pH (above 6.0) (Greaser, 2001). This species was once almost hunted to extinction but their numbers have recovered due to conservational efforts by wildlife farmers. The annual population growth of 28-33% of black wildebeest makes it a viable meat production species (Hoffman *et al.*, 2009). Like many game species, black wildebeest adapts easily to available forage and various environmental conditions.

Animal sex influences the physical and chemical composition of meat. Male animals are usually heavier and larger than females, however, female animals reach maturity sooner than males (Lawrie & Ledward, 2006). Male animals tend to produce leaner meat compared to females, this is due to the increased physical activity levels in mature male animals. In wild ungulates, male animals are known to lose body weight during the mating and rutting season. The fatty acid content and composition also differs between sexes; female animals tend to have a higher content of saturated fatty acids whereas males have a higher polyunsaturated fatty acid content.

Meat quality also differs with regards to the muscle location in the carcass. Domestic red meat is commonly sold as various cuts regularly made up of more than one muscle as well as value-added products (Paton *et al.*, 2010). The *Longissumus thoracis et lumborum* (loin) muscle is the most sought after muscle in the industry due to its commercial value, whilst other muscles are often not taken into account. Game meat also has potential to be marketed as individual muscles rather than cuts, this warrants more research to be conducted on the various muscles of meat producing species to allow for muscle assortment for different products within the meat industry.

Tenderness is amongst the most important factors which influence meat quality; consumers prefer tender meat over tough meat. The amount and quality of collagen in muscles directly affects meat tenderness; muscles with more total collagen are reported to produce tougher meat than those with less collagen (Ba *et al.*, 2014). On the other hand, a high content of soluble collagen in the muscles leads to more tender meat although the total collagen content is high (Dominik *et al.*, 2012). Game meat was traditionally used to produce dried meat products, during those times meat tenderness was not considered as factor. Now a days, game animals have gained potential for use in fresh meat production which warrants studies to be conducted on the tenderness of various game meat producing species. Ageing is an important process which improves the tenderness of meat, this process is vital for game species which are renowned for producing tougher meat (Lawrie & Ledward, 2006).

Therefore the aim of this study was to investigate the physicochemical (pH, colour, drip loss, cooking loss, tenderness, moisture, protein, fat, ash and fatty acids profile) and microbiological (total viable count and *Enterobacteriaceae*) meat quality attributes of male and female black wildebeest (*Connochates gnou*) muscles [*Longissimus thoracis et lumborum* (LTL), *Biceps femoris* (BF), *Infraspinatus* (IS), *Supraspinatus* (SS), *Semimembranosus* (SM) and *Semitendinosus* (ST)]. The effect of animal sex on the carcass composition of black wildebeest and the aforementioned meat quality attributes was also investigated in the study. As there is interest in using the major muscles from this species as fresh meat, the effect of chilled ageing on the physicochemical quality and microbial safety of aged meat was also investigated.

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Chapter 2: Literature review

2.1 Introduction

Game meat refers to meat obtained from non-domesticated (wild) animals that can be hunted and used for human consumption (Klein, 2005). In countries such as Australia, New Zealand, Europe and America the term venison is used which refers to meat from all game animals, including domesticated and farmed animals. Deer is the common venison species. In Africa, game animals are wild and free-running (Hoffman & Wiklund, 2006). In South Africa, game meat is commonly obtained from springbok, kudu, blesbok, black wildebeest, blue wildebeest, deer, zebra, impala and gemsbok (Hoffman *et al.*, 2004, 2011). The consumption of game meat has increased significantly over the years and this could be attributed to game meat being perceived as a healthier alternative to domestic red meat. Game meat is reported to contain much less fat (2- 3%) and more protein compared to that derived from traditionally domesticated animals (van Schalkwyk & Hoffman, 2010), making it a healthy alternative to domestic red meat. Although game meat possesses these positive attributes there is still a lack of knowledge on the preparation thereof and for this reason, consumers show little interest in purchasing it. Game meat is generally tougher and darker than domestic red meat; the darker colour makes game meat appear less appealing to consumers. However, game meat is traditionally used for the making of biltong and droëwors, biltong being a popular dried meat product whilst droëwors is a dried sausage that has had fat added to it (Jones *et al.*, 2017).

This literature review will cover the overview of the South African game industry followed by the characterization of the game species of interest - black wildebeest (*Connochaetes gnou*). A brief discussion of the quality attributes of game meat will follow, wherein game meat will be compared to domestic red meat in terms of nutritional quality. There are various factors that affect meat quality, of these factors the effect of pre-slaughter/*ante-mortem* stress, pH of meat and microbial counts will also be briefly discussed.

Black wildebeest is reported to run at very high speeds (up to 70 km/h) over long distances and are also fatigue resistant. Running is their escape strategy from predators (Kohn *et al.*, 2011). Due to the stress susceptibility of black wildebeest, this study aims to investigate the quality attributes of the meat taking into account that the species is prone to *ante-mortem* stress. The pH of the meat *post-mortem* is an indication of the stress that the animal experienced *ante-mortem*, the pH remains high (> 6) for a stressed animal. The pH remains high due to the depleted glycogen reserves in the muscle typically as a result of high *ante-mortem* stress, thus insufficient glycogen is anaerobically converted to lactic acid (Lawrie & Ledward, 2006). Such meat is characterised by a dark surface colour and firm texture, this is

known as dark, firm and dry (DFD). The dark colour is a result of absorption of light whereas the firm texture is brought about by the retention of water causing the muscle fibres to swell (the water is strongly bound to the protein matrix as a result of the high pH) (Warris, 2000). The meat from a stressed animal is also tougher than that of a normal animal since the shortening of muscle tissues as it develops *rigor mortis* occurs much quicker. At the completion of *rigor mortis* the muscle remains tough and inextensible. Tenderness or toughness can be improved by ageing where processes such as protein denaturation and proteolysis occur to increase tenderness. Ageing or conditioning is the process where meat is stored at temperatures above its freezing point to improve tenderness and flavour (Lawrie & Ledward, 2006). Currently no work has been done on the ageing of black wildebeest meat; the research project will investigate the effect of ageing on black wildebeest meat quality with the main aim of determining the optimum tenderisation period of black wildebeest meat.

2.2 Overview of the South African game industry

The South African game industry is described as a free-market enterprise wherein opportunities are generated for game ranchers as well as game meat producers (Hoffman *et al.*, 2004). The commercial use of game species in South Africa has increased tremendously over the years; this has resulted in farmers playing an imperative role in the conservation of many game species. Most game ranches in South Africa are in Limpopo, Northern Cape, Eastern Cape and Mpumalanga provinces. In 1998, Limpopo had an estimated 2 300 game ranches, which covers approximately 3.6 million hectares of land. Game farmers utilise approximately 17-18 million hectares of the country and this is growing at 2.5% each year. An estimate of 9 000 farms were used for the production of wildlife in the year 2005 and a combination of wildlife production and cattle farming utilised a further 15 000 farms. To date there are approximately 10 000 privately owned game ranches in South Africa; these accommodate up to 12 million head of game (WRSA, 2016). Game farmers use approximately 20 million hectares of land. The Limpopo province contains approximately 49% of the wildlife ranches, then the Northern Cape at 19.5% and lastly the Eastern Cape Province at 12.5%. The average size of a game ranch in the Northern Cape is 4 920 hectares and that in the Limpopo province is around 1 340 hectares (Hoffman, 2007). Game (or wildlife) can be used in both consumptive and non-consumptive ways. Examples of the consumptive utilisation of game include trophy-hunting, biltong hunting, culling for the venison/game meat market, as well as live capture and sale. The hunting industry generated an estimated R400 million in the year 1995, the Limpopo province alone generating approximately R221 million annual turnover. The amount of revenue generated from the game industry has grown

dramatically the past number of years with 2016 data indicating that trophy hunting alone contributed nearly R2 billion to the South African economy. It is also estimated that the local "biltong" hunters contribute a further R8 billion to the economy (Netwerk24). As indicated, hunting contributes the most in the generated amounts, followed by live sales and ecotourism. Formal (defined as meat moving through registered abattoirs) game meat production only contributes 3.7% towards the annual turnover (Hoffman, 2007). Game ranching currently contributes approximately R20 billion each year towards the economy of the country through trophy hunting, biltong hunting, culling for game meat marketing, live capture for breeding as well as ecotourism (WRSA, 2016).

Game meat in supermarkets is only available during the winter season (June to September) when hunting occurs more frequently (Hoffman *et al.*, 2004). The most limiting factor concerning the purchasing of game meat is the availability of the meat that has been passed through an approved abattoir and is therefore legal to sell to the public. Game meat is frequently purchased as entire carcasses that are cut in the supermarkets where they are sold (Hoffman *et al.*, 2004). However, when there is a prohibition on the export of game meat (usually due to outbreaks of Foot and Mouth Disease), then the large export companies typically supply processed (deboned, primal cuts as well as other processed products) game meat into the formal supply chain. Meat quality, seasonal availability as well as supplier reliability are the main factors that affect the purchase of game meat. Winter is the traditional hunting season, since the cooler conditions help to prevent the carcasses from spoiling after cropping and dressing (Hoffman *et al.*, 2004). In fact, it is estimated that the game ranching industry provides more than 20% of red meat consumed in South Africa during the hunting season (WRSA, 2016).

The game industry in South Africa has shown tremendous growth and development throughout the years (Cloete, 2015). In the 1900s, the game ranching industry focused more on the consumptive and non-consumptive ways to use wildlife such as hunting, ecotourism and other related activities. However, in the recent years (2000s), the focus has shifted more towards breeding higher value, and or colour and morphological variations (Cloete, 2015). The value of game animals sold at auctions has showed an increase from R93 million in the year 2005 to approximately R1.8 billion in the year 2014, an estimated annual increase of about 26% (Cloete, 2015). However, as the prices are increasing and demand seems to be leaning towards exceeding the supply, such tremendous growth is not likely to occur in the near future. In fact, data from sales in 2016-2017 seem to indicate that there has been a decrease in the number of animals sold on public auction as well as the prices paid for some of the more exotic colour variants (Fig 2.1 and Fig 2.2).

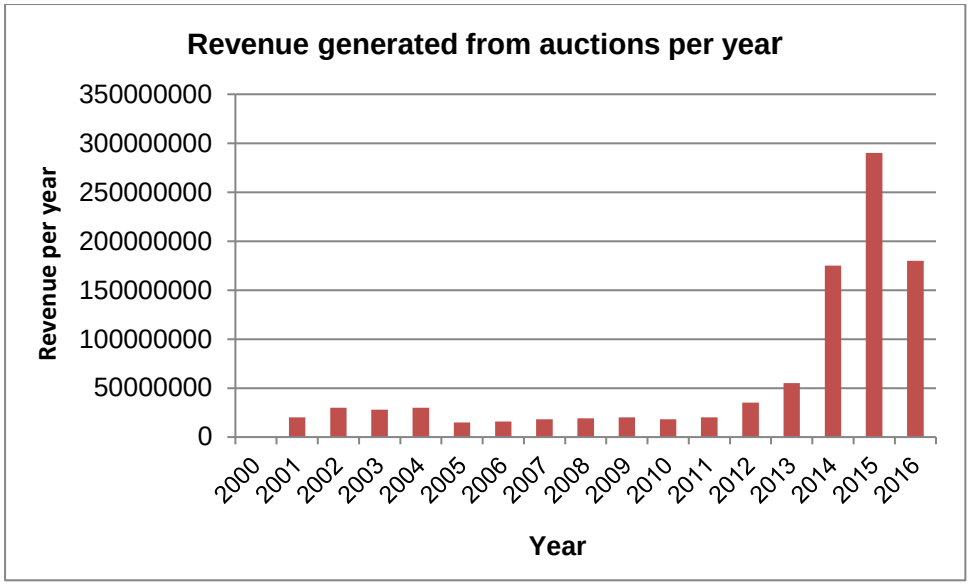

Figure 2.1 Revenue generated from plains game species sold at auctions between the years 2000-2016 (Anon, 2017).

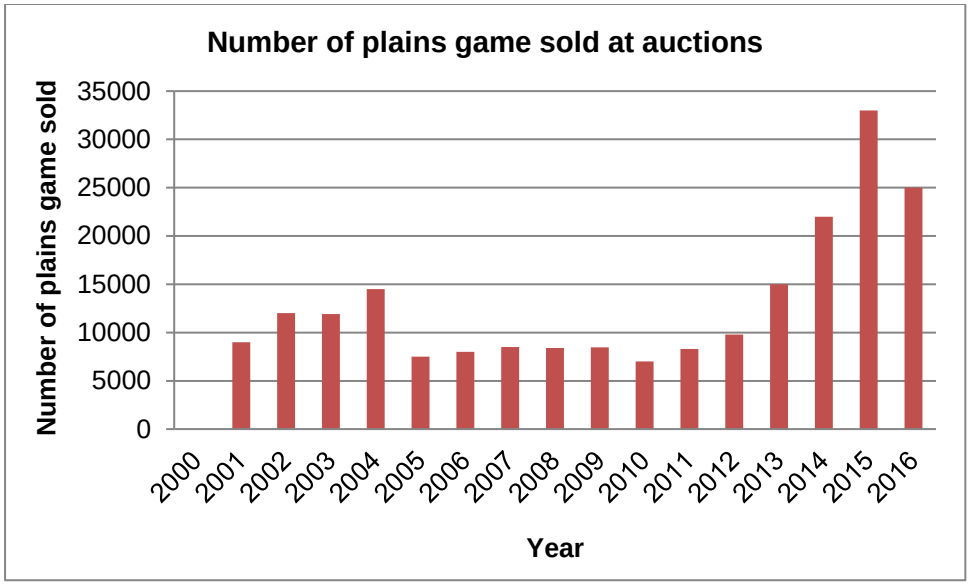

Figure 2.2 Number of plains game species sold at auctions between the years 2000-2016 (Anon, 2017).

Although live breeding seems to be the centre of economic contribution and successive growth of the game ranching industry, the growth rate is likely to decline in the near future. There has been a decline in the number of animals hunted for biltong or trophy hunting between the years 2005 and 2013. This is attributed to the economic crisis in the country as well as globally, which resulted in a decline of foreign hunters coming to South Africa (Cloete,

2015). Also, with the rise in breeding of colour variants, the price of the normal coloured animals has also risen sharply resulting in the local hunters being unable to afford to hunt the numbers of animals that they had hunted in the past. The decline is accompanied by the changes in the firearm act of 2004, the successive growth of the Namibian hunting industry and the economic crisis in South Africa.

None the less, the consumptive use of game animals in South Africa is increasing; the number of game animals is also increasing as more hectares of land become dedicated to game ranching. In order to ensure that game ranching remains an economically viable option of land use, the consumptive markets should be well established and new consumptive market opportunities in the industry should be developed further. Although game meat is consumed at noticeable volumes in South Africa, game meat remains inadequately marketed and many consumers lack adequate knowledge about game meat. This shows that there is a potential for new developments within the game ranch industry in South Africa. The South African game ranch industry offers a unique range of game species such as springbok, impala, blesbok, duiker, Cape eland, blue and black wildebeest; all suitable for meat production.

2.2.1 Marketing of game meat

In South Africa, game farming is utilised through hunting, ecotourism, breeding of rare species, and game meat sales; hunting making the largest contribution towards the economy of the game farm tourism (van der Merwe & Saayman, 2003). In South Africa, one of the more profitable ways to market wildlife is through the production of game meat (van Schalkwyk & Hoffman, 2010). This plays a significant role in increasing the financial viability of game farms. Hunting of surplus stock for the production of biltong is the major consumptive use of wildlife in Southern Africa. Game species adapt well to available food as well as the various environmental factors that affect their growth and development. In comparison to domestic animals, these animals show resistance to illnesses and parasites. Game species also have a higher meat yield than the domestic animals (van Schalkwyk & Hoffman, 2010). Game meat can be marketed as biltong, dried sausage (droëwors), fresh sausage, fresh game meat, meat cuts, roasts, fillets and salami (Hoffman *et al.*, 2004). Van der Merwe and Saayman (2003) highlighted the potential of game meat being sold as an exotic product to the modern health conscious consumer. Game meat has the potential to be marketed as a healthier alternative to domestic red meat due to its very low fat content (2-3%) and high protein per gram of meat (van Schalkwyk & Hoffman, 2010). In comparison to domesticated animals, game offers a wider range of species to suit the different needs and tastes of consumers (van der Merwe & Saayman, 2003).

2.2.2 Harvesting of game species

Hoffman and Wiklund (2006) describe harvesting as the killing of animals for the purpose of meat production. Due to their wild nature, game animals are harvested using different systems to the domestic livestock. The handling of animals during harvesting plays an important role in the overall quality of meat obtained. Harvesting or cropping of animals should be performed in such a manner that it reduces pre-slaughter stress levels in animals as well as damage from bullets and wounds; these factors have an effect on the quality of meat as well as other by-products obtained from the animals after harvesting. For plains game species such as the black wildebeest night cropping is the most suitable harvesting system since the animals are less stressed. However, due to the dark head as well as shape of the horns, skill is required to be able to shoot these animals in the head. Animals are shot at distances up to 150 m away from the vehicle (Hoffman & Laubscher, 2009). Methods such as the use of vehicles, boma capture or helicopters are used during commercial harvesting of plains game species (Hoffman & Wiklund, 2006). Each method is more suitable for a certain species. During night cropping spotlights are used to immobilise the animals prior to being shot. Trained and experienced expert marksmen are preferred when harvesting, this is to ensure minimal bullet damage to the animal/meat. There are desired shooting sites on the animals when harvesting animals especially for meat production. Head and neck shots ensure instantaneous death of animals and less wounds on the carcass. A light calibre silenced rifle is often used during night cropping and apparently has little effect on meat quality (Hoffman & Laubscher, 2009b, 2010, 2011). After being shot, animals are immediately exsanguinated using a sterile knife and hung on the side of the vehicle. It is important that each animal get its own tag for labelling purposes (Hoffman & Wiklund, 2006). Harvesting continues until a set number of animals is obtained. The carcasses are then transported to the field abattoir where further processing takes place. Carcasses can be transported to the processing facility either skin-on or off, but the skin should be removed prior to any cutting of the meat (van Schalkwyk & Hoffman, 2016). In the field abattoir, the skins are removed in the dirty area, then completion of mid-ventral incision, removal of intestines, thoracic organs, liver and pluck are removed from the clean carcass, although the removal of the brown offal is allowed in the field after exsanguination if the killing area is far from the field depot (van Schalkwyk & Hoffman, 2016). It is important that the dirty and clean working areas are kept separate to ensure less contamination of the carcass. At the completion of dressing, carcasses are then loaded into a chilling facility; this must be done not more than 2 hours after shooting the animal (Hoffman & Wiklund, 2006; van Schalkwyk & Hoffman, 2016). Game carcasses should be clearly separated from domestic carcases to avoid cross contamination; facilities should be cleaned thoroughly before and after

handling the carcass (van Schalkwyk & Hoffman, 2016). An ideal harvesting system is depicted in the Figure 2.3 below, as adapted from van Schalkwyk & Hoffman (2010, 2016).

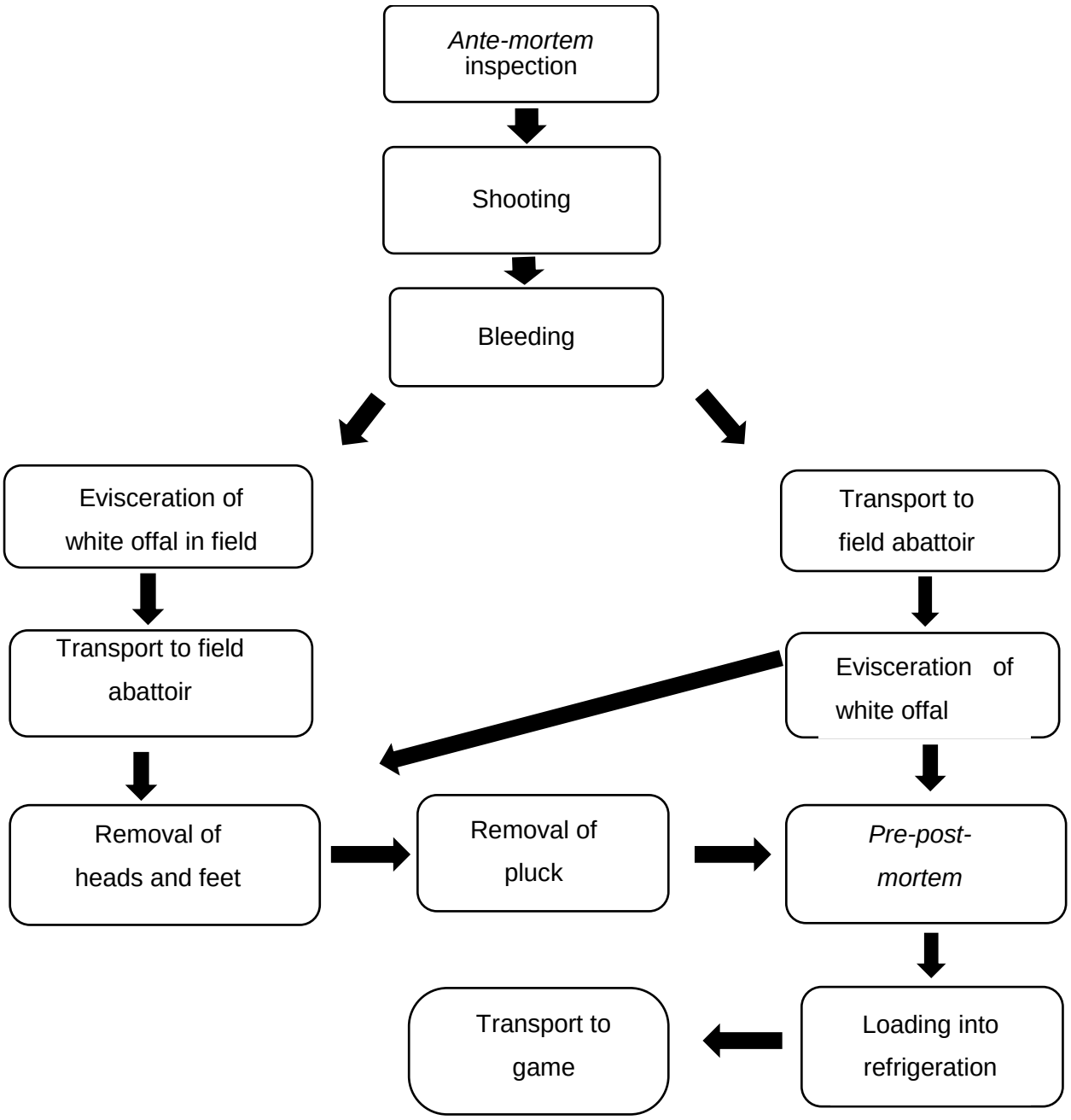

Figure 2.3 The ideal harvesting process (van Schalkwyk & Hoffman, 2010, 2016).

2.3 Characterisation and production potential of black wildebeest (*Connochaetes gnou*)

Black Wildebeest (*Connochaetes gnou*) is one of the two African wildebeest species and is also commonly known as the white-tailed gnu (Booyse & Dehority, 2012; Oberem & Oberem,

2016). *Connochates gnou* belongs to the Alcelaphinae subfamily under the family Bovidae (Smithers, 1983). The characteristic feature of the Black wildebeest is its tail that is dark at the base with the remainder having long off-white hair that almost reaches the ground. Although the colour black is not descriptive of its appearance, at a distance the Black wildebeest appears darker in comparison to its close relative the Blue Wildebeest (*C. taurinus*) which has a silvery-grey colour (Smithers, 1983). The black wildebeest have a dull brown colour, with the older males being almost black. The male black wildebeest have an average weight of 180 kg while the females are lighter (160 kg). The black wildebeest are smaller than the blue wildebeest (Oberem & Oberem, 2016). The horns from both sexes arise from expanded bases, sweep downwards and forwards then curve upwards. The male horns are heavier than those of the females. The face appears darker in colour than the rest of the body. Black wildebeest are the most unusual existing antelopes with the mane of a horse, the face of a steer as well as the delicate legs of a buck. They possess a distinct beard of long hair, particularly on the chest extending from between the forelegs almost to the stomach. The front feet have slightly larger hooves compared to those on the hind feet. The large size of the front feet is attributed to the extra weight of the shoulders and head (Smithers, 1983). Black wildebeest are endemic to the South African sub region. The species used to be distributed over the central, northern as well as north-eastern parts of the Cape Province in many numbers. However their number had declined over the years due to over-exploitation and agricultural development, this almost brought the species to extinction; it is estimated that in the 1950's there were less than 500 animals left. The animals were only protected on farms and reserved areas. Conservation by farmers has increased the number to more than 20 000 animals with 80% being found on privately owned land (Oberem & Oberem, 2016). Open grass veld is the preferred habitat for this species, tall grass areas and thick vegetation are mostly avoided.

The production potential of an animal is an important trait to consider for meat producing species. Various environmental and intrinsic factors such as species, diet, reproduction rate and sex influence the production potential of an animal. For successful farming of a game meat producing species, it is required that the animal should have high fecundity; possess an exceptional food conversion ratio and be flexible in the diet they consume; be preferably polygamous instead of monogamous and show consumer acceptance. The chosen species for meat production should provide meat of good yield and nutritional quality (Hoffman & Cawthorn, 2013) as the modern consumer is health conscious and prefers lean meat due to the health benefits associated with lean meat (Hoffman & Wiklund, 2006). Black wildebeest have an annual population growth between 28-33%; this is of significant value in meat production (Hoffman *et al.*, 2009b; Fustenberg, 2010). This species also adapts easily to various environments (Hoffman *et al.*, 2009b).

2.4 Factors that influence meat quality

Meat quality is influenced by various factors which include geographical location, *ante-mortem* stress, sex, muscle type (anatomical location), microbial counts, content and composition of intramuscular lipids, to list but a few. Those of particular interest in this study include the effect of *ante-mortem* stress as well as sex and muscle type.

2.4.1 *Ante-mortem* stress

An important *ante-mortem* factor is the stress undergone by the animal during the harvesting process. The level of stress experienced by the animal during harvesting has a direct effect on its meat quality. Stress experienced during harvesting normally involves excessive physical activity that depletes the glycogen reserves in the muscles and thereby causing insufficient glycogen to be converted to lactic acid in the muscle *post-mortem*. This results in a high ultimate pH (>6) in the muscle, causing the meat to appear darker than normal, a phenomenon known as dark, firm and dry (DFD) meat. The eating quality of DFD meat is reported to be inferior (Lawrie & Ledward, 2006), the meat becomes susceptible to microbial spoilage (Shange *et al.*, 2018) and the flavour is reduced (Silva *et al.*, 1999). In a stressed animal, the muscles shorten much quicker than normally, this results in the production of tougher meat (Herrera-Mendez *et al.*, 2006). However, DFD meat is reported to have a higher tenderization rate compared to meat of normal pH (Silva *et al.*, 1999).

Living organisms under stress release signals that are directed to the cells; the first signal being released are hormonal. The most common phenomenon caused by *ante-mortem* stress that has been researched widely is pale soft and exudative (PSE) meat typically found in pigs. Under severe stress, the cells receive death-inducing signals through receptors of cellular death. On the other hand, under less intense stress conditions the cells adapt a rapid defence mechanism by synthesis of different proteins know as heat shock proteins. These heat shock proteins slow down the process of cell death and create a hurdle to good quality meat (Ouali *et al.*, 2006) . Black wildebeest is renowned for showing running outbursts during harvesting and thus commonly producing DFD meat due to the high ultimate pH in the muscle (Kohn *et al.*, 2011; Shange *et al.*, 2018).

2.4.2 Sex

Animal sex is reported as one of the most important factors that influence meat quality. Male animals are heavier and larger than female animals, although female animals reach maturity sooner than males (Lawrie & Ledward, 2006). Sexual dimorphism on the carcass

characteristics (live weight, carcass weights and dress-out percentage) has been studied and reported on certain game species such as impala, fallow deer, blue wildebeest and greater kudu (Hoffman *et al.*, 2005, 2007, 2009; Mostert & Hoffman, 2007; Ludwiczak *et al.*, 2016; North *et al.*, 2016). No sex differences were found in body and carcass weights of black wildebeest, although males had higher a dressing percentage than females (Hoffman *et al.*, 2009b). Stanisz *et al.* (2015) also did not find sex differences in the mean body and carcass weights as well as the dressing percentages of male and female fallow deer (*Dama dama*). Blesbok (*Damaliscus dorcas phillipsi*) also did not show sex differences in the carcass components and chemical composition of the meat (Hoffman *et al.*, 2008).

Male game species tend to have higher pH values in the muscles than females (Hoffman, 2000). The higher pH has been attributed to the higher activity levels in male animals compared to females which spend most of their days lying in the shade (Smithers, 1983). Male animals are reported to have a higher myoglobin content as a result of their increased physical activity than females. As discussed, a consequence of a high pH has been reported to be the production of DFD meat which typically results from *ante-mortem* stress (Lawrie & Ledward, 2006). No sex differences were observed in the pH_u values of roe deer (*Capreolus capreolus*) (Daszkiewicz *et al.*, 2012). On the other hand, female springbok were found to have higher pH values than males, although the values were below those reported for DFD meat (North *et al.*, 2016).

Water holding capacity (WHC) is an important factor that influences the purchasing intent of consumers, meat with higher fluid losses has a less appealing appearance to consumers. Sex differences in the water holding capacity are inconclusive; a lower drip loss percentage was reported for male roe deer than that of females. No sex differences were reported for water holding capacity measurements of female and male fallow deer, kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus*) (Hoffman *et al.*, 2009a; Stanisz *et al.*, 2015). It would therefore seem as if the WHC is more a factor of *ante-mortem* stress and its effect on the muscle pH (and the effect thereof related to the iso-electric point of muscle protein) than of sex, except where *ante-mortem* activity is linked to sex.

Female animals tend to have a higher intramuscular fat content than males due the increased level of physical activity in male animals. The lower intramuscular fat content in male animals is influenced by seasonal behavioural patterns, the males utilise most of their energy during the mating and rutting season which leads to a lower fat content and leaner meat than females (Hoffman *et al.*, 2009b). For example, male springbok had a higher moisture content and consequently lower fat content than females (Hoffman *et al.*, 2007b). Females black wildebeest are reported to spend most of their time feeding as opposed to males (Smithers, 1983). The chemical composition of meat from female animals is also

influenced by gestation and lactation. Female roe deer were found to have higher dry matter, protein, and fat content than males (Daszkiewicz *et al.*, 2012). The fatty acid composition of female game species typically consists of monounsaturated fatty acids (MUFA; mainly C18:1) whereas males have higher levels of saturated fatty acids (SFA) such as C14:0, C16:0, C18:0, C20:0 and a higher level of polyunsaturated fatty acids (PUFA; C18:2, C18:3, C20:3) (Hoffman *et al.*, 2005; Mostert & Hoffman, 2007; Daszkiewicz *et al.*, 2012).

Differences in tenderness have also been noted between sexes; female black wildebeest were reported to produce more tender meat than males (Hoffman *et al.*, 2009b). However, Daszkiewicz *et al.* (2012) did not find sex differences in the tenderness of male and female roe deer.

From the afore-mentioned studies it is evident that sex has a significant influence on the physicochemical quality attributes of meat, although this differs between species. Thus this warrants the investigation of the effect of sex on meat quality of various meat producing game species.

2.4.3 Muscle type

Beef muscles are marketed as fillets, loin or muscles from the forequarters and hindquarters, while others are processed further to produce mince and sausages (Paton *et al.*, 2010). There is limited information about the marketing of the different muscles from game meat, and availability of information on the muscle types would allow the game meat industry to select which muscle to use for which cuts or process further. Also, it is well established that different domesticated and farmed species (e.g. beef vs lamb vs pork) differ in their meat quality attributes at a muscle level. Strangely, it is assumed by many that game species all have the same meat quality although from a scientific viewpoint this cannot be so when all the factors that influence meat quality are taken into account. Therefore, research on specific game species will provide information that can improve consumer knowledge with regards to the positive aspects of game meat as well as add value to the meat industry.

Skeletal muscles differ in their overall size, shape, anatomical location in the animal, level and type of activity in the animal, blood and nerve supply, association with other tissues as well as action (fast or slow) (Lawrie & Ledward, 2006). The aforementioned differences between muscles occur due to the different functions of the muscles. Skeletal muscles' properties which distinguish between the different muscle types include contractile protein integrity, sarcomere length, connective tissue content, endogenous protease activity, and intramuscular fat levels. The aforementioned factors influence meat quality; particularly the

tenderness, meat colour, flavour, as well as juiciness (Taylor, 2004; Lawrie & Ledward, 2006). Muscles are composed of different types of muscle fibres which differ in their contractile and metabolic properties (Lee *et al.*, 2010). Muscle fibres influence meat quality in three ways; firstly the larger the muscle fibre the tougher the meat, secondly red muscle fibres are rich in lipids and red colour due to their higher myoglobin content, thereby influencing taste and colour, and lastly affect meat quality by either having an oxidative or glycolytic metabolism (Taylor, 2004). There are three main types of muscle fibres, namely type I, type IIA and type IIB (Taylor, 2004; Choi & Kim, 2009; Lee *et al.*, 2010). According to Taylor (2004), type I muscle fibre types are used for endurance and maintaining posture, type IIA are used for rapid activity with slow fatigue and type IIB fibres are used for sprinting or lifting of weights. The *Infraspinatus* (IS) muscle contains a high percentage of type I fibres, the *Longissimus thoracis et lumborum* (LTL) and *Semitendinosus* (ST) muscles contain more type IIB fibres, the *Supraspinatus* (SS) muscle contains more type I and the *Semimembranosus* (SM) muscle contains more type IIA fibres. A high content of type I muscle fibres is indicative of improved meat (particularly beef) tenderness and more redness and that of type II represents tougher and light red meat (Lee *et al.*, 2010). The LTL muscle is located on the rib section of the carcass while the IS and SS are located on the forequarter of the carcass and the *Biceps femoris* (BF), SM and ST muscles are located on the round of the carcass (Rhee *et al.*, 2004). Due to the differences between muscle types, it is necessary to conduct research on each to enable the profiling of the muscle types according to the different meat quality parameters. Research on meat quality attributes is often conducted on one or two muscle types (such as the LTL muscle) and does not take into account other muscle types that are metabolically different (Mungure *et al.*, 2016a). Profiling of the various muscles will allow the meat industry to select which muscle to use for the various cuts as well as value added meat products.

2.4.4 Microbial growth

Red meat obtained from warm-blooded animals contains a mixed microbial flora typically consisting of mesophilic and psychrotrophic bacteria (Johnston & Tompkin, 1992). Sources of these microorganisms include the animal itself, the soil, water, people as well as equipment involved in the processing (Gouws *et al.*, 2017). Most of the microorganisms found in meat are mesophilic; this is due to the ambient conditions under which the processing occurs. The mesophilic bacteria are indicative of the degree of sanitation during the slaughtering process. Cooking and refrigerating the meat destroys most of the microorganisms in the meat, with the exception of spore formers. Pyschrotrophic bacteria can grow and increase in numbers in the refrigerated post-processing conditions (Johnston & Tompkin, 1992). Psychrotrophs are a

sub-group of mesophiles and defined as microorganisms that grow at temperatures of 7 ± 1°C within 7 to 10 days. Since psychrotrophic bacteria grow at refrigerated conditions, they eventually spoil the product therefore, their counts will indicate the shelf life of a product.

The type of microbial growth in fresh and processed meat and poultry products is determined by factors including pH, addition of salt, nitrite, sugar, smoke or acidulants as well as the state of the meat (Johnston & Tompkin, 1992). Psychrotrophs involved in the spoilage of meat and meat products include species of *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Chromobacterium*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Klebsiella, Lactobacillus*, *Microbacterium*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Serratia*, *Sthaphylococcus* and *Streptococcus* (Cousin *et al.*, 1992; Carrizosa *et al.*, 2017).

Packaging meat, fish and other foods in vacuum or modified atmospheric packaging promotes the growth of facultative anaerobes as well as true anaerobes. The main bacterial genera found in these packaging conditions include *Brochothrix*, *Lactobacillus*, *Leuconostoc*, *Pediococccus* as well as members of the *Entererobacteriaceae* family (Cousin *et al.*, 1992; Carrizosa *et al.*, 2017).

Total bacterial counts and *Enterobactericeae* counts are regarded as the standard methods to indicate microbial contamination in the carcass and can thus be referred to as meat quality indicators (Magwedere *et al.*, 2013). The aim of microbiological analysis in the current study is to determine the degree of microbial contamination in aged vacuum-packed meat. This study focuses on the shelf stability of black wildebeest (*Connochaetes gnou*) meat and thus will focus on the microorganisms that affect meat quality; Total viable count (TVC) and *Enterobacteriaceae*.

The Total Viable Count (TVC) encompasses the entire microflora count of a sample. The Meat Industry Guide (2015) describes TVC as the measure of bacteria that can grow in the conditions on the surface of the carcass or in processed meat, can be collected using a sampling procedure and be grown on an agar plate. Since TVC includes microorganisms that are responsible for meat spoilage, it will thus provide insight on the shelf life and quality of the meat.

Enterobacteriaceae is a family of gram-negative, oxidase-negative, non-spore forming, non-acid fast, straight, rod-shaped bacteria (Brenner, 1991). This family is the most studied group of microorganisms as they are of medical and economic value, easy to isolate and cultivate, have a rapid generation time and show ease of genetic manipulation. Sources of *Enterobacteriaceae* include water, soil, human intestinal flora, as well as many animals. *Enterobacteriaceae* are non-halophilic, facultative anaerobes that grow well at temperatures

between 20°C and 35°C. *Enterobacteriaceae* (EB) are different from *Vibrionaceae* and *Pasteurellaceae* families that also contain facultative anaerobes in gram-negative rods. *Enterobacteriaceae* differ by their straight cell shape, motility, lateral flagella, negative oxidase test, no requirement or stimulation from sodium and the production of enterobacterial common antigen (Brenner, 1991). According to the Meat Industry Guide (2015), this group of microorganisms can be found primarily in the intestines of animals. The group includes most of the major food-borne pathogens of animal origin such as *Salmonella*, *Yersinia* and *Escherichia coli* O157. The presence of these organisms on the surface of a carcass normally indicates faecal and environmental contamination. Analysis of *Enterobacteriaceae* will give an indication of the risk of pathogens occurring in the sample.

Microbial growth limits are imperative as they give an indication of the level of safety in food products; Table 2.1 shows the recommended criteria for the TVC and *Enterobacteriaceae*.

Table 2.1: Recommended microbiological criteria for the microorganisms enumerated in the current study (Heinz & Hautzinger, 2016)

Microorganism	**Good microbiological standard**	**Critical microbiological standard**	**Not acceptable**
Total Colony Count (TCC)	<10 000 cfu/g (10^4)	10 000-10 0000 (10^4 -10^5) cfu/g	>10 0000 (10^5) cfu/g
Enterobacteriaceae	<100 cfu/g	>100- <1 000 cfu/g	>1 000 cfu/g

Spoilage becomes evident to consumers through off-odours, off-flavours as well as discoloration at total bacterial counts exceeding 6.0 log cfu/g (Fernández-López *et al.*, 2008). Dainty and Mackey (1992) suggested microbial counts of 7.0 log cfu/g as the level at which meat becomes completely unacceptable.

2.5 Consumer perception of meat quality

Upon purchasing meat, consumers consider two important attributes of meat; colour and tenderness; although it is still unclear how a consumer can measure the level of tenderness from a visual appraisal.

2.5.1 Colour

Colour is the most common trait used by consumers upon deciding to purchase meat (Honikel, 2004; Ouali *et al.*, 2006). A bright red colour is associated with freshness as opposed to a paler colour (Mancini & Hunt, 2005). Colour is a meat characteristic that is influenced by the pH of the meat (Honikel, 2004). Colour can also indicate microbial spoilage in meat; growth of certain microorganisms shows changes in the pigmentation on the surface of the meat. The sarcoplasmic protein known as myoglobin is the main protein responsible for meat colour. Other haem proteins such as haemoglobin and cytochrome C also contributed to meat colour, but to a lesser extent than myoglobin (Mancini & Hunt, 2005).

Myoglobin structure

Myoglobin is a water soluble protein which consist of numerous amino acid residues and 8 alpha helices (named A to H) which are linked by non-helical sections (Mancini & Hunt, 2005). Histidine is the most important amino acids residue in myoglobin because it affects its structure and function. The hydrophobic pocket of myoglobin contains a prosthetic group located in it. The heme ring of myoglobin has a centred iron atom that can form six bonds; four bonds can be formed with nitrogen's of the pyrrole while the fifth bond is formed with a histidine -93 residue. The sixth bond can be formed reversibly with ligands. Meat colour is dictated by the ligand bound to the iron atom as well as the valence of the atom. Myoglobin is a protein, and like any other protein its structure and function tends to alter under certain pH temperature conditions.

Myoglobin chemical forms

The chemical state of myoglobin determines the colour of red meat muscles (Ercolini *et al.*, 2006). The colour change in meat is affected by the concentration of the myoglobin in the muscle, which depends on breed, animal age as well as the animal's nutritional status. Other sources of colour change include *ante-mortem* and *post-mortem* handling of the animal which affect colour through the rate of pH and temperature declination. Variation in meat colour is influenced by oxygenation and oxidation of the haem iron of myoglobin, which take place during storage, distribution and display of meat (Honikel, 1998; Mancini & Hunt, 2005; Ouali *et al.*, 2006).

There are three main chemical/ redox forms of myoglobin which influence meat colour; these are deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb). Deoxymyoglobin occurs when no ligand in bound in the sixth site of iron and the iron exists in its ferrous state (Fe^{2+}). The resulting meat colour is purplish-red or purplish-pink; this is the colour of vacuum packed or freshly cut meat that has not been exposed to oxygen. Upon exposure to oxygen myoglobin turns into oxymyoglobin, this typically happens during

blooming where meat is exposed to oxygen to produce the bright cherry-red colour that is desirable to consumers. The bright red colour is associated with freshness and wholesomeness, thus desirable to consumers (Lindahl, 2011). Oxymyoglobin forms as a result of a diatomic oxygen being bound on the sixth coordination site of iron. The oxymyoglobin layer thickens and penetrates deeper in the surface of meat with longer exposure to oxygen. Temperature, pH, oxygen partial pressure and competition for oxygen by other respiratory processes are factors that influence the thickness and penetration of the oxymyoglobin layer. Oxidation of iron from the myoglobin ferrous state to ferric state (Fe^{3+}) which cannot bind oxygen leads to the formation of metmyoglobin, this form of myoglobin causes meat discoloration (Mancini & Hunt, 2005; Ouali *et al.*, 2006). The brownish-red meat colour is unappealing to consumers and is regarded as that of inferior quality resulting in product rejection. Formation of metmyoglobin is influenced by pH, oxygen partial pressure, reducing activity of the meat, temperature as well as microbial growth (Mancini & Hunt, 2005).

Hoffman *et al.* (2007a) found that springbok (*Antidorcas marsupalis*) meat samples with higher ultimate pH values had more redness (increased a* values) than those of lower ultimate pH values. Extended ageing period resulted in meat discoloration; the L*, a* and b* values of beef *longissimus lumborum* muscle decreased with increasing ageing period (Colle *et al.*, 2015). A high myoglobin content in muscles influences their colour stability; the *Psoas major* muscle of *Bos indicus* contained a higher myoglobin content, lower redness (a*), high lipid and protein oxidation levels compared to the more colour stable *Longissimus lumborum* muscle (Canto *et al.*, 2016).

2.5.2 Tenderness

In addition to colour, the tenderness of meat is another important attribute that influences the perception of meat quality and often causes unacceptability of meat by consumers (Riley *et al.*, 2009). Tenderness of meat occurs as a result of the *post-mortem* breakdown of myofibrillar proteins by enzymes such as cathepsins and calpains. Tenderness of meat is reported to increase during ageing/conditioning.

Warner-Braztler shear force (WBSF) is one of the two most common objective methods used to measure meat tenderness. The Warner-Bratzler shear force measurement is a tenderness test that intends to mimic the force produced during biting and mastication (Hoffman, 2015). Low WBSF values represent more tender meat whereas higher values represent tough meat (Riley *et al.*, 2009). The consumer's eating experience of beef (and meat from other species) is influenced by the tenderness (Ouali *et al.*, 2006; Riley *et al.*, 2009). The amount of connective tissue in a muscle as well as the orientation of muscle fibres also

has an effect on the tenderness of meat (Yadata *et al.*, 2009). It was reported that consumers would rather pay more for a tender steak than a tough one; Riley *et al.* (2009) further reported factors that influence tenderness include processing, ageing, food preparation as well as animal production system. Other factors such as initial pH (pH_0), ultimate pH (pH_u) as well as the rate of pH decline also have an effect on the tenderness of meat (Hoffman, 2007).

Ageing of meat

Game meat was traditionally utilised for the production of dry meat products such as biltong (Jones *et al.*, 2017) where tenderness was not taken into account. With the growing interest for using game species for fresh meat production, research on the tenderness of the meat has become a necessity and ageing is the most important process used in improving tenderness. Game species are known for producing tougher meat as a result of their increased activity levels, ageing is useful in recovering some of the tenderness lost during *rigor mortis* (Lawrie & Ledward, 2006). Although the South African game industry mainly focuses on tourism and hunting, fresh meat production from game species also has potential to make a valuable contribution to the game industry. In order for this to occur, meat of good quality must be provided; this is possible through proper handling of the carcass and meat. Ageing, particularly of the more valuable primal cuts, is reported to be the most crucial part of this process (North & Hoffman, 2015).

Ageing or conditioning of meat not only improves tenderness but also enhances the flavour and aroma of the meat. Storage of unprocessed meat at conditions above its freezing point with the aim of enhancing tenderness and flavour is termed conditioning or ageing (Lawrie & Ledward, 2006). *Post-mortem* glycolysis is the dominant process amongst the degradative changes that occur within the first 12-36 h *post-mortem*. Conditioning/ ageing continues until ideal tenderness and flavour is obtained; however, prolonged storage results in gross denaturation and dehydration of proteins and microbial spoilage, which render meat unfit for consumption. The supply of energy in the form of ATP in the muscle helps to prevent muscle proteins from disorientation; however, during death, this energy depletes and protein denaturation takes place (Lawrie & Ledward, 2006). Two most important degradative processes that occur during ageing are protein denaturation and proteolysis.

At a high ultimate pH, the rate of proteolysis increases (Ouali *et al.*, 2006). Temperature also plays a role in the extent of proteolysis; with higher temperatures correlated with greater activity than lower temperatures. Amino acids and peptides generated during proteolysis combined with lipid oxidation contribute enormously to flavour development of meat (Ouali *et al.*, 2006). Ageing of springbok (*Antidorcas marsupialis*) *Longissimus thoracis*

et lumborum (LTL) muscle was found to increase tenderness and juiciness, however the flavour and aroma were altered and off-flavours developed as a result of prolonged storage. The optimum ageing period for springbok meat was found to be eight days (North & Hoffman, 2015).

Extended ageing time increased tenderness, purge loss, level of lipid oxidation and microbial counts in beef *Semimembranosus* muscle (Colle *et al.*, 2016). The L* and pH value of the meat also increased with longer ageing time, however colour stability was found to decrease during ageing (Mungure *et al.*, 2016b). Neethling (2016) investigated the colour stability of springbok, blesbok (*Damaliscus pygargus phillipsi*) and fallow deer (*Dama dama*) LTL, *Infraspinatus* (IS) and *Biceps femoris* (BF) muscles. From the study it was found that colour stability lessened during the eight-day storage period at 2.0 ± 0.6°C, however the IS muscle was found to be more colour stable than the LTL and BF muscles whilst the pH and lipid oxidation levels increased with increased storage time. Ageing of beef LTL muscle was found to decrease toughness from day 1 to day 6 of storage, no significant changes in toughness occurred after 13 days of storage. The ultimate pH and cooking loss were also found to increase with increased ageing time (Silva *et al.*, 1999). Bykowska *et al*. (2016) studied the meat quality of fallow deer *Supraspinatus* (SS), LTL and SM muscles stored under vacuum storage for 14 days and found that drip loss and cooking loss percentages decreased with longer storage time, whilst the L* parameter of colour was unaffected by storage time. Longer storage time also resulted in increases in dry matter and crude protein content of the samples, this was mainly due to the increase of weep in the packaging. Ageing of beef LD and ST muscles for 28 days was found to decreased WBSF and L* values, while increasing the level of lipid oxidation in the muscles (Ba *et al.*, 2014). A decrease in WBSF values with longer ageing time is attributed to the destruction of myofibrillar components that occurs during ageing.

2.6 Conclusion and study objectives

Game species readily stress during harvesting due to their inherent wild nature. The contribution of black wildebeest to the game industry is thought to be minimal; this is attributed to poor perceptions of its quality as well as inadequate information about its nutritional value. This study aims to provide information on the carcass yield and meat quality attributes of the major muscles for utilisation of this species in the meat industry as well as enhance the knowledge of consumers. Black wildebeest is known to be more prone to stress, it is a plains game species that typically notices a predator from a distance and uses running as an escape strategy. This study therefore aims to investigate the shelf life stability of black wildebeest

meat taking into account that the species is prone to stress during harvesting. Due to high stress levels, DFD meat is often produced as a result of a high ultimate pH in the meat. The microorganisms that are responsible for spoilage at this high pH will be investigated. Currently no work has been done on ageing of black wildebeest meat, thus the study will investigate the effect of ageing on the physicochemical and microbiological attributes of black wildebeest meat with the aim of determining the optimum tenderization period of the meat. The effect of sex on the physicochemical and microbiological attributes of black wildebeest meat will also be investigated in the study.

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Chapter 3: Influence of sex on carcass composition of black wildebeest (*Connochaetes gnou*)

Abstract

This study aimed to determine the effect of sex on the carcass composition of mature male (n = 17) and female (n = 10) black wildebeest (*Connochates gnou*). Live weights of black wildebeest harvested in March 2016 were lower than those harvested in March 2017 (149.5 kg and 163.4 kg respectively) due to the long drought period prior to the 2016 harvest. Live weights, warm and cold carcass weights of male black wildebeest (141.4 kg, 89.8 kg and 85.8 kg respectively) were significantly higher than those of females (117.4, 71.0 and 68.6 kg respectively). The males had heavier ($p<0.05$) hides, heads, trotters, stomachs and intestines, hearts and spleens than females. Consumable offal contributed 12.7% to male live weight and 11.2% to the female live weights. From this study it was shown that black wildebeest has meat production potential and can thus contribute towards combating the growing food security challenges.

3.1 Introduction

Over the past number of years, South Africa has been experiencing a dramatic increase in wildlife ranching, partly driven by the increase in the commercial value of these animals as well as by climate changes such as increased frequency of droughts which are becoming detrimental to traditional livestock farming. Commercial farmers have resorted to using their land for either mixed livestock-wildlife ranching or converting their farms to only wildlife ranching. Wildlife ranching has been recognised as one of the fastest growing agricultural branches (Otieno & Muchapondwa, 2016). Although game animals are reported to adapt more readily to various environmental conditions, the recent climate changes have been reported to be potentially dangerous for them too (Dominik *et al.*, 2012; Otieno & Muchapondwa, 2016). Game species provide an alternative high quality protein source to compensate for the ever-growing human population (and thus food demand) as well as combat food security challenges. However, consumers are still not educated about the positive attributes of game meat which makes it a priority for researchers to provide such information.

Black wildebeest (*Connochates gnou*) is an endemic Southern African antelope commonly known as the white-tailed gnu (Smithers, 1983). Black wildebeest are short grass grazers which require water daily; open grasslands are their preferred habitat (Smithers, 1983; Booyse & Dehority, 2012; Lease *et al.*, 2014). Mature male black wildebeest are larger and heavier (stand approximately 120 cm at the shoulders and weigh around 180 kg) than females whose height is 110 cm and weight between 120-160 kg (Smithers, 1983). Black wildebeest were almost hunted to extinction but due to protection on farms and private areas their numbers have recently recovered (Smithers, 1983; Oberem & Oberem, 2016).

Black wildebeest have been reported to show meat production potential due to their population growth of between 28-32% per annum (Hoffman *et al.*, 2009b). The meat production potential of some common game species has been investigated. In order for a species to compete with others in terms of meat production potential it needs to have a high fecundity, must be adaptable to the region in which it will be farmed, should preferably be found in a herd (to ease of harvesting), must have an acceptable meat yield and its meat must also be acceptable to consumers (Hoffman & Cawthorn, 2013).

South Africa has distinct summer and winter seasons and some game species are harvested in different seasons which may have an influence on their carcass yield and composition (physical or chemical). Within a season, the amount of rainfall may also influence these parameters; with more rainfall more food may be more available compared to drought periods (Hoffman *et al.*, 2009b). Male animals also differ from females in their feeding as well as their activities. The effect of sex on carcass characteristics of various game species has been studied, but there is limited research on black wildebeest carcass composition and yield. Also, the offal of an animal as a food source is frequently overlooked, yet it forms an important component of many South Africans diet. The aim of this study was to determine the effect of black wildebeest sex on the various carcass components as well as to determine the contribution of each component to the overall animal live weight.

3.2 Materials and methods

3.2.1 Black wildebeest harvesting

A first group of seventeen (n = 9 males and n = 8 females) black wildebeest (*Connochaetes gnu*) were harvested at the Bredasdorp farm in the Western Cape in March 2016. The harvest was done after a long period of drought. A second group of ten of black wildebeest (n = 8 males and n = 2 females) was harvested in March 2017. Both harvests were performed using similar methods as outlined by Hoffman and Laubscher (2009) and adhered to the ethical

norms and standards as prescribed by the University of Stellenbosch (ethical clearance number: SU-ACUM14-001SOP). Table 3.1 shows the number of animals from both harvests. Experienced marksmen performed harvesting at night to reduce pre-slaughter stress experienced by the animals. Target animals were shot in the head or high neck; this was followed by exsanguination immediately.

The study area is reported to have an average rainfall of 496 mm annually (de Jager, 2016). Figure 3.1 shows the monthly rainfall patterns for the study area (SAWS, 2017), the arrow shows the period when black wildebeest harvested.

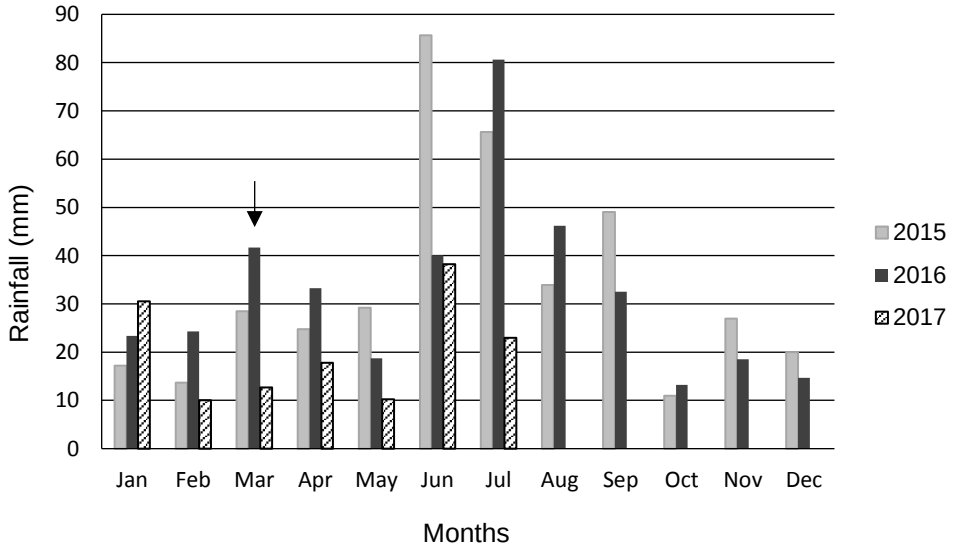

Figure 3.1 The 2015 and 2016 rainfall data (per month) for the study area showing the month when black wildebeest were harvested.

Whole carcasses were transported to the abattoir facilities where they were weighed individually (live weight) followed by skinning and dressing. The hide, head, and trotters, and the internal offal and organs (heart, liver, kidneys, spleen, and tongue) were removed and weighed individually in kg and expressed at percentages of the live animal weight. The dressed carcasses were weighed (warm carcass weight), followed by chilling (4°C) in a cold truck overnight and transported to the meat laboratory at the department of Animal science at Stellenbosch University. Approximately 16 h *post-mortem* the chilled carcasses were removed from the cold room and weighed (cold carcass weight).

The dressing percentage was calculated from the cold carcass weight and live weight as follows:

$$Dressing\ \% = \left(\frac{cold\ carsass\ weight\ (kg)}{live\ wight\ (kg)}\right) \times 100$$

Table 3.1 Number of male and female black wildebeest harvested in March 2016 and March 2017

Year	Month	Males	Females	Total
2016	March	9	8	17
2017	March	8	2	10
Total		17	10	27

3.2.2 Meat and bone yield

The entire carcass of one bull was first weighed to obtain the total cold weight prior to deboning. After removal of the individual larger muscles, the remainder of the carcass was deboned. The total meat, bones and trimmings were obtained from an individual carcass and weighed.

3.2.3 Statistical analysis

Statistica 13.2 (Statsoft, 2016) was used for the statistical analysis of the differences between the various carcass components. One-way ANOVA was used to analyse the carcass characteristics, with sex and harvesting year as the main effects. Differences were regarded as significant at a 5% level ($p \leq 0.05$).

3.3 Results

Animal sex had an effect ($p<0.001$) on the live weights, warm and cold carcass weights as well as the weights of hides, heads, trotters and hearts, but there was no sex effect ($p>0.05$) on the dressing percentages of both males and females. The live weights, warm and cold carcass weights of male black wildebeest were found to be higher than those of females; the weights of hides, heads, trotters and hearts followed the same trend.

Harvesting year did not have an effect ($p \leq 0.05$) on the live weights, warm and cold carcass weights and dressing percentages; however an influence was observed on the weights of hides and trachea and lungs; where the 2017 showed heavier weights than 2016. The effect of sex and harvesting year on the weighed carcass components is shown in Table 3.2.

Table 3.2 Effect of sex and year on the mean (± SEM[1]) carcass composition of black wildebeest (*Connochates gnou*)

Parameter	Sex		p-value	Year		p-value
	Male (n=17)	Female (n=10)		2016 (n=17)	2017 (n=10)	
Live weight (kg)	141.4 ± 5.92	117.4 ± 4.23	**0.000**	149.5 ± 4.23	163.4 ± 5.92	0.066
Warm carcass weight (kg)	89.8 ± 1.91	71.0 ± 2.67	**0.000**	78.0 ± 1.91	82.9 ± 2.67	0.144
Cold carcass weight (kg)	85.8 ± 1.99	68.6 ± 2.79	**0.000**	74.1 ± 1.99	80.3 ± 2.79	0.083
Dressing %	50.2 ± 0.62	48.6 ± 0.87	0.136	49.6 ± 0.62	49.1 ± 0.87	0.671
Hide (kg)	14.5 ± 0.35	10.6 ± 0.49	**0.000**	11.6 ± 0.35	13.4 ± 0.49	**0.006**
%	8.3 ± 0.21	7.2 ± 0.23	**0.003**	7.8 ± 0.19	8.2 ± 0.26	0.193
Head (kg)	14.6 ± 0.30	10.1 ± 0.42	**0.000**	11.9 ± 0.30	12.9 ± 0.42	0.064
%	8.8 ± 0.18	7.0 ± 0.19	**0.000**	7.9 ± 0.16	7.8 ± 0.23	0.815
Tongue (kg)	0.4 ± 0.01	0.4 ± 0.02	0.751	0.4 ± 0.01	0.4 ± 0.02	0.905
%	0.2 ± 0.01	0.3 ± 0.01	0.250	0.3 ± 0.01	0.2 ± 0.01	0.214
Trotters (kg)	3.0 ± 0.06	2.7 ± 0.08	**0.002**	2.8 ± 0.06	2.9 ± 0.08	0.130
%	1.8 ± 0.04	1.9 ± 0.04	0.884	1.9 ± 0.03	1.8 ± 0.04	0.296
Gastrointestinal tract (kg)	36.6 ± 1.22	36.4 ± 1.71	0.910	34.6 ± 1.22	38.3 ± 1.71	0.088
%	21.0 ± 0.87	28.7 ± 0.92	**0.002**	23.3 ± 0.62	22.9 ± 0.86	0.628

Liver & gall bladder (kg)	1.8 ± 0.07	1.7 ± 0.10	0.279	1.8 ± 0.07	1.8 ± 0.10	0.991
%	1.1 ± 0.05	1.2 ± 0.06	0.340	1.2 ± 0.04	1.1 ± 0.05	0.236
Heart (kg)	1.2 ± 0.03	0.9 ± 0.04	**0.000**	1.0 ± 0.03	1.1 ± 0.04	0.913
%	0.7 ± 0.03	0.7 ± 0.03	0.510	0.7 ± 0.02	0.7 ± 0.03	0.135
Trachea & lungs (kg)	2.8 ± 0.12	2.7 ± 0.17	0.561	2.4 ± 0.12	3.1 ± 0.17	**0.003**
%	1.5 ± 0.10	1.7 ± 0.11	0.153	1.6 ± 0.08	1.9 ± 0.11	**0.047**
Kidneys (kg)	0.4 ± 0.03	0.4 ± 0.04	0.409	0.3 ± 0.03	0.4 ± 0.04	0.226
%	0.2 ± 0.02	0.2 ± 0.02	0.763	0.2 ± 0.02	0.3 ± 0.02	0.451
Spleen (kg)	0.4 ± 0.01	0.3 ± 0.02	**0.005**	0.4 ± 0.01	0.4 ± 0.02	0.842
%	0.2 ± 0.01	0.2 ± 0.01	0.654	0.2 ± 0.01	0.2 ± 0.01	0.277

SEM[1]= Standard error of the mean; % = of the live weight

The single bull that was totally deboned had a cold carcass weight of 90.6kg (dressing % = 50.17%) and yielded 45.7 kg (50.3% of cold carcass weight) total meat, 39.2 kg (36.3% of total carcass weight) trimmings and 12.1 kg (13.3% of cold carcass weight) bone.

3.4 Discussion

Black wildebeest that were harvested in March 2017 were heavier than those harvested in the previous year (163.7 ± 5.92 kg and 149.5 ± 4.23 kg, respectively), although not statistically so. Harvesting year did not show a significant influence on the animal live weights as was expected. However, as the animals had been randomly (within sex) shot, the slightly lighter weights of the animals that were harvested in 2016 may have been due to the long drought period prior to the harvest; in 2015 South Africa experienced the lowest rainfall (average of 403 mm) since 1903 (de Jager, 2016) and is thus amongst the most water stressed African countries (Otieno & Muchapondwa, 2016). The availability of food is greatly influenced by rainfall so drought conditions would likely result in body weight loss in the animals (Hoffman

et al., 2009b). The black wildebeest in the current study were also harvested during the peak mating season which is reported to be March/April (Skinner *et al.*, 1974; Hoffman *et al.*, 2009b). Another factor that may have had an influence on the animal weights is age; the exact age of the animals was unknown thus some animals may not have been of the same maturity level. Game species that are wild and roam freely and are usually referred to as young or mature; these animals were all mature. Owing to the heavier weights of the animals harvested in 2017, the hides and trachea combined with lungs were heavier than those harvested in 2016 ($p=0.006$ and $p=0.003$, respectively).

The live weights of male black wildebeest in the current study were found to be higher ($p<0.001$) (141.4 ± 5.92 kg) than those of females (117.4 ± 4.23 kg); this was expected as male black wildebeest are generally heavier than females. As expected the warm and cold carcass weights were also significantly influenced by sex; the male had heavier carcass weights compared to females. There was no influence of sex in an earlier study on black wildebeest (Hoffman *et al.*, 2009b). Male impala (*Aepyceros melampus*) was also found to be heavier than females (Hoffman, 2000; Hoffman & Laubscher, 2009a). Fitzhenry (2016) also found male fallow deer (*Dama dama*) to be heavier than females (47.4 kg and 41.9 kg respectively). Kudu (*Tragelaphus strepsiceros*) was reported to show strong sexual dimorphism where males had live weights of approximately 250 kg and female weighed a lighter 180 kg (Mostert & Hoffman, 2007). Female animals are reported to grow at a faster rate and reach maturity sooner than males, but the males are heavier than females at the mature stages (Lawrie & Ledward, 2006). Live weights, carcass weights and dressing percentages of springbok (*Antidorcas marsupialis*) and blesbok (*Damaliscus dorcas phillipsi*) did not differ between sexes ($p>0.05$) but were rather influenced by animal age (Van Zyl & Ferreira, 2004).

The dressing percentage is an important attribute taken into account when determining the meat production potential of a meat species. In the current study the mean dressing percentages were not significantly influenced by animal sex. Male black wildebeest in an earlier study had higher dressing percentages than females (Hoffman *et al.*, 2009b). Sex was found to have an effect ($p\leq0.05$) on fallow deer dressing percentage; males had higher dressing percentage than females (61.6 ± 0.52% and 59.0 ± 0.57%, respectively). Impala rams also had a higher dressing percentage (60.9%) than ewes (58.6%); (Hoffman *et al.*, 2009a). Male Kudu was also found to have a higher dressing percentage than females (58.3% and 55.9%, respectively). In an earlier study in a different region (Hoffman *et al.*, 2009), male black wildebeest was also found to have a significantly higher (53.1 ± 2.22%) dressing percentage than females (50.7 ± 1.41%); these values are higher than those obtained in the

current study although they are comparable. The dressing percentages of Bonsmara, Nguni and Aberdeen Angus steers that were raised on natural pasture (Muchenje *et al.*, 2008) were similar to those of black wildebeest in the current study.

The mean weights of the skins, heads and trotters in the current study were significantly influenced by animal sex; the male weights were heavier than those of females. The contributions of the skins and heads (%) to the live weight also differed significantly between sexes, with males having higher contribution than the females. The contribution of the trotters did not differ between sexes. The weights of the heart and spleen differed between the sexes, although sex did not have a significant on their contributions to live weight. The mean weights and contributions (%) to live weight of the liver, trachea and lungs, kidneys and tongues did not differ significantly between the sexes. The stomach and intestines were weighed with their contents thus an appropriate comparison between the sexes cannot be made, although that of males would be expected to be heavier than that of females. The stomach and intestines were thus not added to the total weight of consumable offal which includes heads, liver, kidney, heart and lungs.

Consumable offal is an important and nutritious food source to most African and parts of Asia and as a result is highly priced (Magwedere *et al.*, 2013). According to Hoffman and Cawthorn, (2013) edible offal and internal organs are consumed by most South Africans and an increased supply thereof would provide a low cost source of protein and micronutrients. Thus it is important that the edible portions be extracted from the game carcass as much as possible, including the internal offal as it is more edible than external offal (Van Zyl & Ferreira, 2004). In the current study, consumable offal of the male black wildebeest contributed to approximately 12.8% of the live weight and that of females contributed 11.2% of the live weight. Consumable offal of male fallow deer contributed 9.6% to the live weight and females contributed 8.9% to the live weights (Fitzhenry, 2016). Van Zyl and Ferreira (2004) also found sex differences in external offal contribution to live weight; the males had higher contribution (%) than females. Aduku *et al.* (1991) reported that offal contributed approximately 33% of the edible parts of slaughtered animals.

The meat obtained from one animal in the current study contributed 50.3% to the total cold carcass weight. Fitzhenry (2016) obtained 57.9% and 60.5% from male and female fallow deer (*Dama dama*). Bones contributed 13.3% to the total cold carcass weight, this is lower than the contribution of fallow deer bones (26.2%) (Fitzhenry, 2016). Meat yield is important as it indicates how much meat can be obtained from an animal, although it can be surprising when considering the live weight of the animal and one expects to obtain a lot more. Meat yield calculations exclude the head, trotters, skin and gut fill hence the low values.

Game species are reported to have better meat production than domestic livestock when considering their dressing percentages and production of lean meat (Van Zyl & Ferreira, 2004). Dressing percentages on Nguni, Bonsmara and Angus cattle species were found to be in the range of 52.1%-56.9%; these values are higher than those of black wildebeest obtained in the current study. Total carcass weights of the cattle breeds contributed 53%-59% to the slaughter weight (Muchenje *et al.*, 2008), in the current study the contribution of black wildebeest total carcass weight was 63%. However, higher dressing percentages were obtained for impala and kudu (58.6-60.9% and 55.9-58.3%), and their carcass weights contributed 58.1%-65.0% and 54.7%-59.4% to the live weights, respectively (Hoffman *et al.*, 2009a).

3.5 Conclusion

The current study was conducted to determine whether animal sex had an influence on the carcass characteristics; it was found that male black wildebeest are heavier than the females. Dressing percentage of black wildebeest is comparable with other game species as well as domestic livestock (cattle), thus black wildebeest is a valuable meat producing game species. Offal obtained from black wildebeest meat could be a valuable low cost protein source to most South Africans, thus it is imperative that handling is done in a hygienic manner. This study has shown that black wildebeest has a carcass yield that would make it suitable for game meat export. Information on the physicochemical attributes of the meat will be of value for the local as well as for the meat export market.

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Chapter 4: Physicochemical meat quality attributes of black wildebeest (*Connochaetes gnou*) muscles

Abstract

The physicochemical attributes of six muscles: *Longissimus thoracis et lumborum* (LTL), *Biceps femoris* (BF), *Infraspinatus* (IS), *Supraspinatus* (SS), *Semimembranosus* (SM) and *Semitendinosus* (ST), obtained from male (n = 9) and female (n = 8) black wildebeest (*Connochaetes gnou*) were investigated. The muscles were profiled according to pH_u, colour (*CIE* L, *CIE* a*, *CIE* b*, Chroma, hue angle), drip loss and cooking loss percentages, Warner-Bratzler shear force (WBSF), proximate composition (moisture, total protein, total fat, ash) and fatty acids content. Sex had no effect ($p>0.05$) on the physical attributes and proximate composition of black wildebeest muscles. Male black wildebeest had higher levels of polyunsaturated fatty acids (PUFA) than females ($p<0.05$). Female black wildebeest contained higher levels of saturated fatty acids than males. Mean pH_u values of the muscles ranged from 6.50 to 6.59 thus indicative of dark firm and dry meat (DFD). Muscle type had no effect ($p>0.05$) on the pH_u and drip loss percentage. However, muscle type had an effect ($p<0.05$) on the colour coordinates (*CIE* L*, *CIE* b*, Chroma, hue angle), cooking loss percentage and WBSF values. Mean drip loss and cooking loss percentages ranged from 1.10% to 2.09% and 25.9% to 33.5%, respectively. The SS muscle had the lowest mean WBSF (3.11 kg/cm ø), whereas the SM had the highest (5.09 kg/cm ø). The SS muscle had the highest mean moisture content (78.1%) whereas the LTL muscle had the highest protein content (22.6%). The ST muscle had the lowest fat (1.3%) and the lowest ash content (1.1%). The major saturated fatty acids (SFA) of black wildebeest muscles were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and tetracosanoic acid (C24:0); C18:0 was at the highest composition (%). Monounsaturated fatty acids (MUFA) included palmitoleic acid (C16:1), oleic acid (C18:1*ω-9c*) and nervonic acid (C24:1); C18:1*ω-9c* acid being at the highest composition. The major PUFA were linolenic acid (C18:3*ω-3*), conjugated linolenic acid (C18:2*ω-6c*), arachidonic acid (C20:4*ω-6*) and docosahexaenoic acid (C22:6*ω-3*); the latter being the highest composition (%). PUFA:SFA of black wildebeest muscles ranged from 0.4 to 1.0 and *ω-6:ω-3* ranged from 1.2 to 4.5. Although black wildebeest muscles can be seen as being healthy due to their low fat and favourable fatty acids profile, the physical quality attributes tend to be negative due to the DFD phenomenon. This phenomenon is normally linked to *ante-mortem* stress and is thus a factor that could be controlled.

4.1 Introduction

Red meat is an important part of the human diet due to its high nutritional value; it is high in protein, iron, zinc, B-vitamins (niacin, cobalamin, riboflavin) to list but a few components (Czerwonka & Tokarz, 2017). However, excessive consumption of red meat has been associated with increased health risks such as cardiovascular diseases, diabetes and colon cancer, which can be attributed to the high fat content and iron levels exceeding the suggested limit (Hoffman & Wiklund, 2006; Czerwonka & Tokarz, 2017), this has resulted in a negative impact on consumers' perception of red meat. The modern consumer is inquisitive about the health and safety aspects of the food they consume (Hoffman & Wiklund, 2006). Game meat can be classified as an organic product since animals are wild and free-living and no growth promoters are used on the animals (Mostert & Hoffman, 2007). Furthermore, game species easily adapt to harsh environmental conditions such as drought and limited nutrition. Game meat can thus serve as a healthier alternative due to its lower intramuscular fat content (1-3%) as well as higher protein content (~20%); game meat has received popularity over the years due to these attributes. In addition, game meat has been previously found to be free of pathogenic microorganisms such as *Salmonella spp* (Magwedere *et al.*, 2013; Gouws *et al.*, 2017). In South Africa, game meat is commonly obtained from springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus dorcas phillipsi*), impala (*Aepyceros melampus*), greater kudu (*Tragelaphus strepsiceros*), and black (*Connochaetes gnou*) and blue (*Connochaetes taurinus*) wildebeest (Hoffman & Laubscher, 2009; Hoffman *et al.*, 2009).

Although the consumption of game meat has increased over the years, there still remains a lack of knowledge about the nutritional information of game meat and as a result, game meat contributes little to the formal South African meat industry (Hoffman *et al.*, 2005). Another factor contributing to the low contribution of game meat is that game meat can sometimes be classified as dark, firm and dry (DFD) due to the higher pH values in the muscles caused by stressful conditions under which the animals are sometimes harvested.

Internationally, game meat is sold similar to ostrich meat, as whole muscles (Hoffman, 2008) whereas beef muscles are marketed as fillets, loin or whole muscles from the forequarters and hindquarters, while others are processed further to produce mince and sausages (Paton *et al.*, 2010). There is limited information about the quality and therefore marketing potential of the different muscles from game meat, and availability of information about the muscle types would allow the game meat industry to select which muscle to use for which cuts or to process further. Moreover, research on the specific game species will help provide information that can improve consumer knowledge with regards to the positive aspects of game meat as well as add value to the meat industry.

Skeletal muscles differ in their overall size, shape, anatomical location in the animal, level and type of activity in the animal, blood and nerve supply, association with other tissues as well as action (fast or slow) (Purslow, 2017). The aforementioned differences between muscles occur due to the different functions of the muscles. Skeletal muscles' properties which distinguish between the different muscle types include contractile protein integrity, sarcomere length, connective tissue content, endogenous protease activity, and intramuscular fat levels. The aforementioned factors influence meat quality; particularly the tenderness, meat colour, flavour, as well as juiciness (Taylor, 2004; Lawrie & Ledward, 2006). The *Longissimus thoracis et lumborum* (LTL) muscle is located on the back (both the rib and lumbar sections) of the carcass while the *Infraspinatus* (IS) and *Supraspinatus* (SS) muscles are located on the chuck (forequarter) of the carcass and the *Biceps femoris* (BF), *Semimembranosus* (SM) and *Semitendinosus* (ST) are on the round of the carcass (Rhee *et al.*, 2004). The differences between the muscle types warrant further research to be conducted in order to profile the muscle types according to their various meat quality attributes.

The quantification of quality attributes of game meat is necessary to improve its quality as well as to make it competitive on the market with other types of meat (Kohn *et al.*, 2005). Due to the increased interest in utilising game species for fresh meat production, more research is necessary on specific game species to provide the physical and nutritional information about meat from each species. Research on meat quality attributes is often conducted on one or two muscle types and does not take into account other muscle types that are metabolically different (Mungure *et al.*, 2016). Therefore the aim of this study is to profile six major muscles (LTL, BF, IS, SS, SM, ST) of black wildebeest in terms of their physical (pH_u, colour, drip loss, cooking loss and Warner-Bratzler shear force) and chemical (moisture, total fat, total protein and ash, fatty acid content) meat quality attributes. The effect of sex on the above mentioned attributes will also be investigated.

4.2 Materials and methods

4.2.1 Harvesting and sample collection

Seventeen (n = 9 males, n = 8 females) black wildebeest (*Connochaetes gnu*) were harvested as part of the farm's annual wildlife management strategy near Bredasdorp in the Western Cape in March 2016. Harvesting was performed at night to reduce the pre-slaughter stress experienced by the animals. Head and upper neck shots were utilised as they result in reduced carcass damage. Animals were exsanguinated a few minutes after being shot. Skinning and dressing of the carcasses was performed at the abattoir facilities on the farm, the carcasses were then chilled in a cold truck and transported (<4 °C) to the meat laboratory at Animal sciences in Stellenbosch University for analysis (see Chapter 3 for more detail).

Deboning of the chilled carcasses was performed in the meat laboratory at the Department of Animal Science at Stellenbosch University. Six muscles [*longissimus thoracis et lumborum* (LTL), *biceps femoris* (BF), *infraspinatus* (IS), *supraspinatus* (SS), *semimembranosus* (SM), *semitendinosus* (ST)] were removed from the back, hind and fore quarters of the chilled carcasses. Samples were vacuum packed and stored at 4°C until analysis commenced. Samples that were used for the determination of proximate composition and fatty acid content were stored and frozen at -18°C until analysis.

4.2.2 Physical analyses

Ultimate pH (pH_u)

The ultimate pH of the muscles was measured at 36 h post mortem. The pH was measured using a calibrated Crison portable pH meter with a knife electrode. The pH meter was calibrated after every six measurements using standard buffers at pH 4.0 and 7.0 (Lasec SA, Cape Town, South Africa).

Surface colour measurement

The muscle surface colour was measured on freshly cut steaks using a colour-guide 45°/0° colorimeter (aperture size 11 mm; illuminant/observer of D65/10°) (Catalogue number 6801; BYK-Gardner, Geretsried, Germany). The colorimeter was calibrated using the standards provided (BYK-Gardner). Before the colour measurements were taken muscles were removed from vacuum bags and cut into steaks. The steaks were bloomed for 30 min at 4°C. The measurements were taken at five different sites on the steaks. Measurements consisted of L* (lightness), a* (redness) and b* (yellowness) values. From these a* and b*values, the hue angle and Chroma were calculated according to equation 1 & 2, respectively (AMSA, 2012).

Equation 1: Hue angle ($\mathbf{h_{ab}}$)=$\tan^{-1}\left(\frac{b^*}{a^*}\right)$

Equation 2: Chroma ($\mathbf{C^*}$)= $\sqrt{(a^*)^2+(b^*)^2}$

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

Drip loss %

Drip loss percentage was determined according to Honikel (1998). Drip loss percentage was calculated as the percentage of the initial weight of the sample to that of the sample after suspension in the fridge for 24 h.

Cooking loss %

Cooking loss was determined according to Honikel (1998). Cooking loss was expressed as a percentage of the initial weight of the samples to that of samples after cooking in a water bath at 75°C for 1h, followed by cooling down to 4°C.

Warner-Bratzler Shear force

The samples that were used for cooking loss determination were used to measure shear force using the Universal Instron Testing Machine fitted with the Warner-Bratzler shear attachment (Honikel, 1998). Six rectangles (1 x1 cm surface area, 3 cm length) were cut from the steaks. The pieces were sheared at right angles to the direction of muscle fibres using a Warner–Bratzler shear attachment (V-notch blade) connected to an Instron® Universal Testing Machine (Model 4201, Instron Corp., Canton, MA) at crosshead speed of 250 mm/min. Visible connective tissue was avoided when cutting the rectangles. At least six measurements were taken for each steak from each animal. The unit of measurement used was N, however the data was converted to kg/cm diameter using the factor 9.8066 for easy comparison to literature sources.

4.2.3 Chemical composition

Moisture content

The moisture content of the samples was determined according to AOAC Official Method 934.01 (2002a). Briefly, empty and moisture-free crucibles were weighed and recorded (A). The scale was then tared, subsequently 2.5 g of homogenised meat samples was weighed into the crucibles, this weight was also recorded (B). The samples were analysed in duplicate. The crucibles were placed in an oven at 100°C for 48 h. Afterwards the crucibles were cooled in desiccators the weights of the moisture-free samples and crucibles (C) were recorded. The moisture content was then calculated as follows:

$$\%\, moisture = \frac{[(A + B) - C]}{B} \times 100$$

% Ash

The weighed moisture-free samples that were used for moisture content determination were incinerated in the furnace at 500°C for 6 h. The incinerated samples were then allowed to cool for 2 h and then placed in a desiccator for 30 min. These were then weighed (D). Variables A, B and C were already determined for moisture content. Ash content was then calculated as follows (AOAC Official Method 942.05, 2002):

$$\%\, ash = \frac{(D - A)}{sample\ mass} \times 100$$

Total fat

A chloroform/methanol extraction method was used to determine total fat/crude fat of the ground meat samples (Lee *et al.*, 1996). Since the samples were of game meat, which contains fat levels <5%, a 1:2 chloroform/methanol ratio was used. Fat beakers which had been placed in the oven at 100 °C overnight and cooled in a desiccator for 30 min were weighed (weight was noted). Five grams of homogenised meat were weighed into 800 ml beakers, this was followed by adding 50 ml of the chloroform and methanol mixture and mixed using a Bamix for 1 min. The homogenate was filtered through Whatman no. 1 paper into separation funnels. Twenty ml of NaCl (0.5% w/v) was subsequently added to the filtrate and gently shaken. The mixture was left for 45 min to separate, then the clear bottom layer was collected into Erlenmeyer flasks. Five mL of the bottom layer was transferred into the pre-weighed fat beakers. The fat beakers were placed on a sand bath at 80°C for 45 min. After all the solvents had evaporated, the fat beakers were cooled in a desiccator for 30 min before being weighed again. Total/crude fat was calculated as follows:

$$total\ fat\ \% = \frac{\left(w_{fat\ beaker+fat} - w_{fat\ beaker}\right)}{sample\ mass} \times \frac{chloroform\ volume^{*}}{5} \times 100$$

Total protein

The Dumas combustion method was used to determine the protein content of the dried, defatted and ground meat samples. Sub-samples (0.15 g) of the ground meat samples were encapsulated in a LecoTM foil sheet and were analysed in a Leco Nitrogen/Protein analyser (FP – 528, Leco Corporation, St. Joseph, Michigan, USA). Ethylene-diamine-tetra-acetic acid (EDTA) (Leco Corporation) was used to calibrate the Leco analyser prior to analysis per batch of samples. To ensure accuracy and recovery rate of the method, a calibration sample of unknown protein content was run after every 10 test samples. The obtained results presented as % nitrogen (N), were multiplied by a conversion factor of 6.25 in order to determine the total crude protein (%) values.

Calculation of crude protein was as follows:

$$\%\ Nitrogen\ = \frac{Leco\ N\ value\ \times (100\ - Moisture\ \%\ - Fat\ \%)}{100}$$

$$\%\ Crude\ protein = \%\ Nitrogen\ \times 6.25$$

Fatty acid content

The fat from a 1 g sample of raw muscle homogenate was extracted using a 2:1 (v/v) chloroform:methanol solution (Folch *et al.*, 1957), which contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The samples were homogenised in the extraction solvent for 30 sec using a polytron mixer (WiggenHauser D-500 Homogeniser, fitted with a standard shaft 1, speed setting D). Heptadecanoic acid (C17:0) was employed as an internal standard (catalogue number H3500, Sigma-Aldrich, Gauteng, South Africa) to quantify the individual fatty acids present in each muscle sample.

A 250 µl sub-sample of the extracted lipids was subsequently transmethylated at 70°C for 2 h using 2 ml of a 19:1 (v/v) methanol:sulphuric acid solution as the transmethylating agent. After allowing the resultant mixtures to cool to room temperature, the fatty acid methyl esters (FAME) were extracted with water and hexane. Following separation of the distilled water and FAME-containing hexane fluids, the top hexane layer was transferred to a spotting tube and dried under nitrogen. Fifty µl hexane was then added to each dried FAME sample, of which 1 µl was injected into the gas chromatograph. The FAMEs were analysed using a Thermo TRACE 1300 series gas-chromatograph (Thermo Electron Corporation, Milan, Italy) equipped with a flame-ionisation detector, using a 30 m TR-FAME capillary column with an internal diameter of 0.25 mm and a 0.25 µm film (Cat. No. HY260M142P, Anatech, Cape Town, South Africa) and a run time of *ca*. 40 min. The following oven temperature settings were utilised: initial temperature of 50°C (maintained for 1 min) and final temperature of 240°C attained after three ramps (initial increase at a rate of 25°C/min until a temperature of 175°C was reached; thereafter an immediate increase at a rate of 1.5°C/min to reach 200°C and maintenance of this temperature for 6 min; lastly an increase at a rate of 10°C/min to reach 240°C and maintenance of this temperature for a minimum of 2 min). The injector temperature was set at 240°C and the detector temperature at 250°C. The hydrogen gas flow rate was 40 ml/min. The FAME of each sample was identified by comparing the retention times with those of a standard FAME mixture (Supelco™ 37 Component FAME mix, Cat no. 47885-U, Supelco, USA), with results being expressed as mg fatty acid/g meat or as a % of identified fatty acids.

4.2.4 Statistical analysis

Statistical analysis of the data was performed with Statistica 13.2 (StatSsoft, 2016) using the General Linear Model (GLM) procedure. Analysis of variance (ANOVA) was used to compare the means of the physicochemical attributes with sex and muscle type being treated as the main effects. The animals were treated as a random effect. Fisher's Least Significant Differences (LSD) was used for post hoc tests. Differences between the means were considered significant at a 5% ($p \leq 0.05$) significance level.

4.3 Results

There was no significant interaction between sex and muscle type with regards to the physical attributes and proximate composition, the factors were thus considered individually. However, sex did not have a significant effect on any of the aforementioned attributes. With regards to the fatty acid composition, there was a significant sex by muscle type (G*M) interaction on the mean % composition of the C22:0 and C18:3*ω-3* fatty acids, total monounsaturated fatty acids (MUFA) and total *ω-6* polyunsaturated fatty acids (PUFA). Sex showed a significant influence on the mean % composition of PUFA and the PUFA to saturated fatty acids (SFA) ratio (PUFA:SFA). Muscle type had a significant

influence on the mean % composition of the SFA which included C14:0, C15:0, C18:0, C21:0, C22:0 and C24:0, MUFA (C15:1, C16:1, C18:1*ω-9c* and C24:1) and PUFA (C18:2*ω-6c*, 18:2*ω-6t*, C18:3*ω-3*, C20:3*ω-3*, C20:4*ω-6*, C20:5*ω-3* and C22:6*ω-3*).

4.3.1 Physical analyses

The effect of muscle type on the measured physical attributes of black wildebeest muscles is shown in Table 4.1. Ultimate pH values did not differ significantly between the muscles although all mean values were >6.5 indicative of DFD meat (Table 4.1). The CIE L* and b*, Chroma and hue angle values differed significantly between black wildebeest muscles. The IS muscle had the highest value of L* (although not significantly different from the ST muscle) and the SM muscle had the lowest value. The ST muscle had the highest b* followed by the forequarter muscles (IS and SS) whilst the LTL and SM muscles had the lowest values. There were no significant differences in CIE a* values between the muscles, however, the forequarter muscles (IS and SS) had slightly higher values than the hindquarter muscles (BF, SM, ST) and the LTL muscle had the lowest value. The Chroma value was the highest and did not differ between the IS, SS and ST muscles, followed by BF, SM and lastly the LTL muscle. The hue angle values did not differ between the IS and SS muscles as well as between LTL, BF, SS and SM muscles. A significant muscle type effect was found on the cooking loss percentage and average WBSF values. The LTL, BF and IS muscles had lower cooking loss percentages than the SS, SM and ST muscles. The IS and SS muscles had the lowest WBSF values whilst the SM and ST muscles had the highest WBSF values.

Table 4.1 The effect of muscle type on the mean (± SEM) physical attributes values of black wildebeest muscles

Parameter	LTL	BF	IS	SS	SM	ST	P-value
pH_u	6.58 ± 0.18	6.65 ± 0.17	6.59 ± 0.11	6.57 ± 0.11	6.51 ± 0.16	6.50 ± 0.16	0.819
CIE* L	28.7^{cd} ± 1.06	30.4^{bc} ± 0.64	33.4^{a} ± 0.92	30.6^{b} ±0.76	27.0^{d} ±1.01	32.7^{a} ±0.74	**0.000**
CIE* a	11.4 ± 0.62	12.2 ± 0.49	12.5 ± 0.45	13.0 ± 0.36	12.0 ± 0.82	12.2 ± 0.52	0.063
CIE* b	8.0^{d} ± 0.63	8.6^{bc} ± 0.43	9.7^{a} ± 0.29	9.1^{ab} ± 0.39	8.3^{cd} ± 0.65	10.2^{a} ± 0.51	**0.000**
Chroma	14.0^{c} ± 0.85	14.9^{b} ± 0.64	15.8^{a} ± 0.50	15.9^{a} ± 0.47	14.6^{b} ± 1.02	15.9^{a} ± 0.69	**0.005**
Hue angle	34.4^{b} ± 0.92	35.1^{b} ± 0.64	38.1^{a} ± 0.74	34.8^{b} ± 0.87	34.3^{b} ± 0.90	39.7^{a} ± 0.84	**0.000**

Drip loss (%)	2.1 ± 0.92	1.8 ± 0.78	1.6 ± 0.16	1.1 ± 0.31	1.9 ± 0.45	1.2 ± 0.54	0.708
Cooking loss (%)	27.4^{b} ± 1.48	26.9^{b} ± 1.78	25.9^{b} ± 0.89	32.0^{a} ± 1.02	32.4^{a} ± 1.76	33.5^{a} ± 1.34	**0.000**
WBSF (kg/cm ø)	5.0^{b} ± 0.65	5.5^{b} ± 0.36	3.9^{c} ± 0.40	3.9^{c} ± 0.33	6.5^{a} ± 0.52	6.3^{a} ± 0.51	**0.000**
WBSF (N)	44.6^{b} ± 5.45	42.2^{b} ± 2.79	30.8^{c} ± 3.07	30.5^{c} ± 2.59	50.3^{a} ± 4.03	49.1^{a} ± 3.97	**0.000**

$^{a, b, c, d}$ Row means with different supper scripts differ significantly at p ≤0.05. CIE L* = black to white (0 to 100); CIE a* = negative green, positive red; CIE b* = negative blue, positive yellow. LTL = *Longissimus thoracis et lumborum*, BF = *Biceps femoris*, IS = *Infraspinatus*, SS = *Supraspinatus*, SM = *Semimembranosus*, ST = *Semitendinosus*. SEM= standard error of the mean.

Six animals had normal pH values (<6.0) and 11 had higher values (>6.0) which showed susceptibility to being DFD. As not all muscles were DFD, the LTL muscle was further classified according to pH values, where values ≥6.0 were classified as DFD and those <6.0 were classified as normal (Table 4.2). The DFD meat showed a darker colour as a result of the lower L*, a*, b*, Chroma and hue angle values in comparison to the normal meat. Drip loss and cooking loss percentages of the DFD meat were lower than those of the normal meat. The normal meat had higher WBSF value than the DFD meat.

Table 4.2 The physical attributes of the LTL muscle as influenced by pH classification

pH class	*CIE* L*	*CIE* a*	*CIE* b*	Chroma	Hue angle	Drip loss %	Cooking loss %	WBSF (kg/cm ø)
Normal (n=6)	32.7^{a} ± 0.95	14.4^{a} ± 0.45	10.9^{a} ± 0.41	18.1^{a} ± 0.57	37.0 ± 0.95	0.75^{a} ± 0.16	32.2^{a} ± 1.38	6.2^{a} ± 0.49
DFD (n=11)	29.3^{b} ± 0.70	11.0^{b} ± 0.33	7.9^{b} ± 0.30	13.6^{b} ± 0.42	35.5 ± 0.70	1.4^{b} ± 0.12	27.7^{b} ± 1.03	4.6^{b} ± 0.37

$^{a, b, c, d}$ Column means with different supper scripts differ significantly at p ≤0.05.

4.3.2 Chemical analyses

The effect of muscle type on the proximate composition of the black wildebeest muscles is presented in Table 4.3. The LTL muscle had the lowest moisture content whilst the IS and SS muscles had the highest moisture content. The SS muscle had the lowest protein content whilst the LTL muscle had the highest protein content. The fat content was low and not significantly different between the BF and IS muscles, and that of LTL, SS and SM muscles was the highest and did not differ significantly

between the muscles. The ash content was low in the LTL, IS and ST muscles and was the highest in the BF muscle.

Table 4.3 Mean (± standard error) chemical meat quality attributes of black wildebeest muscles

Parameter	LTL	BF	IS	SS	SM	ST	P-value
Moisture (%)	75.6^{c} ± 0.18	76.5^{b} ± 0.18	77.4^{a} ± 0.25	78.1^{a} ± 0.20	76.5^{b} ± 0.25	77.7^{a} ± 0.35	**0.000**
Protein (%)	22.6^{a} ± 0.20	21.9^{bc} ± 0.12	21.3^{c} ± 0.31	19.4^{e} ± 0.24	20.1^{a} ± 0.23	20.3^{d} ± 0.33	**0.000**
Fat (%)	1.8^{a} ± 0.10	1.6^{b} ± 0.07	1.4^{bc} ± 0.11	1.8^{a} ± 0.09	1.8^{a} ± 0.12	1.3^{c} ± 0.05	**0.001**
Ash (%)	1.1^{b} ± 0.02	1.3^{a} ± 0.02	1.1^{b} ± 0.01	1.2^{ab} ± 0.04	1.2^{ab} ± 0.03	1.1^{b} ± 0.03	**0.055**

[a, b, c, d] Row means with different supper scripts differ significantly at p ≤0.05. LTL = *Longissimus thoracis et lumborum*, BF = *Biceps femoris*, IS = *Infraspinatus*, SS = *Supraspinatus*, SM = *Semimembranosus*, ST = *Semitendinosus*.

Fatty acid composition (%)

Figures 4.1 and 4.2 below display the influence of sex on the mean total PUFA and PUFA:SFA, respectively. Male black wildebeest contained a higher proportion of PUFA than females, consequently PUFA:SFA was higher in male black wildebeest than in females.

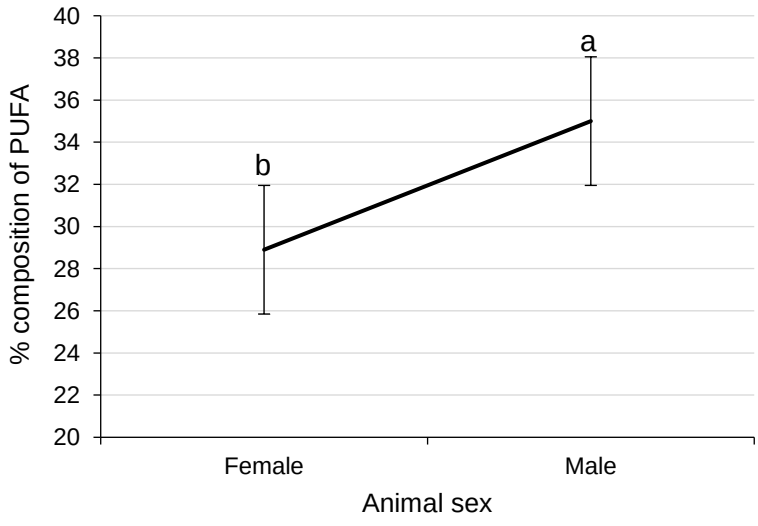

Figure 4.1 Influence of animal sex on the mean % composition of PUFA of black wildebeest muscles (p=0.040).

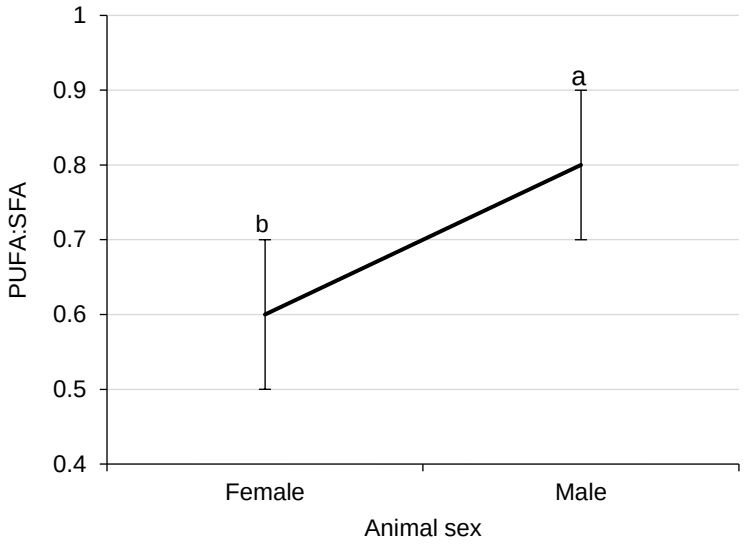

Figure 4.2 Influence of animal sex on the PUFA:SFA ratio of black wildebeest muscles (p=0.049). Different letters show significant differences.

The influence of muscle type on the fatty acid profile of black wildebeest muscles is shown in Table 4.4. Of the SFA, C18:0 (stearic acid) had the highest % composition (12.2-19.4%), followed by C16:0 (palmitic acid) which ranged from 11.3-15.7%, then C24:0 (tetracosanoic acid), C14:0 (myristic acid), whilst C15:0 (pentadecanoic acid) had the lowest % composition (1.3-2.3%). The IS muscle had the highest mean % composition of stearic acid, followed by the SM muscle and the ST muscle had the lowest mean % composition. The ST muscle had the highest mean % composition of tetracosanoic acid, followed by the IS and the SM muscle had the lowest mean % composition. The IS muscle had the highest mean % composition of myristic acid followed by the SM muscle and the SS had the lowest composition. Of the MUFA, C18:1ω-9c (oleic acid) had the highest mean % composition (1.7-7.7%), this was followed by C16:1 (palmitoleic acid while C24:1 (nervonic acid) had the lowest mean % composition which ranged from 1.0-4.2%. The SS muscle had the highest mean % composition of oleic acid, followed by the BF, ST and the SM muscle had the lowest % composition. The ST muscle contained the highest mean % composition of nervonic acid, followed by the SS and the SM muscle had the lowest composition. The SM contained the highest mean % composition of palmitoleic acid, followed by IS, BF, and the SS muscle had the lowest % composition. Of the PUFA, C22:6*ω-3* (docosahexaenoic acid) had the highest mean % composition (2.6-13.0%), followed by C18:2*ω-6c* (conjugated linoleic acid), C18:3*ω-3* (α-linolenic acid), while C20:4*ω-6* (arachidonic acid) was at the lowest composition which ranged from 0.7-4.3%. The ST and LTL muscles had the highest mean % composition of C22:6*ω-3* and the BF muscle had the lowest % composition. The ST and LTL muscles also had the highest mean % composition of conjugated linoleic acid, and the SM muscle

had the least % composition. The BF and ST muscles had the highest mean % composition of α-linolenic acid, whilst the SM and IS muscles had the lowest mean % composition.

Total SFA had the highest proportion (47-60%), followed by PUFA (23-40%) and lastly the MUFA had the lowest proportion (8.1-12.9%). The IS muscle had the highest proportion of total SFA, followed by the SM muscle, and the ST muscle had the lowest % proportion. The LTL and ST muscles had the highest proportion of PUFA and the IS muscle had the lowest proportion. The SS muscle had the highest total MUFA proportion, this was followed by the ST muscle and lastly the IS muscle had the lowest proportion. The PUFA:SFA ratios of the muscles ranged from 0.4 to 1.0 and *ω-6:ω-3* ranged from 1.2 to 4.5. The LTL muscle had the highest PUFA:SFA while the SM had the lowest ratio. The *ω-6:ω-3* ratio was the highest in the BF muscle and lowest in the SM muscle.

The significant interaction between sex and muscle type (G*M) is shown in Table 4.5. Male black wildebeest muscles had a higher % composition of C22:0 (docosanoic acid) (0.3-2.0%) while the females had a lower % composition which ranged from 0.2-0.8%. The ST muscle of male black wildebeest had the highest % composition of docosanoic acid while the IS muscle of female black wildebeest had the lowest composition. The mean % composition of C18:3*ω-3* (α-linolenic acid) for male black wildebeest muscles ranged from 1.3-3.5%, while that of females ranged from 1.4-3.1%. Male BF and ST muscles had the highest % composition of α-linolenic acid while the female LTL muscle had the lowest. The total MUFA proportion of male black wildebeest muscles ranged from 5.9-15.8% and that of female muscles ranged from 7.1-26.1%. Female SS muscle had the highest MUFA proportion and male SM muscle had the lowest proportion. Total *ω-6* PUFA of male muscles ranged from 5.7-36.5% and while that of female muscles ranged from 7.4-24.5%. Male BF and ST muscles contained a higher proportion of *ω-6* PUFA than the female LTL muscle, whilst the male SM muscle had the lowest proportion of *ω-6* PUFA.

Table 4.4 The effect of muscle type on the mean (± standard error) % composition of fatty acid profile of six black wildebeest muscles

Fatty acid profile	Muscle type						P-value
	LTL	BF	IS	SS	SM	ST	
SFA							
C14:0	$3.4^{e} \pm 0.65$	$3.9^{d} \pm 0.67$	$6.1^{a} \pm 0.64$	$2.1^{f} \pm 0.64$	$5.6^{b} \pm 0.80$	$5.2^{c} \pm 0.58$	**0.000**
C15:0	$2.2^{b} \pm 0.22$	$2.2^{b} \pm 0.23$	$2.6^{a} \pm 0.25$	$1.3^{c} \pm 0.21$	$2.1^{b} \pm 0.27$	$2.3^{ab} \pm 0.19$	**0.002**
C16:0	$11.3^{d} \pm 1.05$	$12.4^{c} \pm 0.96$	$15.7^{a} \pm 1.48$	$15.0^{a} \pm 1.10$	$14.6^{b} \pm 1.17$	$12.7^{c} \pm 1.01$	0.064
C18:0	$14.5^{c} \pm 1.09$	$14.8^{c} \pm 1.23$	$19.6^{a} \pm 1.41$	$13.4^{d} \pm 1.23$	$17.1^{b} \pm 1.09$	$12.2^{e} \pm 1.02$	**0.002**
C20:0	0.56 ± 0.07	0.61 ± 0.06	0.61 ± 0.07	0.49 ± 0.07	0.4 ± 0.07	0.61 ± 0.05	0.348
C21:0	$0.2^{b} \pm 0.06$	$0.3^{a} \pm 0.06$	$0.1^{c} \pm 0.06$	$0.2^{b} \pm 0.06$	$0.3^{a} \pm 0.06$	$0.3^{a} \pm 0.06$	**0.030**
C22:0	$0.8^{b} \pm 0.23$	$0.5^{c} \pm 0.23$	$0.2^{e} \pm 0.22$	$0.4^{cd} \pm 0.22$	$0.1^{ef} \pm 0.23$	$1.3^{a} \pm 0.23$	**0.005**
C24:0	$3.1^{d} \pm 1.1$	$4.3^{bc} \pm 0.94$	$4.7^{b} \pm 1.47$	$3.8^{c} \pm 0.96$	$2.0^{e} \pm 1.47$	$8.1^{a} \pm 1.10$	**0.015**
MUFA							
C15:1	$0.7^{b} \pm 0.21$	$0.2^{d} \pm 0.21$	$0.8^{ab} \pm 0.22$	$1.1^{a} \pm 0.22$	$0.4^{c} \pm 0.21$	$1.2^{a} \pm 0.23$	**0.018**
C16:1	$1.5^{d} \pm 0.17$	$1.9^{ab} \pm 0.18$	$2.0^{ab} \pm 0.16$	$1.1^{e} \pm 0.16$	$2.2^{a} \pm 0.19$	$1.7^{c} \pm 0.15$	**0.000**
C18:1*ω-9c*	$3.3^{cd} \pm 0.72$	$5.6^{b} \pm 0.65$	$1.7^{e} \pm 0.71$	$7.7^{a} \pm 1.05$	$1.5^{ef} \pm 0.68$	$4.12^{c} \pm 0.65$	**0.000**

C20:1	0.7 ± 0.08	0.8 ± 0.08	0.8 ± 0.08	0.7 ± 0.08	0.7 ± 0.09	0.6 ± 0.07	0.813
C24:1	1.9^{c} ± 0.49	ND	ND	2.8^{b} ± 0.50	1.0^{d} ± 0.49	4.2^{a} ± 0.51	**0.000**
PUFA							
C18:2*ω-6c*	8.5^{b} ± 1.90	2.2^{d} ± 3.65	2.4^{d} ± 1.90	7.4^{c} ± 1.80	1.5^{e} ± 1.72	11.4^{a} ± 1.72	**0.001**
C18:2*ω-6t*	0.0^{d} ± 0.05	0.1^{c} ± 0.05	0.3^{a} ± 0.05	0.1^{c} ± 0.05	0.0^{d} ± 0.05	0.2^{b} ± 0.04	**0.003**
C18:3*ω-6*	1.4 ± 0.14	1.5 ± 0.16	1.4 ± 0.14	1.4 ± 0.13	1.3 ± 0.16	1.5 ± 0.13	0.900
C18:3*ω-3*	2.6^{bc} ± 0.30	2.9^{a} ± 0.29	1.5^{d} ± 0.29	2.5^{bc} ± 0.30	1.6^{d} ± 0.29	2.7^{b} ± 0.28	**0.002**
C20:2*ω-6*	1.5 ± 0.17	1.6 ± 0.17	1.6 ± 0.17	1.5 ± 0.17	1.3 ± 0.18	1.3 ± 0.16	0.682
C20:3*ω-3*	1.6^{c} ± 0.34	1.8^{b} ± 0.32	0.2^{f} ± 0.31	1.2^{d} ± 0.32	0.7^{e} ± 0.31	2.2^{a} ± 0.35	**0.000**
C20:4*ω-6*	3.1^{c} ± 0.52	4.3^{a} ± 0.60	0.8^{e} ± 0.52	2.6^{d} ± 0.52	0.7^{e} ± 0.51	3.9^{b} ± 0.51	**0.000**
C20:5*ω-3*	0.6^{bc} ± 0.23	0.4^{d} ± 0.24	0.7^{b} ± 0.25	0.6^{bc} ± 0.21	0.7^{b} ± 0.34	1.4^{a} ± 0.21	**0.030**
C22:2*ω-6*	0.9 ± 0.27	0.6 ± 0.27	0.2 ± 0.27	0.1 ± 0.27	0.3 ± 0.27	0.6 ± 0.28	0.305
C22:6*ω-3*	11.5^{b} ± 2.45	2.6^{f} ± 2.38	6.7^{d} ± 2.45	5.5^{e} ± 2.38	9.4^{c} ± 2.47	13.0^{a} ± 2.38	**0.030**
Fatty acid totals							
SFA	48.3^{d} ± 3.71	53.1^{b} ± 3.71	68.8^{a} ± 3.71	52.6^{c} ± 3.70	66.0^{a} ± 3.71	47.0^{de} ± 3.70	**0.000**
MUFA	11.7^{bc} ± 1.57	9.5^{d} ± 1.56	8.1^{e} ± 1.57	20.9^{a} ± 1.57	9.5^{d} ± 1.57	12.9^{b} ± 1.56	**0.000**
PUFA	40.0^{a} ± 3.30	37.4^{b} ± 3.30	23.0^{e} ± 3.30	26.5^{c} ± 3.30	24.6^{d} ± 3.30	40.0^{a} ± 3.29	**0.000**

ω-6	20.6^{b} ± 3.15	28.5^{a} ± 3.85	10.8^{d} ± 2.41	15.3^{c} ± 2.65	8.2^{e} ± 2.22	20.4^{b} ± 2.47	**0.000**
ω-3	19.5^{a} ± 2.89	9.4^{e} ± 1.63	12.5^{c} ± 2.78	10.5^{d} ± 2.23	16.4^{b} ± 3.24	19.8^{a} ± 2.05	**0.010**
Fatty acid ratios							
PUFA:SFA	1.0^{a} ± 0.10	0.8^{ab} ± 0.11	0.4^{cd} ± 0.11	0.6^{bc} ± 0.11	0.5^{c} ± 0.11	0.9^{a} ± 0.11	**0.000**
ω-6:ω-3	2.4^{c} ± 0.72	4.5^{a} ± 0.72	2.2^{cd} ± 0.72	3.3^{b} ± 0.72	1.2^{f} ± 0.77	1.5^{e} ± 0.72	**0.030**

$^{a, b, c, d}$ Row means with different supper scripts differ significantly at p ≤0.05. ND- not detected.

Table 4.5 Influence of sex and muscle type on the mean (± standard error) fatty acid composition of black wildebeest muscles

	LTL	BF	IS	SS	SM	ST	P-value
C22:0							**0.023**
Male	$0.9^{a} \pm 0.32$	$0.3^{c} \pm 0.32$	ND	ND	ND	$2.0^{b} \pm 0.34$	
Female	$0.6^{b} \pm 0.34$	$0.8^{a} \pm 0.34$	$0.3^{c} \pm 0.30$	$0.8^{a} \pm 0.32$	$0.2^{c} \pm 0.32$	$0.6^{b} \pm 0.32$	
C18:3*ω-3*							**0.010**
Male	$2.0^{b} \pm 0.39$	$3.5^{a} \pm 0.39$	$1.7^{c} \pm 0.41$	$2.2^{b} \pm 0.39$	$1.3^{d} \pm 0.39$	$3.5^{a} \pm 0.39$	
Female	$3.1^{a} \pm 0.44$	$2.4^{c} \pm 0.42$	$1.4^{e} \pm 0.41$	$2.7^{b} \pm 0.44$	$1.8^{d} \pm 0.42$	$1.9^{d} \pm 0.42$	
Total MUFA							**0.000**
Male	$7.8^{e} \pm 2.15$	$10.4^{c} \pm 2.15$	$9.2^{d} \pm 2.15$	$15.7^{a} \pm 2.15$	$5.9^{f} \pm 2.15$	$15.8^{a} \pm 2.15$	
Female	$15.7^{b} \pm 2.28$	$8.7^{e} \pm 2.28$	$7.1^{f} \pm 2.28$	$26.1^{a} \pm 2.29$	$13.0^{c} \pm 2.28$	$10.0^{d} \pm 2.29$	
Total *ω-6*							**0.010**
Male	$17.1^{c} \pm 3.67$	$36.5^{a} \pm 3.67$	$13.8^{e} \pm 3.67$	$15.7^{d} \pm 3.67$	$5.7^{f} \pm 3.67$	$25.8^{b} \pm 3.67$	
Female	$24.5^{a} \pm 3.90$	$19.5^{b} \pm 3.89$	$7.4^{f} \pm 3.89$	$17.0^{c} \pm 3.89$	$11.0^{e} \pm 3.89$	$14.5^{d} \pm 3.89$	

[a, b, c, d] Row means with different supper scripts differ significantly at $p \leq 0.05$. ND- not detected.

4.4 Discussion

One of the most important meat quality determinants is muscle/meat pH, the post-mortem ultimate pH gives an indication of the future meat quality parameters such as colour, water holding capacity, tenderness, and shelf-life (Honikel, 2004; van Schalkwyk & Hoffman, 2010). The time at which pH is measured post mortem gives an indication of future meat quality characteristics, pH_u values taken at least 24 h *post-mortem* that are lower than 6.0 indicates normal meat whereas values >6.0 indicates dark, firm and dry (DFD) meat. *Ante-mortem* stress in bovine results in pH decline stopping at values above 6.0 due to the depleted glycogen stores in the live animal and thus less lactic acid is formed (Honikel, 2004; Hoffman *et al.*, 2007). High ultimate pH values are indicative of dark, firm and dry (DFD) or dark-cutting beef (Honikel, 2004).

Animal sex did not have a significant effect on the pH_u values in the current study; the same was found in the pH values of roe deer (Daszkiewicz *et al.*, 2012). North *et al.* (2016) reported that female springbok had higher pH values than males. Male black wildebeest in the current study were expected to have higher pH values than female as male game species generally have higher pH values (Hoffman, 2000).

The pH_u values from the black wildebeest muscles (LTL, BF, IS, SS, SM, ST) in the current study fell within the range of 6.50-6.59, these values are higher than the normal ultimate pH of meat which falls between 5.3-5.8, and could thus be classified as DFD. The higher values obtained in the black wildebeest muscles are attributed to higher levels of *ante-mortem* stress experienced by the animals. The animals (black wildebeest) in the study thus may have had lower glycogen levels at point of death. The lower levels of glycogen are attributable to the level of physical activity in the animals; game animals are much more active compared to domestic livestock. Of most of the game species researched, the black wildebeest is renowned for producing DFD meat due to its behaviour during harvesting/culling operations (Shange *et al.*, 2018). High pH values have also been reported from springbok (*Antidorcas marsupialis*) and greater kudu which are also planes species susceptible to pre-slaughter stress (Hoffman *et al.*, 2007; Hoffman & Laubscher, 2009).

Meat colour in the current study was measured after blooming the muscles for 30 minutes, so differences obtained are mainly attributable to muscle type. The LTL and SM muscles b* values compared to other muscles. A combination of highest L*, lowest a* and b* values is characteristic of an overall lighter colour of the muscle as it diverges the most from the true red axis (Brewer *et al.*, 2001). However, the L* values reported in this study were

lower than those reported for other game species (Hoffman *et al.*, 2007b; Neethling *et al.*, 2016) and are once again indicative of DFD meat. Onyango *et al.* (1998) found that desirable game meat colour consists of high a* and b* values, which leads to higher colour saturation giving a brighter appearance and pure colour to the muscle. Less colour saturation results in a dull appearance in meat. An inverse relationship between the pH and lightness of meat from young bovine bulls was observed by Vestergaard *et al.* (2000). The forequarter muscles (IS and SS) had the highest Chroma value than the rest. The colour coordinate values obtained in the study are noticeably lower than those of control LTL, BF, SS, and ST beef muscles from a study conducted by Hoffman *et al.* (2012).

In addition to meat colour, water holding capacity is another meat quality determinant that is influenced by the ultimate pH, particularly where the pH declines at a rapid or slow rate and remains either too high or low (Brewer *et al.*, 2001). The hindquarter muscles BF and SM had higher drip loss percentages than those of the forequarter (IS and SS), and the LTL muscle had the highest drip loss percentage (although the differences were not significant). Drip loss is also undesirable with regards to meat quality as it shows loss of imperative sarcoplasmic proteins such as myoglobin which is responsible for the bright red colour in meat. Although not significant, drip loss percentages of the muscles in the current study ranged from 1.1-2.15% while cooking loss percentages differed significantly between the muscles and ranged from 25.9-33.5%.

When comparing black wildebeest to other game species in terms of water holding capacity attributes (drip loss and cooking loss), springbok meat had higher drip loss percentages and comparable cooking loss percentages (Hoffman *et al.*, 2007a). Impala (*Aepyceros melampus*) cropped during the day and at night had higher drip loss percentage (2.78%-3.66%) and lower cooking loss percentage ranging from 27.41% to 27.99% (Hoffman & Laubscher, 2009b). Both these species had pH_u values <6.0 and could be classified as normal. Blue wildebeest (*Connochates taurinus*) also showed a lower ultimate pH of 5.41, a higher drip loss (4.9%) and cooking loss (39.4%) (Hoffman *et al.*, 2011) than the black wildebeest in this study. Day and night cropped greater kudu had a slightly higher drip loss percentage (1.36%-2.76%) than black wildebeest (Hoffman & Laubscher, 2009b). When the data of the present study was categorized (Table 4.2) into Normal and DFD meat, the differences in WBC became even more clear.

Fluid loss due to cooking are reported to be higher than those from uncooked meat, this is attributed to an increased temperature. Anatomical location of samples also has an influenced on the cooking loss, sirloin steaks loss less moisture on cooking than those of the topside; this is attributable to an increased amount of intramuscular fat in the sirloin (Lawrie & Ledward, 2006). Meat with higher cooking loss percentages is reported as being of inferior

quality. Higher cooking losses (and in turn a lower water holding capacity) can lead to dry and less juicy meat, giving the perception that the meat is also tough. The ST muscle in the current study had the highest cooking loss percentage and thus a lower water holding capacity compared to the other muscles. When Rhee *et al.* (2004) compared 11 beef muscles, they also found that the ST muscle had the highest cooking loss percentage compared to other muscles.

There were significant differences in the mean Warner-Bratzler shear force values between the muscles. The SM muscle had the highest mean Warner-Bratzler shear force value (and thus least tender), followed by the LTL, whilst the forequarter muscles (IS and SS) had the lowest mean Warner-Bratzler shear force values and thus were most tender. There are threshold values reported in literature to measure beef tenderness, Warner-Bratzler shear force values below 42.28 N (4.87 kg/cm ø) are representative of tender meat whereas those above 58.76 N (5.99 kg/cm ø) represent tough meat (Destefanis *et al.*, 2008). All of the mean WBSF values obtained from the muscles fall within the threshold range of 42.28- 58.76 N which seems to indicate that they are intermediate in tenderness/toughness. Arranging the muscles from most to least tender the following ranking is noted: SS > IS > LTL > BF > ST > SM. Rhee *et al.* (2004) also found beef IS muscle amongst the most tender of the major muscles. In addition to meat colour, tenderness is another attribute that influences consumer perception of meat quality (Lawrie & Ledward, 2006; Henchion *et al.*, 2017). Tenderness of meat is also influenced by the ultimate pH of meat which in turn affects the colour and water holding capacity thereof, increased pH results in increased toughness in meat, this is attributable to a reduced sarcomere length in the pH range 5.58-6.2 (Hoffman & Laubscher, 2009b). Neethling *et al.* (2012) studied blesbok (*Damaliscus pygargus phillipsi*) and obtained similar results to those in the current study; muscles arranged from most to least tender for the blesbok were: IS > SS > BF > SM > LD > ST. Fitzhenry (2016) found in fallow deer that the LTL was most tender followed by BF, whilst the ST muscle was the least tender. Differences in tenderness results can be attributed to either apparatus used to measure tenderness or sites in the muscles that were used in the analysis. The IS and SS were expected to be the least tender since they contain more connective tissue than other muscles (Ba *et al.,* 2014), but the connective tissue is avoided when measuring tenderness so those parts of the muscles may have been more tender as a result. The IS and SS have a higher total collagen content than other muscles but they also have a high content of soluble collagen (Dominik *et al.*, 2012), which could also explain the results found in the current study. Ba *et al.* (2014) further reported that the ST muscle had a higher total collagen content which resulted in an increased WBSF value in the muscle. Another factor to take into account is that the larger muscles such as the SM have variation within the muscle, these variations are both

in the colour and tenderness (Sawyer *et al.*, 2007) and would result in variation in WBSF readings, even when a large number of samples have been sheared.

When comparing tenderness particularly of the LTL muscle of black wildebeest with that of other game species such as springbok, impala (*Aepyceros melampus*) and greater kudu; springbok meat is more tender than that of black wildebeest. Impala that were cropped during the day and at night had lower mean WBSF values (4.27 kg/cm ø to 4.69 kg/cm ø) than the black wildebeest in this study (Hoffman & Laubscher, 2009a). Blue wildebeest showed a WBSF value of 4.91 kg/cm ø (Hoffman *et al.*, 2011) whilst day and night cropped greater kudu had lower WBSF values (3.45 kg/cm ø to 4.06 kg/cm ø) (Hoffman & Laubscher, 2009a) than the black wildebeest. Interestingly, greater kudu investigated by Mostert & Hoffman (2007) showed much higher WBSF values for both male (13.93 kg/cm ø) and female (14.27 kg/cm ø) than the black wildebeest; this could be attributed to exercise as well as activities which will influence the muscle fibre types. Black wildebeest are typically found in savannahs whilst kudu are found in bushveld, with the former typically running more than the kudu who tend to jump more. Beef *longissimus lumborum* and BF muscles that were cooked in a water bath showed WBSF values of 3.23 kg/cm ø and 4.53 kg/cm ø, respectively (Obuz *et al.*, 2004); these values are comparable to those obtained in the current study for the same muscles.

The LTL muscle is the most sought after muscle in the industry due to its commercial value. This muscle is also regarded as the most representative of the carcass. In the current study six LTL samples were normal (pH <6.0) and 11 were found to be DFD (pH >6.0). The results obtained from the pH classification were as expected; the DFD meat had an overall darker red colour than the normal meat. The DFD meat also showed a higher water holding capacity than the normal meat. DFD meat is reported to have a higher tenderization rate than meat of normal pH (Greaser, 2001; Lawrie & Ledward, 2006); this was observed in the current study as the DFD meat had lower WBSF values than the normal meat (Table 4.2).

Muscle type had an effect (p=0.000) on the chemical components of black wildebeest. The chemical composition of the black wildebeest muscles in the current study consists of a moisture content between 75-80%, total protein between 19-23%, total fat content between 1.3-1.9% as well an ash content ranging from 1.1-1.3%. These values are comparable to those obtained for black wildebeest in an earlier study (Hoffman *et al.*, 2009) where the moisture content ranged between 74-76%, protein 20-24%, fat content 0.9-1.2% although a higher ash content ~2.4% was reported. The LTL, BF, SS, and ST beef muscles investigated by Hoffman *et al.* (2012) contained almost double the level of lipid (2.28%, 3.04%, 2.95% and 2.10% respectively), comparable protein (19.78%, 20.21%, 20.28% and 20.71% respectively) and moisture content (73.84%, 73.17%, 75.22%, and 75.10% respectively) to the black

wildebeest muscles in the current study. These results are in agreement with the general perception that game meat is a lower fat alternative to domesticated red meat. Blue wildebeest (*C. taurinus*) evaluated by Hoffman *et al.* (2011) showed comparable values for chemical composition; 76.04% moisture, 22.28% protein, 1.06% lipid as well as 2.35% ash. Game meat is generally perceived as healthier than domesticated livestock meat due to its lower fat content (1% to 3%) which is attributed to higher levels of physical activity in the animals than pork, lamb and beef, and higher protein content per gram of meat (~20%) (Hoffman & Ferreira, 2004; Hoffman *et al.*, 2005; van Schalkwyk & Hoffman, 2010).

The fatty acid content and composition is mainly influenced by animal diet; where a grass based diet is reported to contain high levels of stearic acid as well as α-linolenic acid (Vestergaard *et al.*, 2000b; Wood *et al.*, 2004). Animal sex and muscle type showed an influence on the fatty acid profile of black wildebeest muscles in the current study. Male black wildebeest contained higher levels of PUFA than females, while females had higher levels of MUFA and SFA than males. Male impala also had higher levels of PUFA (with high proportions of α-linolenic acid, *γ*-linoleic acid and linolenic acid) compared to females. The sex by muscle type interaction (G*M) observed in the current study ($p<0.05$) showed that male black wildebeest muscles contained a higher proportion of C18:3*ω-3* than females. On the other hand, female black wildebeest muscles contained a higher proportion of MUFA than those of male black wildebeest. Muscles of male black wildebeest contained a higher total *ω-6* PUFA than those of female black wildebeest.

Muscles are composed of different fibre types and thus their fatty acid composition is expected to vary between muscles; red muscle fibres have a higher proportion of phospholipids than white fibres and consequently contain higher levels of PUFA compared to the white fibres (Wood *et al.*, 2004). The fatty acid profile of black wildebeest muscles in the current study consisted of a higher proportion of SFA, followed by PUFA and lastly the MUFA with the lowest proportion. Impala also contained a higher proportion of SFA, followed by PUFA and the MUFA had the lowest fatty acid proportion. Higher levels of SFA were also found in beef *longissimus dorsi* muscle, this was followed by PUFA which ranged from 5-16% (Rule *et al.*, 2002). PUFA are susceptible to oxidation and this is reported to induce flavor development in meat during cooking (Wood *et al.*, 2004). The major SFA of the muscles in the current study included C14:0 (myristic acid), C15:0 (pentadecanoic acid), C16:0 (palmitic acid), C18:0 (stearic acid), C24:0 (teracosanoic acid); stearic acid had the highest composition (%). The major MUFA were C16:1 (palmitoleic acid), C18:1*ω-9c* (oleic acid) and C24:1 (nervonic acid). The main PUFA were found to be C18:2*ω-6c* (linoleic acid), C18:3*ω-3* (α-linolenic acid), C20:4*ω-6* (arachidonic acid) and C22:6*ω-3* (docosahexaenoic acid). The main

SFA of impala were myristic acid, palmitic and stearic acid (Hoffman *et al.*, 2005). The main fatty acids of the *longissimus dorsi* muscle of young bulls included myristic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid; oleic acid had the highest proportion which ranged from 33.97% to 38.48% (Ladeira *et al.*, 2014). The SM muscle of grass-fed lamb contained high proportions of α-linolenic, eicosapentaenoic and docosahexaenoic acids (Wood *et al.*, 2004). The major fatty acids of beef LD muscle included myristic, pentadecanoic, palmitic, stearic, oleic, linolenic, α-linolenic and arachidonic acids (Rule *et al.*, 2002). Palmitic and stearic acids were the major fatty acids found in the LD muscle of blesbok (Hoffman *et al.*, 2008).

The PUFA:SFA ratios in the current study ranged from 0.4 to 1.0; although the highest ratio was higher than the recommended value of 0.7 by Raes *et al.* (2004) the values obtained fell within the range of 0.58-1.81 reported by Hoffman and Wiklund (2006) for African game meat species. The *ω-6:ω-3* ratio in the current study ranged from 1.2 to 4.5; the highest value is greater than the value of <4 recommended by Wood *et al.* (2004) as values that exceed 4 are at a risk of cancers as well as heart diseases. Game meat is a considered as a health product, this is attributed to its low intramuscular fat content and high levels of PUFA (Hoffman *et al.*, 2005).

4.5 Conclusion

This study is one of the first to be conducted on the physicochemical meat quality attributes of black wildebeest as influenced by muscle type and sex. Sex did not have a significant effect on the physical attributes and proximate composition of black wildebeest meat, although a sex effect might be observed if harvesting were conducted on different seasons and at different location. Male black wildebeest contained higher levels of PUFA than females, while females had higher levels of MUFA than males. Female black wildebeest had higher proportions of SFA than males. The results obtained from this study demonstrate that black wildebeest meat compares favourably with other commonly used game species as well as domestic livestock species (such as cattle) as pertaining to the chemical composition and thus nutritional value. Black wildebeest contains comparable moisture content as that of beef but a higher protein and lower fat content. However, all of the muscles analysed in the study showed susceptibility to DFD meat due to their higher pH_u (>6.2). Muscle type showed a significant effect on the measured physical (pH, drip loss, cooking loss, Warner-Bratzler shear force) and chemical (moisture, total fat, total protein, ash) meat quality attributes, which could be valuable to the game meat industry in terms of selecting which muscle type will be marketed as prime cuts or

which muscle is used for further processing eg. mincing. This information will also add to consumer knowledge about the positive aspects of game meat. The results on chemical attributes obtained in the study can be utilised to make possible nutritional information claims on the meat; such as being high in protein, low in fat as well as possessing a favourable fatty acid profile.

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Addendum

Table 4.6 Mean (± standard error) fatty acid content (mg/g meat) of six black wildebeest muscles

	Muscle type						
Fatty acid profile	LTL	BF	IS	SS	SM	ST	**P-value**
SFA							
C14:0	0.75 ± 0.160	0.77 ± 0.160	1.03 ± 0.151	0.60 ± 0.151	1.21 ± 0.172	0.67 ± 0.151	0.064
C15:0	0.49 ± 0.062	0.44 ± 0.062	0.51 ± 0.060	0.27 ± 0.062	0.49 ± 0.066	0.30 ± 0.060	**0.017**
C16:0	2.05 ± 0.257	1.90 ± 0.257	2.43 ± 0.272	2.64 ± 0.285	2.67 ± 0.271	1.93 ± 0.237	0.130
C18:0	2.58 ± 0.256	2.54 ± 0.267	2.96 ± 0.28	2.64 ± 0.275	2.83 ± 0.256	1.60 ± 0.240	**0.005**
C20:0	0.16 ± 0.021	0.13 ± 0.021	0.11 ± 0.02	0.13 ± 0.021	0.14 ± 0.021	0.08 ± 0.021	0.126
C21:0	0.06 ± 0.011	0.06 ± 0.011	0.01 ± 0.011	0.04 ± 0.011	0.04 ± 0.011	0.04 ± 0.011	**0.041**
C22:0	0.20 ± 0.065	0.13 ± 0.054	0.02 ± 0.019	0.07 ± 0.040	0.05 ± 0.039	0.20 ± 0.044	**0.031**
C24:0	1.04 ± 0.208	0.67 ± 0.208	1.38 ± 0.222	0.66 ± 0.214	1.26 ± 0.229	1.23 ± 0.208	0.075
MUFA							
C15:1	0.12 ± 0.045	0.02 ± 0.014	0.12 ± 0.041	0.24 ± 0.063	0.06 ± 0.032	0.22 ± 0.033	**0.000**
C16:1	0.28 ± 0.043	0.33 ± 0.042	0.31 ± 0.039	0.23 ± 0.039	0.40 ± 0.047	0.22 ± 0.039	**0.035**
C18:1*ω-9c*	0.96 ± 0.155	0.87 ± 0.155	0.32 ± 0.165	2.00 ± 0.171	0.17 ± 0.172	0.53 ± 0.155	**0.000**
C20:1	0.19 ± 0.036	0.16 ± 0.026	0.13 ± 0.016	0.16 ± 0.019	0.19 ± 0.038	0.08 ± 0.007	**0.048**

C24:1	0.32	±	0.07	±	ND		0.55	±	0.15	±	0.56	±	**0.000**
	0.111		0.073				0.128		0.082		0.089		
PUFA													
C18:2*ω-6c*	0.67	±	1.42	±	0.53	±	0.88	±	0.23	±	1.51	±	**0.015**
	0.349		0.406		0.273		0.307		0.262		0.253		
C18:2*ω-6t*	0.03	±	0.04	±	0.06	±	0.03	±	0.03	±	0.02	±	0.622
	0.015		0.016		0.014		0.013		0.021		0.005		
C18:3*ω-6*	0.26	±	0.33	±	0.20	±	0.24	±	0.26	±	0.19	±	**0.023**
	0.054		0.045		0.026		0.016		0.041		0.017		
C18:3*ω-3*	0.45	±	0.45	±	0.24	±	0.43	±	0.25	±	0.34	±	**0.002**
	0.050		0.047		0.047		0.049		0.047		0.047		
C20:2*ω-6*	0.30	±	0.29	±	0.24	±	0.30	±	0.17	±	0.17	±	**0.007**
	0.034		0.033		0.033		0.033		0.040		0.033		
C20:3*ω-3*	0.33	±	0.30	±	0.02	±	0.24	±	0.10	±	0.34	±	**0.000**
	0.055		0.053		0.053		0.053		0.053		0.053		
C20:4*ω-6*	0.60	±	0.70	±	0.10	±	0.45	±	0.10	±	0.49	±	**0.000**
	0.123		0.129		0.033		0.113		0.055		0.070		
C20:5*ω-3*	0.37	±	0.34	±	0.32	±	0.10	±	0.55	±	0.19	±	**0.047**
	0.128		0.134		0.085		0.256		0.138		0.029		
C22:2*ω-6*	0.21	±	0.07	±	0.02	±	0.02	±	0.05	±	0.11	±	**0.027**
	0.073		0.038		0.023		0.025		0.032		0.045		
C22:6*ω-3*	2.05	±	0.45	±	1.09	±	0.99	±	1.64	±	1.67	±	0.070
	0.494		0.316		0.420		0.378		0.469		0.335		
Fatty acid totals													
SFA	8.90	±	8.50	±	10.10	±	9.40	±	12.16	±	6.15	±	**0.004**
	0.937		0.826		1.106		0.801		1.568		0.530		
MUFA	2.21	±	1.49	±	1.19	±	3.81	±	1.58	±	1.64	±	**0.000**
	0.363		0.143		0.244		0.564		0.272		0.178		
PUFA	7.33	±	5.82	±	3.09	±	4.69	±	3.82	±	5.09	±	**0.000**
	0.671		0.604		0.439		0.701		0.546		0.220		
ω-6	4.03	±	4.28	±	1.42	±	2.84	±	1.29	±	2.55	±	**0.000**
	0.723		0.625		0.291		0.449		0.342		0.301		

ω-3	3.31 ±	1.54 ±	1.67 ±	1.85 ±	2.53 ± 0.401	2.55 ±	**0.021**
Fatty acid ratios							
PUFA:SFA	1.01 ± 0.137	0.85 ± 0.131	0.40 ± 0.074	0.61 ± 0.118	0.48 ± 0.108	0.91 ± 0.076	**0.000**
ω-6:ω-3	2.40 ± 0.725	4.47 ± 0.724	2.19 ± 0.725	3.29 ± 0.725	1.23 ± 0.772	1.51 ± 0.725	**0.030**

[a, b, c, d] Row means with different supper scripts differ significantly at p ≤0.05. ND- not detected.

Chapter 5: Influence of *post-mortem* ageing on the physicochemical and microbiological attributes of black wildebeest (*Connochaetes gnou*) *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles

Abstract

This study investigated the effect of *post-mortem* ageing on the physicochemical and microbiological attributes of black wildebeest (*Connochaetes gnou*) *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles. Randomised samples of the LTL and BF muscles from male (n = 9) and female (n = 8) mature black wildebeest were stored in vacuum packaging for 26 days at 4.0 ± 0.05°C. Shelf life stability of the samples was based on pH, weep loss and cooking loss percentages, Warner-Bratzler shear force (WBSF), lipid oxidation (TBARS value), fatty acid composition and microbial growth. Sex had no significant effect on the physical attributes, lipid oxidation and microbial counts of both muscles. Muscle type showed an effect on the weep loss percentage (p=0.003) and TBARS values (p=0.001); the LTL had higher weep loss % than the BF and the BF had higher TBARS values than the LTL. Ageing time had an effect (p≤0.05) on all the measured meat quality attributes. pH, cooking loss percentage and WBSF values decreased whilst the TBARS value, total viable count (TVC) and *Enterobacteriaceae* increased with longer ageing time. It was concluded that black wildebeest LTL and BF muscles should be aged for at least 12 days under chilled vacuum packaging.

5.1 Introduction

Wild life species have been traditionally utilised for the production of dried meat products (such as biltong and dröewors) (Jones *et al.*, 2017) where the tenderness of the meat is not considered as a factor. Recently consumers have shown interest in using game species for fresh meat production thus warranting research to be conducted on the tenderness, particularly that of the primal cuts. Black wildebeest (*Connochaetes gnou*) is renowned for its running outbursts during harvesting thus producing dark, firm and dry (DFD) meat due to the high ultimate pH in the meat *post-mortem* (Shange *et al.*, 2018).

Ageing or conditioning of meat is a process of storing meat under controlled conditions (temperature, relative humidity, controlled atmosphere) to enhance its flavour and tenderness (Huff & Parrish, 1993; Lawrie & Ledward, 2006; Farouk *et al.*, 2012). Ageing of meat can be performed either in the traditional dry ageing method where no packaging is applied or wet ageing where the meat is stored in packaging (Li *et al.*, 2013). Wet aged meat is reported to be easier to handle, uses less storage space, ensures a better yield and produces better quality product compared to dry aged meat (Lepper-Blilie *et al.*, 2016). Meat is reported to be less tender at the onset of *rigor mortis*, thus it is aged to regain some of the tenderness lost at *rigor mortis* (Huff & Parrish, 1993; Lawrie & Ledward, 2006; Lana & Zolla, 2016). Game meat in particular is aged for this purpose (Lawrie & Ledward, 2006). The shelf life stability of meat stored in vacuum packaging has been noted to increase (Vázquez *et al.*, 2004; Hur *et al.*, 2013). Vacuum packaging is also reported to prevent growth of pathogenic microorganisms in meat (Pennacchia *et al.*, 2011).

Studies have been conducted on the ageing and the effects thereof on the meat quality parameters of beef, mutton as well as some game species such as springbok (*Antidorcas marsupialis*), fallow deer (*Dama dama*) and blesbok (*Damaliscus pygargus phillipsi*) (Watanabe *et al.*, 1996; Buys *et al.*, 1997; Vázquez *et al.*, 2004; Colle *et al.*, 2015; North & Hoffman, 2015; Lepper-Blilie *et al.*, 2016; Neethling, 2016). A high number of studies have been conducted on the ageing of meat since it is the main factor that affects tenderness of meat (Hur *et al.*, 2013), although most of these studies are on the traditionally farmed ruminant species. It is known that the ageing requirements of beef and sheep vary; in fact, there are also breed variations in ageing outcomes within a specific species. However, very little research has been done on the ageing effects of various game species. Ageing of meat has a positive influence on the tenderness and flavour development of meat, however prolonged ageing leads to loss of colour stability (or discoloration) (Neethling *et al.*, 2016b), development of off-flavours and odours from other changes that take place such as lipid oxidation and microbial growth (Ba *et al.*, 2014). Game meat is reported to have a high content of polyunsaturated fatty acids; these are known to be susceptible to lipid oxidation which ultimately influences meat colour and flavour. Aerobic plate counts and *Enterobacteriaceae* are known as the standards methods to indicate the microbial contamination of carcasses (Magwedere *et al.*, 2013).

Currently no ageing studies have been performed on black wildebeest (*C. gnou*) meat, a species that has shown to have great game meat production potential. The aim of this study was therefore to investigate the physicochemical and microbiological changes of black wildebeest *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) that occur during

chilled ageing under vacuum in order to determine the optimal tenderization period. The effect of sex on the aforementioned attributes was also investigated.

5.2 Materials and methods

5.2.1 Study design and sample preparation

The ageing study was performed on the *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles of the black wildebeest (*Connochaetes gnou*), from all harvested animals (n= 17). The trial was conducted over 26 days (days 4, 6, 8, 12, 18, and 26) with samples under chilled vacuum storage (~4°C). Analyses performed at each time points included pH, weep loss %, cooking loss %, Warner-Bratzler shear force (WBSF), lipid oxidation and microbiological counts. The proximate analyses (moisture, ash, fat and protein) were performed on day 4 for all samples. The fatty acid content of the LTL and BF muscles were analysed on the first (day 4) and last day (day 26) of the ageing study. Samples that were used for fatty acids and lipid oxidation were stored at -80°C. The muscles were cut into portions from the anterior side and were randomly allocated to the different ageing time points, at each time point the portions were removed from storage and cut into 2.5 cm thick steaks which were used for the various analyses.

5.2.2 Physical analyses

pH

The pH of the samples was measured at all ageing time points (4, 6, 8, 12, 18, 26 days). The same methodology as outlined in Chapter 4.2.2 was used for pH measurement.

Weep loss %

Weep loss was measured on days 4, 6, 8, 12, 18, and 26 of the ageing trial. The steaks were weighed (initial weight) immediately after cutting and then packed in vacuum bags and stored at 4°C until analysis. At each ageing time point, the steaks were removed from vacuum storage, dried and re-weighed (final weight) prior to further analysis. Weep loss was measured according to the following equation:

$$weep\ loss\ \% = \frac{weight_{before} - weight_{after}}{wieght_{before}}\ x\ 100$$

Cooking loss %

Cooking loss percentage was measured at all the time points using the same procedure outlined in Chapter 4.2.2.

Warner-Bratzler Shear force

Warner-Bratzler shear force was measured at all the time points using the same procedure outlined in Chapter 4.2.2.

Combined water loss %

Combined water loss % was calculated as a sum of weep loss and cooking loss percentages.

5.2.3 Chemical analyses

Lipid oxidation

Lipid oxidation in the meat samples was measured at all ageing time points using a modified version of the 2-thiobarbituric acid (TBARS) extraction method (Lynch & Frei, 1993). One gram of each meat sample was weighed into 50 ml centrifuge tubes; this was followed by the addition of 10 ml of 0.15 mol/L KCl buffer. This mixture was then homogenised (P-8; Kinematica, Littau, Switzerland) for 20 s. A 0.5 ml volume of the homogenate was transferred to 15 ml test tubes, subsequently equal volumes (0.25 ml) of 1% (w/v) 2-thiobarbituric acid (TBA) + 50 mM NaOH mixture and 2.8% (w/v) Trichloracetic acid (TCA) were added to the test tubes. The samples were then mixed with the vortex and incubated in the water bath at 100°C for 1 h. After incubation, the samples were removed from the water bath and allowed to cool to room temperature. Subsequently, 2 ml of n-butanol was added to and mixed with the homogenate. This was then centrifuged for 30 min at 4°C, 4000 rpm (Allegra X22R; Beckman Coulter, Germany). An amount of 200 µl of the extract was pipetted into separate wells of a microplate and absorbance was measured at 532 nm (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). The concentration of thiobarbituric acid reactive substances (TBARS) was calculated from a standard curve of 1,1,3,3-tetramethoxypropane (TMP) and expressed as mg malondialdehyde (MDA)/kg of meat.

Fatty acids composition

The fatty acid composition of the aged LTL and BF samples was analysed on day 4 and day 26 of vacuum storage, the same methodology as outlined in Chapter 4.2.3 was used.

5.2.4 Microbiological analyses

Samples from the LTL and BF muscles were obtained at each ageing time point and stored at -20°C until analysis was performed. Microbiological analysis was done on days 4, 8, 12, 18 and 26 of vacuum storage at 4°C. The media that were used in the analysis included plate count agar (PCA) (C6.500, Merck, Modderfontein, South Africa), violet red bile glucose agar

(VRBG) (C23.500, Merck, Modderfonterin, South Africa), peptone water buffered (C134.500, Modderfontein, South Africa). Growth media were prepared according to the manufacturer's specifications. Ten grams of meat was weighed aseptically followed by the addition of 90 ml of buffered peptone water (Merck, South Africa). The mixture was homogenised in a stomacher (BagMixer 400CC, Interscience) for 60 s. A dilution series was then prepared for the meat samples using buffered peptone water, then 1 ml of the corresponding dilution were aseptically transferred into labelled sterile petri dishes. The methods, incubation times and temperatures for the microorganisms enumerated in the study are displayed in Table 5.1.

Table 5.1 Methods, incubation times and temperatures for the enumerated microorganisms

Microorganism	**Media**	**Method**	**Incubation time & temperature**
Total viable count (TVC)	PCA	ISO 4833	24 h at 37°C
Enterobacteriaceae (EB)	VRBG	ISO 21528	24 h at 37°C

At the end of the incubation period, plate counts were done and the results were expressed as colony forming units (cfu) per gram of meat sample (cfu/g).

5.2.5 Statistical analysis

Statistica software, version 13.2 (Statsoft, 2016) was used for the statistical analysis of the data. Mixed models of ANOVA were used to investigate the differences between the muscles; sex and ageing time effects were taken into account. Sex, muscle type and ageing time were treated as the main effects and animal numbers were the random effects. Fischer's Least Significant Difference (LSD) was used for post hoc tests. Differences were considered significant at a 5% ($p \leq 0.05$) significance level.

5.3 Results

The interactions between the main effects for the different analyses are shown in Table 5.2. There were no interactions ($p > 0.05$) observed between sex and muscle type (S*M); sex and ageing day (S*D); muscle type and sex (M*S) and between sex, muscle type and ageing time (S*M*D) for the physical analyses, lipid oxidation and microbiological analyses that were performed. The main factors and the effects thereof on the meat quality attributes were thus considered individually. Sex did not have an effect ($p > 0.05$) on all the afore-mentioned analyses. Muscle type had an effect ($p \leq 0.05$) on the weep loss %, level of lipid oxidation and

% composition of C18:2*ω-6c*. An interaction between muscle type and ageing day (M*D) was observed on the % composition of C21:0, C22:0, C16:1, C18:1*ω-9c*, C24:1, C22:2*ω-6* and C22:6*ω-3* as shown in Table 5.7. An interaction between animal sex, muscle type and ageing day (S*M*D) was observed in the composition of C16:0, C18:3*ω-3*, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA) and PUFA:SFA; this interaction is shown in Table 5.8.

Ageing time had an influence (p≤0.05) on the physical analyses, lipid oxidation, composition of C21:0 C22:0, C24:0, C18:3*ω-3*, C20:3*ω-3*, C20:4*ω-6*, C22:2*ω-6* and on the microbial counts. Only the significant interactions and individual main effects will be discussed further.

Table 5.2 The P-values[1] indicating the effect of sex, muscle type and ageing day on the physicochemical attributes of black wildebeest LTL and BF muscles

Parameter	S*M[2]	S*D[3]	M*D[4]	S*M*D[5]	Sex	Muscle	Day
pH	0.259	0.247	0.214	0.659	0.539	0.322	**0.000**
Weep loss	0.850	0.068	0.076	0.784	0.553	**0.003**	**0.000**
Cooking loss %	0.369	0.871	0.719	0.799	0.186	0.312	**0.002**
Total water loss %	0.730	0.991	0.710	0.640	0.230	0.690	**0.005**
WBSF (kg/cm ø)	0.676	0.811	0.919	0.377	0.119	0.148	**0.000**
TBARS (mg MDA/kg)	0.501	0.545	0.400	0.279	0.655	**0.001**	**0.000**

[1]P-values in bold specify significant effect/interaction (p≤0.05). [2]Interaction between sex and muscle type. [3]Interaction between sex and ageing day. [4]Interaction between muscle type and ageing day. [5]Interaction between sex, muscle type and ageing day.

Table 5.3 The P-values[1] indicating the effect of sex, muscle type and ageing time on the fatty acid composition of black wildebeest LTL and BF muscles

Fatty acid profile	S*M[2]	S*D[3]	M*D[4]	S*M*D[5]	Sex	Muscle	Day
SFA							
C14:0	0.440	0.200	0.900	0.210	0.840	0.460	0.250
C15:0	0.840	0.560	0.920	0.660	0.210	0.930	0.540
C16:0	0.090	0.350	0.150	**0.010**	0.630	0.850	0.900
C18:0	0.330	0.790	0.770	0.060	0.200	0.900	0.530
C20:0	0.730	0.410	0.960	0.520	0.250	0.560	0.960

C21:0	0.160	0.950	**0.050**	0.080	0.530	0.470	**0.000**
C22:0	0.410	0.170	**0.040**	0.870	0.500	0.310	**0.010**
C24:0	0.940	0.750	0.200	0.790	0.780	0.880	**0.000**
MUFA							
C15:1	0.690	0.210	0.110	0.200	0.110	0.160	0.120
C16:1	0.110	0.850	**0.040**	0.960	0.950	0.550	0.660
C18:1*ω-9c*	0.980	0.440	**0.020**	0.140	0.630	0.910	0.420
C20:1	0.740	0.240	0.400	0.500	0.270	0.550	0.980
C24:1	0.390	0.130	**0.010**	0.620	0.890	0.190	0.520
PUFA							
C18:2*ω-6c*	0.910	0.990	ND	ND	0.840	**0.020**	0.080
C18:2*ω-6t*	0.280	0.480	0.560	0.970	0.950	0.120	0.670
C18:3*ω-6*	0.240	0.550	0.590	0.540	0.240	0.130	0.170
C18:3*ω-3*	0.530	0.470	0.260	**0.010**	0.510	0.880	**0.050**
C20:2*ω-6*	0.460	0.490	0.330	0.810	0.730	0.930	0.120
C20:3*ω-3*	0.440	0.180	0.250	0.850	0.840	0.650	**0.000**
C20:4*ω-6*	0.470	0.850	0.130	0.080	0.890	0.750	**0.030**
C20:5*ω-3*	0.950	0.920	0.320	0.780	0.580	0.890	0.080
C22:2*ω-6*	0.930	0.360	**0.050**	0.250	0.440	0.290	**0.000**
C22:6*ω-3*	0.110	0.350	**0.000**	0.150	0.120	0.430	0.100
Fatty acid totals							
SFA	0.320	0.210	0.270	**0.020**	0.440	0.630	0.100
MUFA	0.200	0.370	0.750	**0.000**	0.060	0.100	0.760
PUFA	0.540	0.080	0.250	0.090	0.090	0.850	**0.050**
Fatty acid ratios							
PUFA:SFA	0.390	0.140	0.190	**0.030**	0.280	0.870	0.440
ω-6:ω-3	0.110	0.570	**0.020**	0.150	0.320	0.720	0.920

[1]P-values in bold specify significant effect/interaction (p≤0.05). [2]Interaction between sex and muscle type. [3]Interaction between sex and ageing day. [4]Interaction between muscle type and ageing day. [5]Interaction between sex, muscle type and ageing day. SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω3 PUFA, omega-3

polyunsaturated fatty acids; ω6 PUFA, omega-6 polyunsaturated fatty acids; PUFA:SFA, polyunsaturated to saturated fatty acids ratio; ω6:ω3, omega-6 to omega-3 polyunsaturated fatty acids ratio. ND, not determined.

Table 5.4 The P-values[1] indicating the effect of sex, muscle type and ageing day on the microbiological attributes of black wildebeest LTL and BF muscles

Parameter	S*M[2]	S*D[3]	M*D[4]	S*M*D[5]	Sex	Muscle	Day
Total Viable Count (log cfu*l*g)	0.478	0.239	0.719	0.597	0.413	0.873	**0.000**
Enterobacteriaceae (log cfu*l*g)	0.252	0.997	0.109	0.489	0.870	0.237	**0.000**

[1]P-values in bold specify significant effect/interaction (p≤0.05). [2]Interaction between sex and muscle type. [3]Interaction between sex and ageing day. [4]Interaction between muscle type and ageing day. [5]Interaction between sex, muscle type and ageing day.

5.3.1 Physical analyses

The LTL muscle had a weep loss of 0.56 ± 0.073% whereas the BF muscle had 0.38 ± 0.073% (p=0.002).

The pH values of both muscles gradually decreased until day 12 of storage, after which a gradual increase was observed on day 18 (Table 5.5). The pH on day 26 was significantly lower than that of the day 4. The weep loss percentage decreased significantly with longer ageing time (Table 5.5). Cooking loss % decreased from day 4 to day 6 of storage, an increase from day 6 to day 8 was observed and thereafter the values decreased until day 26 of storage. Total water loss % decreased from day 4 to day 6 of storage, after which an increased occurred until day 8. Total water loss % decreased from day 8 to day 26 of storage. WBSF values increased from day 4 to day 8 of storage, there was a significant decrease from day 8 to day 12 after which another increase was observed until day 26 (Figure 5.1).

Table 5.5 Effect of ageing time (days) on the mean (± standard error) physical analyses of LTL and BF muscles of black wildebeest

	Ageing time (days *post-mortem*)					
	4	**6**	**8**	**12**	**18**	**26**
pH_u	6.53^{a} ± 0.123	6.44^{b} ± 0.092	6.45^{ab} ± 0.083	6.44^{b} ± 0.082	6.55^{a} ± 0.122	6.37^{c} ± 0.080
Weep loss %	0.2^{cd} ± 0.089	0.3^{c} ± 0.089	0.4^{bc} ± 0.090	0.5^{b} ± 0.090	0.7^{a} ± 0.089	0.7^{a} ± 0.089
Cooking loss %	26.9^{a} ± 1.04	24.1^{bc} ± 0.84	26.7^{a} ± 0.93	25.8^{a} ± 0.77	24.7^{b} ± 0.82	23.7^{c} ± 0.83

Total water loss (%)	$27.2^{a} \pm 1.14$	$24.8^{c} \pm 1.14$	$27.3^{a} \pm 1.13$	$26.8^{ab} \pm 1.13$	$25.6^{b} \pm 1.13$	$24.7^{c} \pm 1.13$

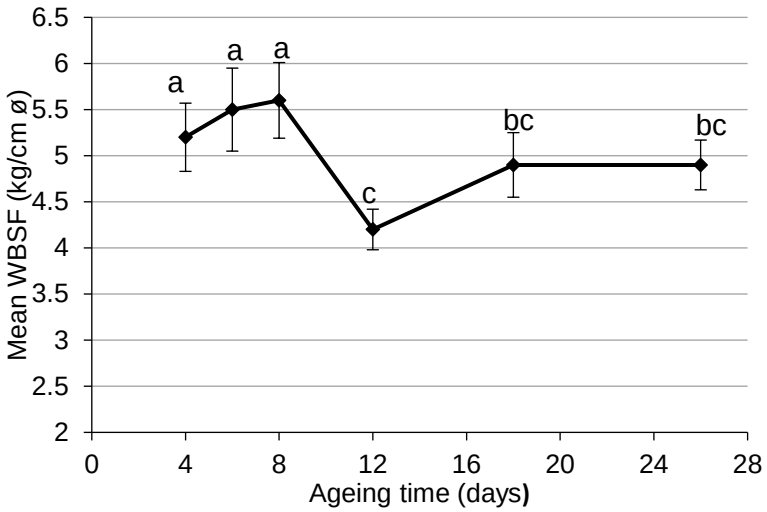

Figure 5.1 Timeous change in the mean WBSF values of black wildebeest *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles. Significant differences (p≤0.05) are indicated by different letters.

5.3.2 Chemical analyses

Lipid oxidation

The level of lipid oxidation on the aged LTL and BF muscles was influenced (p≤0.05) by muscle type and ageing time. The mean concentration of TBARS (mg MDA/kg) of the LTL muscle was lower (1.11 ± 0.056 mg MDA/kg meat) than that of the BF muscle (1.32 ± 0.055 mg MDA/kg meat); p=0.001.

The level of lipid oxidation showed an overall increase from day 4 to day 26 (1.1-1.80 mgMDA/kg meat) of storage, although there was also an up and down pattern on the days in between (Figure 5.2).

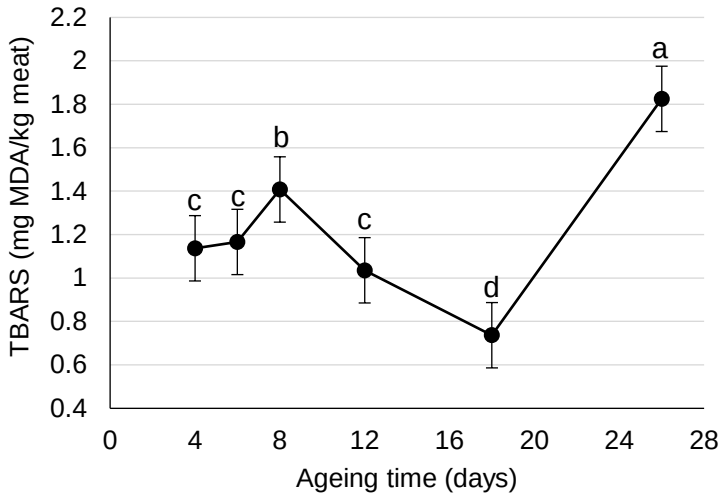

Figure 5.2 Effect of ageing time on the mean concentration of TBARS (mg MDA/kg meat) of *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles of black wildebeest. Significant differences (p≤0.05) are indicated by different letters.

Fatty acid composition

Table 5.6 shows the influence of ageing time on the fatty acid profiles of black wildebeest LTL and BF muscles. C16:0 and C18:0 were the major SFA in the LTL and BF muscles. The dominant MUFA in both muscles was found to be C18:*1ω-9c.* PUFA with the highest proportion were C18:2*ω-6c* followed by C22:6*ω-3* andC20:4*ω-6*. Total SFA increased with ageing time from 50.6% to 45.5%, there was a slight decrease in the total MUFA from 10.6% to 10.3% and the total PUFA increased from 38.7% to 44.2%. The PUFA:SFA increased slightly from 0.9 to 1.0 at the end of the ageing period and the *ω-6:ω-3* remained unchanged.

Muscle type showed an influence on the composition (%) of C18:2*ω-6*c (p=0.020); the LTL muscle contained 12.4 ± 1.78% whereas the BF muscle had 5.7 ± 2.08%.

The composition (%) of C21:0, C22:0, C24:0, C18:3*ω-3*, C20:3*ω*-3, C20:4*ω-6* and C22:6*ω-3* increased from day 4 to day 26 of ageing.

Table 5.7 shown an interaction between muscle type and ageing time (M*D) in the % composition of C16:1, C18:1*ω-9c*, C24:1, C22:2*ω-6*, C22:6*ω-3* and *ω-6:ω-3* ratio (Table 5.7). The C16:1 composition of the LTL muscle decreased with longer ageing time from 1.8% to 1.5% whereas that of BF muscle increased from 1.5% to 1.7%. Composition of C18:1*ω-9c* increased with longer ageing time in LTL muscle (from 3.4% to 6.1%), the opposite was observed in the BF muscle (5.6% decreased to 4.1%). Composition of the C24:1 fatty acid in the LTL muscle decreased from 1.9% to 0.3% as the ageing period lengthened, the oppiside was observd in the BF where the composition increased from 0.0% to 1.0%. Composition of

the C22:2*ω-6* fatty acid increased with loner ageing period in both LTL and BF muscles; from 0.9% to 1.6%v and 0.6% to 2.7% respectively. The C22:6*ω-3* decreased in the LTL muscle (from 1.5% to 0.8%), whereas there was in increase in the BF muscle (from 2.6% to 6.5%). The *ω-6:ω-3* in the LTL muscle decreased from day 4 to day 26 of the ageing period; the opposite was observed in the BF muscle (from 4.5% to 2.6%).

Table 5.8 shows the interaction between sex, muscle type and ageing time (S*M*D) in the composition of C16:0 and C18:3*ω-3* fatty acids, total SFA, total MUFA, and PUFA:SFA. Male LTL muscle showed a higher composition of C16:0 than the female on day 4, however the female LTL had a higher composition than the male on day 26. Female BF muscle had a higher composition of C16:0 on day 4, the opposite was obtained in the male BF muscle. Male LTL muscle had the lowest composition of C18:3*ω-3* (2.0%) on day 4, there was no significant difference between the male and female LTL muscles on day 26 of the ageing period. Female BF muscle had an increase in the composition of C18:3*ω-3* from 2.4% to 3.9%, the opposite was obtained in the male BF muscle (from 3.4% to 2.7%). Total SFA in the male LTL muscle decreased from 50.6% to 44.8%, an increase from 45.8% to 48.1% was found in the female LTL muscle; male BF had an increase from 44.5% to 47.7% and the opposite was obtained in the female muscle where the composition decreased from 61.6% to 41.3% from day 4 to day 26. Total MUFA composition in the male LTL muscle increased from 7.8% to 11.4%, the composition decreased from 15.7% to 10.9% in the female muscle. Total MUFA in the male BF decreased from 10.3% to 7.9%; an increase was found in the female muscle (from 8.7% to 11.2%). The PUFA:SFA ratio remained the same in the male LTL muscle whereas there was a slight decrease from 1.0% to 0.9% in the female muscle. The PUFA:SFA in the male BF muscle decreased with longer ageing from 1.2% to 1.0% and the opposite was obtained in the female muscle.

Table 5.6 Effect of ageing time (days) on the mean (± standard error) % composition of fatty acids in LTL and BF muscles of black wildebeest

	Ageing time (days *post-mortem*)		
Fatty acid profile	4	26	**P-value**
SFA			
C14:0	3.5 ± 0.47	4.2 ± 0.42	0.249

C15:0	2.2 ± 0.13	2.1 ± 0.12	0.543
C16:0	11.8 ± 0.69	11.7 ± 0.63	0.899
C18:0	14.4 ± 0.77	13.8 ± 0.69	0.528
C20:0	0.6 ± 0.03	0.6 ± 0.03	0.955
C21:0	0.3 ± 0.04	0.5 ± 0.04	**0.001**
C22:0	0.7 ± 0.21	1.4 ± 0.23	**0.010**
C24:0	3.7 ± 0.67	7.6 ± 0.66	**0.001**
MUFA			
C15:1	0.4 ± 0.12	0.2 ± 0.12	0.121
C16:1	1.7 ± 0.10	1.6 ± 0.09	0.662
C18:1*ω-9c*	4.5 ± 0.51	5.1 ± 0.55	0.419
C20:1	0.7 ± 0.05	0.7 ± 0.04	0.977
C24:1	0.9 ± 0.33	0.6 ± 0.33	0.517
PUFA			
C18:2*ω-6c*	6.0 ± 2.16	12.0 ± 2.06	0.082
C18:2*ω-6t*	0.1 ± 0.04	0.10 ± 0.04	0.673
C18:3*ω-6*	1.4 ± 0.079	1.6 ± 0.073	0.168
C18:3*ω-3*	2.7 ± 0.23	3.4 ± 0.24	**0.047**
C20:2*ω-6*	1.5 ± 0.10	1.3 ± 0.09	0.121
C20:3*ω-3*	1.7 ± 0.23	2.9 ± 0.25	**0.003**
C20:4*ω-6*	3.7 ± 0.43	5.1 ± 0.41	**0.030**
C20:5*ω-3*	0.5 ± 0.19	0.1 ± 0.17	0.083
C22:2*ω-6*	0.8 ± 0.25	2.2 ± 0.26	**0.001**
C22:6*ω-3*	7.0 ± 1.36	3.7 ± 1.38	0.104
Fatty acid totals			
SFA	50.6 ± 2.08	45.5 ± 2.08	0.100
MUFA	10.6 ± 0.71	10.3 ± 0.71	0.759
PUFA	38.7 ± 1.80	44.2 ± 1.80	**0.048**
Fatty acid ratios			

PUFA:SFA	0.9 ± 0.07	1.0 ± 0.07	0.436
ω-6:ω-3	3.4 ± 0.47	3.4 ± 0.47	0.916

[1]P-values in bold specify significant effect (p≤0.05). SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω3 PUFA, omega-3 polyunsaturated fatty acids; ω6 PUFA, omega-6 polyunsaturated fatty acids; PUFA:SFA, polyunsaturated to saturated fatty acids ratio; ω6:ω3, omega-6 to omega-3 polyunsaturated fatty acids ratio.

Table 5.7 The interaction between muscle type and ageing day (M*D) on the mean (± standard error) % composition of fatty acids in LTL and BF muscles of black wildebeest

	Ageing time (days *post-mortem*)	
	4	26
C21:0		
LTL	0.2^{d} ± 0.05	0.6^{a} ± 0.06
BF	0.3^{c} ± 0.05	0.5^{b} ± 0.05
C22:0		
LTL	0.8^{c} ± 0.29	0.9^{b} ± 0.30
BF	0.5^{d} ± 0.29	2.0^{a} ± 0.35
C16:1		
LTL	1.5^{c} ± 0.13	1.7^{b} ± 0.12
BF	1.8^{a} ± 0.14	1.5^{c} ± 0.12
C18:1*ω-9c*		
LTL	3.4^{d} ± 0.76	6.1^{a} ± 0.78
BF	5.6^{b} ± 0.69	4.1^{c} ± 0.78
C24:1		
LTL	1.9^{a} ± 0.45	0.3^{c} ± 0.45
BF	0.0^{d} ± 0.47	1.0^{b} ± 0.45
C22:2*ω-6*		
LTL	0.9^{c} ± 0.36	1.6^{b} ± 0.36
BF	0.6^{d} ± 0.35	2.7^{a} ± 0.38
C22:6*ω-3*		
LTL	1.5^{c} ± 1.95	0.8^{d} ± 1.89

BF	2.6^{b} ± 1.89	6.5^{a} ± 2.01
ω-6:ω-3		
LTL	2.4^{cd} ± 0.67	4.1^{b} ± 0.67
BF	4.5^{a} ± 0.67	2.6^{c} ± 0.67

$^{a, b, c, d}$ Column means with different supper scripts differ significantly at p ≤0.05. LTL, *Longissimus thoracis et lumborum*; BF, *Biceps femoris*.

Table 5.8 The interaction between sex, muscle type and ageing day (S*M*D) on the mean (± standard error) % composition of fatty acids of male and female black wildebeest LTL and BF muscles

	Ageing time (days *post-mortem*)	
	4	26
C16:0		
LTL		
Male	14.1^{a} ± 1.40	11.6^{c} ± 1.15
Female	8.3^{e} ± 1.40	13.3^{ab} ± 1.22
BF		
Male	10.8^{d} ± 1.15	11.4^{cd} ± 1.15
Female	14.0^{a} ± 1.40	10.4^{cd} ± 1.30
C18:3*ω-3*		
LTL		
Male	2.0^{e} ± 0.41	3.7^{a} ± 0.43
Female	3.1^{bc} ± 0.46	3.5^{ab} ± 0.43
BF		
Male	3.4^{ab} ± 0.41	2.7^{d} ± 0.43
Female	2.4^{de} ± 0.43	3.9^{a} ± 0.46
Total SFA		
LTL		
Male	50.6^{b} ± 4.04	44.8^{de} ± 4.04

Female	45.8^{d} ± 4.29	48.1^{bc} ± 4.29
BF		
Male	44.5^{e} ± 4.04	47.7^{c} ± 4.04
Female	61.6^{a} ± 4.29	41.3^{f} ± 4.29
Total MUFA		
LTL		
Male	7.8^{f} ± 1.39	11.4^{b} ± 1.38
Female	15.7^{a} ± 1.47	10.9^{c} ± 1.47
BF		
Male	10.3^{cd} ± 1.39	7.9^{ef} ± 1.38
Female	8.7^{e} ± 1.47	11.2^{bc} ± 1.47
PUFA:SFA		
LTL		
Male	1.0^{ab} ± 0.14	1.0^{ab} ± 0.14
Female	1.0^{ab} ± 0.15	0.9^{bc} ± 0.15
BF		
Male	1.2^{a} ± 0.14	1.0^{b} ± 0.14
Female	0.5^{d} ± 0.15	1.2^{a} ± 0.15

$^{a, b, c, d, e, f}$ Column means with different supper scripts differ significantly at p ≤0.05. LTL, *Longissimus thoracis et lumborum*; BF, *Biceps femoris*.SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω3 PUFA, omega-3 polyunsaturated fatty acids; ω6 PUFA, omega-6 polyunsaturated fatty acids; PUFA:SFA, polyunsaturated to saturated fatty acids ratio; ω6:ω3, omega-6 to omega-3 polyunsaturated fatty acids ratio. LTL, *Longissimus thoracis et lumborum*; BF, *Biceps femoris*.

5.3.3 Microbiological analyses

Muscle type had no effect on the microbial growth; only ageing time (days) showed a significant effect on the microbiological analyses that were performed. Mean TVC and *Enterobacteriaceae* counts increased with longer ageing time; TVC increased from 4.0-6.9 log cfu/g while the *Enterbacteriaceae* increased from 3.4-6.9 log cfu/g (see Figure 5.3 and 5.4 respectively).

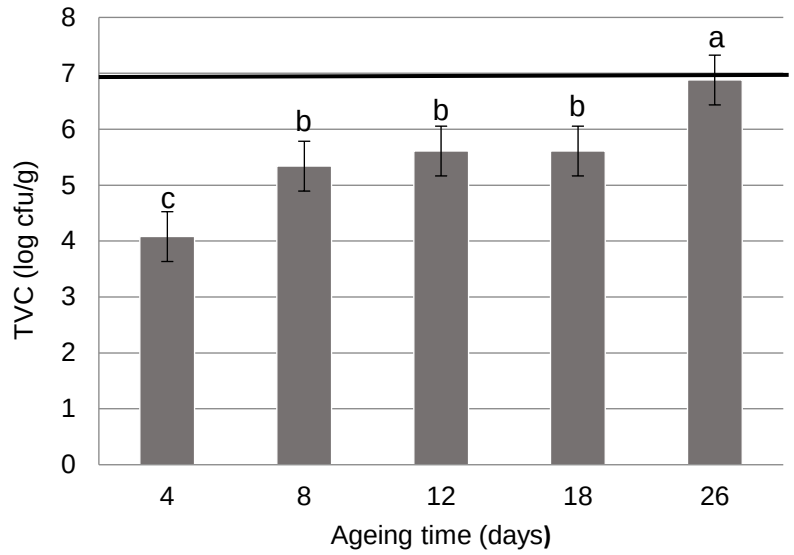

Figure 5.3 Effect of ageing time (days) at 4°C on the mean TVC (log cfu/g) enumerated from *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles of black wildebeest. The bold line at 7.0 log cfu/g is the maximum acceptable limit.

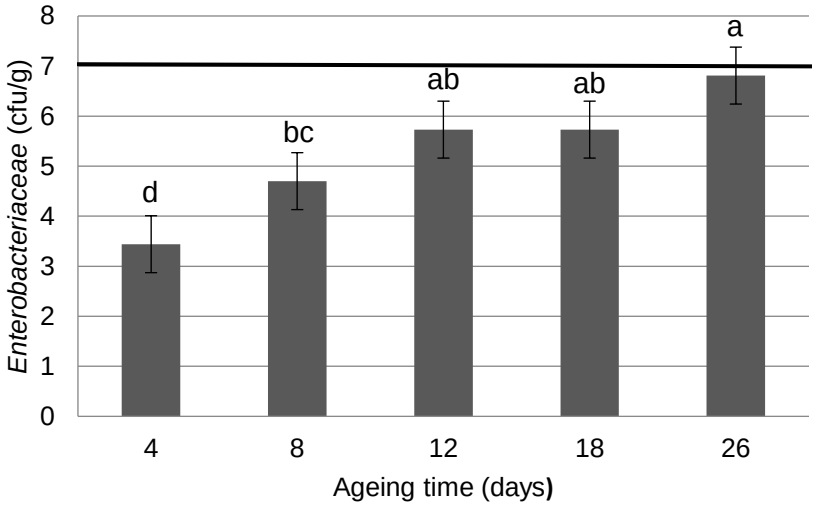

Figure 5.4 Effect of ageing time (days) at 4°C on the mean *Enterobacteriaceae* (log cfu/g) enumerated from *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles of black wildebeest. The bold line at 7.0 log cfu/g is the maximum acceptable limit.

5.4 Discussion

pH is known as the most important meat quality attribute, its measurement is crucial as it is indicative of future changes in meat quality (Honikel, 2004). The pH in the current study was not influenced by muscle type ($p>0.05$). Ageing time showed a significant effect on the pH values of LTL and BF muscles, the values decreased during the 26 day ageing period (Table 5.5). The gradual decline in pH is attributed to the production of lactic acid from glycogen through anaerobic glycolysis *post-mortem* (Lawrie & Ledward, 2006; Bykowska *et al.*, 2012). Although the pH decreased overtime the values were still higher than those of normal meat, indicative of dark, firm and dry (DFD) meat. DFD is a phenomenon that occurs as a result of ultimate pH values that remain high; the high pH values are normally as a result of *ante-mortem* stress which depletes an animal's glycogen reserves. The insufficient glycogen is converted to less lactic acid thus the pH remains high. Meat of inferior quality is produced as a result of the DFD phenomenon. High pH values create an ideal environment for microbial spoilage to occur in the meat and thus shorten the shelf life stability of the meat (Silva *et al.*, 1999; Honikel, 2000; Lawrie & Ledward, 2006; Shange *et al.*, 2017). The pH of the LTL muscle of springbok (*Antidorcas marsupialis*) did not change significantly during the 28 day ageing period and was lower (5.49) than the values obtained in the current study on the LTL muscle (North & Hoffman, 2015). Extended ageing on beef LTL and *Gluteus medius* (GM) resulted in an increase in pH (Colle *et al.*, 2015). Silva *et al.* (1999) found that the ultimate pH of beef LTL muscle increased with longer ageing time. The pH of aged fallow deer (*Dama dama*) *Supraspinatus* (SS), LTL and *Semimembranosus* (SM) muscles also decreased over a 15 day ageing period (Bykowska *et al.*, 2012). Storage time was found to have a significant influence on the pH of blesbok (*Damaliscus pygargus phillipsi*) muscles (*Infraspinatus*, *Supraspinatus*, *Biceps femoris*, *Semimembranosus*, *Semitendinosus*) (Neethling, 2016).

Weep loss in the current study was influenced by muscle type and ageing time ($p\leq0.05$); the LTL muscle had a higher weep loss than BF and the weep losses increased with longer ageing time (Table 5.5). However, the weep loss in both muscles was lower than reported in other studies (North & Hoffman, 2015), a typical phenomenon expected with DFD meat. The increase in weep loss during ageing can be attributed to the structural breakdown by proteolytic enzymes during ageing (Lawrie & Ledward, 2006). Cooking loss is a representative measure of water holding capacity (WHC); the cooking loss percentages in the current study were not influenced by sex or muscles type ($p>0.05$) but were influenced by ageing time ($p\leq0.05$) where the values decreased as the ageing time lengthened. The cooking losses over time can also be explained by the structural degradation by proteolytic enzymes that occurs during ageing. Meat with higher fluid losses is regarded as that of inferior quality (Lawrie & Ledward, 2006). Increasing cooking loss % was observed in beef LTL by Silva *et*

al. (1999); values increased from 14.4-15.3% but these values are lower than those found in the current study. Beef GM muscle aged under vacuum was found to have higher fluid losses than dry-aged meat (Li *et al.*, 2013). Bykowska *et al.* (2012) also found a decrease in cooking loss of aged fallow deer SS, LTL and SM muscles; purge losses decreased over the 15 day ageing period, the cooking loss in this study were influenced by muscle type ($p \leq 0.05$). No significant changes were found in cooking loss of springbok LTL and BF muscles that were aged for 28 days (North & Hoffman, 2015).

Meat becomes tough at the beginning of *rigor-mortis*, so ageing is done to increase the tenderness that is lost during *rigor-mortis*. Game meat in particular is aged for this reason (Lawrie & Ledward, 2006). DFD meat is reported to be more tender than normal pH meat (Silva *et al.*, 1999). In the current study, black wildebeest LTL and BF tenderness increased with ageing time, however no significant increases in tenderness were observed after 12 days of ageing (Table 5.5). Beef LTL that was aged for 13 days also showed decrease in toughness over time although there were no significant decreases after 6 days of *post-mortem* ageing (Silva *et al.*, 1999). Springbok LTL and BF also became tender as ageing time progressed, although no significant changes occurred from day 8 to 28 of the ageing period (North & Hoffman, 2015).

Lipid oxidation is reported to be the main cause of quality deterioration of stored meat products. Lipid oxidation mainly affects the polyunsaturated fatty acids and results in negative changes such off-flavours, off-odours and warmed-over flavour (Ladikos & Lougovios, 1990; Fernández *et al.*, 1997). The level of lipid oxidation is influenced by a number of factors such as the age of the animal *ante-mortem*, animal species, muscle type (or anatomical location), diet status and *post-mortem* processes (Rhee *et al.*, 1996; Guyon *et al.*, 2016). The concentration of TBARS in the current study was influenced by muscle type ($p \leq 0.05$); the BF had a higher concentration of TBARS than the LTL muscle. Muscle type has been reported to influence the TBARS concentration of beef LD and ST muscles; the LD muscle had a higher concentration on day 7 however, on day 28 the ST muscle had a higher TBARS concentration (Ba *et al.*, 2014). The level of lipid oxidation of the black wildebeest muscles was also influenced by ageing time ($p \leq 0.05$); TBARS concentration increased from 1.13 mg MDA/kg meat on day 4 to 1.81 mg MDA/kg meat on day 26. The TBARS values obtained in the current study were below the reported threshold value of 2 mg MDA/kg meat which was reported after 3 days of beef storage (Vatansever *et al.*, 2000). The value of TBARS in blesbok muscles stored at 2°C for 10 days increased from 1.6 mg MDA/kg meat at day 0 to 2.2 mg MDA/kg meat at day 10 of storage (Neethling, 2016), these values are higher than those obtained in the current study. Lipid oxidation is reported to influence colour stability of meat and meat products (Morrissey *et al.*, 1998; Neethling *et al.*, 2016a); this was also observed in the current

study where the meat samples lost the dark red colour and appeared to be brown-red over time. Off-odours were also observed from day 18 of vacuum storage of the samples in the current study. Low grade beef *Longissimus dorsi* aged under vacuum for 21 days had TBARS values from 0.13 mg MDA/kg meat on day 1 to 0.60 mg MDA/kg meat on day 21 of storage (Hur *et al.*, 2013). Mungure *et al.* (2016) suggested that an increase in TBARS value during ageing occurs due to the changes in cellular and tissue structure, the membrane no longer serves as an effective barrier to enzymes and substrates thus resulting in lipid oxidation. During ageing iron is released from its high molecular sources (haemoglobin, myoglobin) thus it becomes liable to reaction with amino acids forming chelates which are reported to be active catalysts of lipid oxidation.

A desirable fatty acid profile consists of unsaturated fatty acids as well as stearic acid (C18:0) (Hoffman *et al.*, 2005a). Fatty acid composition is greatly influenced by the diet; where a grass rich diet contains high levels of stearic (C18:0) and linolenic acids (C18:3*ω-3*) (Wood *et al.*, 2004; Hoffman *et al.*, 2005b; Hoffman, 2008). Animal sex is also reported to influence the fatty acid composition; male animals are generally higher in PUFAs compared to females (Hoffman *et al.*, 2005a) although females generally have higher levels of SFAs than males. Although not significant, female black wildebeest in the current study had a higher % composition of SFA than males whilst the males also had a higher % composition of PUFA than the females. Females had a significantly higher % composition of MUFA than males in the current study (Table 5.8). Male impala were also found to have a higher PUFA level than females. Female springbok also had a higher proportion of MUFA than males (Hoffman *et al.*, 2007). Muscle type showed an influence on the % composition of the linoleic acid (C18:2*ω-6*) where the LTL muscle had a higher % composition (12.36%) than the BF (5.70%) muscle (Table 5.6). Ageing time (Table 5.6) showed a significant influence on the % composition of certain SFA (C20:0, C21:0, C22:0, C24:0), MUFA (C20:1) as well as PUFA (C18:2*ω-6c*, C18:3*ω-3*, C20:2*ω-6*, C20:3*ω-3*, C20:4*ω-6* and C22:2*ω-6*). The % composition of the C21:0, C22:0, C24:0, C18:3*ω-3*, C20:3*ω-3*, C20:4*ω-6* and C22:2*ω-6* fatty acids increased overtime. The total composition of PUFA increased from day 4 to day 26 of ageing. A significant muscle type by ageing day interaction was observed (Table 5.3) on the % composition of palmitoleic acid (C16:1), oleic acid (C18:1*ω-9c*), docosadienoic acid (C22:2*ω-6*) and docosahexaenoic acid (C22:6 *ω-3*); at the end of the ageing period (day 26) the LTL muscle contained higher levels of palmitoleic and oleic acids than the BF muscle whereas the BF muscle contained a higher level of docosahexaenoic acid than the LTL muscle. Another interaction between sex, muscle type and ageing day was observed on the % composition of the palmitic (C16:0) and linolenic acids (C18:3*ω-3*), total SFA, total MUFA and the PUFA:SFA ratio. Natural grass is rich in α-linolenic acid (C18:3ω-3) which is an essential fatty acid with benefits to human health

(Rule *et al.*, 2002; Wood *et al.*, 2004). The diet of black wildebeest has a high proportion of grass (63%) followed by karroid shrubs (Smithers, 1983). High levels of palmitic acid are at the risk of increased cholesterol levels. The PUFA:SFA ratio for healthier meat is recommended to be greater 0.4 (Wood *et al.*, 2004), this was also obtained in the current study with values from 0.55 to 1.17. Springbok that have a similar diet to black wildebeest had a PUFA:SFA that ranged from 0.96 to 1.18 (Hoffman *et al.*, 2007). The ratio of omega 6 to omega 3 PUFA (*ω-6:ω-3*) should not exceed 4, as this increases the risk of cancer, coronary heart disease as well blood clotting which may lead to heart attacks (Wood *et al.*, 2004). In the current study, *ω-6:ω-3* at the end of the ageing period was 4.15 in the LTL muscle and 2.57 in the BF muscle. Springbok had a *ω-6:ω-3* ranging from 3.02 to 3.35. The black wildebeest muscles in the current study contained more SFA than MUFA, and PUFA had the lowest contribution to the total fatty acids.

In addition to lipid oxidation, microbial growth also causes meat quality to deteriorate during storage. In the current study the microbial counts were significantly influenced by ageing time; total viable counts increased from 4.08 log cfu/g on day 4 of vacuum storage to 6.88 log cfu/g on day 26 (Figure 5.3). *Enterobacteriaceae* counts also increased with ageing time; from 3.44 log cfu/g to 6.81 log cfu/g on day 26. Total bacterial counts (TBC) TBC counts ranged from 2.4 log cfu/cm^2 to 3.9 log cfu/cm^2 and *Enterobacteriaceae* ranged between 0.4 log cfu/cm^2 to 0.8 cfu/cm^2 after 14 days of ageing beef under vacuum conditions (Li *et al.*, 2013). The high initial microbial counts in the current study may be attributed to the higher pH which resulted in meat being classified as DFD, DFD meat is known to have a higher spoilage rate than meat of normal pH (Shange *et al.*, 2018). Another cause of the higher initial microbial counts may be contamination during the slaughter process (Gouws *et al.*, 2017). Due to the high pH the meat becomes an ideal environment for microbial growth which results in reduced shelf life stability of the meat (Lawrie & Ledward, 2006). Van Schalkwyk and Hoffman (2016) reported the maximum tolerance limit for aerobic colony counts to be 6.0 log cfu/g; this value indicated meat safety as well as hygiene care taken during the *post-mortem* handling of the carcass that is to be used for human consumption. Swab samples of springbok showed APC counts that were >3.4 log cfu/cm^2 and *Enterobacteriaceae* counts ranged from 0.5 log cfu/cm^2 to >2.5 log cfu/cm^2 3 days *post-mortem*, whilst black wildebeest samples were found to have APC ranging from 2.7 log cfu/cm^2 to 4.09 log cfu/cm^2 and *Enterobacteriaceae* ranged from 0.13 log cfu/cm^2 to 2.62 log cfu/cm^2 (Gouws *et al.*, 2017). These counts are lower than those obtained in the current study due to the shorter storage time compared to that of the current study. Also, the pH values of the springbok and black wildebeest reported in their study were lower (pH<6) than those of black wildebeest in the current study. Aerobic mesophilic bacteria and *Enterobacteriaceae* counts in springbok meat that was aged for 19 days were also

influenced by ageing time; the total count were up to 6.0 log cfu/cm^2 and *Enterobacteriaceae* counts were >5.0 log cfu/cm^2 (Buys *et al.*, 1997). Aerobic plate counts and *Enterobacteriaceae* are reported to be the main indicators of microbial quality, hygiene as well as good handling of meat (Magwedere *et al.*, 2013). According to aerobic bacterial counts storage under vacuum packaging extended the shelf life of ostrich steaks by four days compared to storage under aerobic conditions (Fernández-López *et al.*, 2008). Fernández-López *et al.* (2008) also reported that when spoilage becomes evident through odour, taste, and colour change the bacterial counts would have exceeded 6.0 log cfu/g. Dainty and Mackey (1992) reported 7.0 log cfu/g as the approximate level at which meat would be deemed unacceptable. Shelf life of foal meat was extended to 14 days under vacuum storage, this was the longest shelf life compared to other storage conditions (Gómez & Lorenzo, 2012). Initial total viable counts were 4.3 log cfu/g and at day 14 of vacuum storage the counts were above 5.0 log cfu/g; *Enterobacteriaceae* growth was limited by vacuum packaging, this was seen in the initial counts of 2.1 log cfu/g which did not differ significantly to those at day 14 of storage (Gómez & Lorenzo, 2012). The higher microbial counts of black wildebeest in the current study can be attributed to its higher initial pH which creates an ideal environment for microbial growth; microorganisms are reported to compete better at pH values above 6.0. Red meat carcasses are reported to carry initial microbial loads of 10^2 to 10^4 bacteria/cm^2 whose sources include hide, faecal matter, gut contents as well as hands of personnel and equipment used during slaughter (Dainty & Mackey, 1992). It therefore becomes crucial that good practises are applied during the evisceration and skinning of carcasses so as to minimise the initial bacterial contamination (Gouws *et al.*, 2017).

5.5 Conclusion

This study represents the first work to be done on ageing of black wildebeest meat. Animal sex had no significant influence on the analyses performed in the current study. Although the pH values decreased overtime, the values were still higher than 6.0 and thus the meat could be classified as DFD which shown to produce meat of inferior quality. Tenderness increased as the ageing time lengthened, however there was a decrease in tenderness observed after 12 days of ageing. Microbial counts also increased with ageing time but the counts remained within the acceptable range which was below 7.0 log cfu/ at day 12 of storage. From the results obtained, the optimum tenderization period of black wildebeest meat would thus be at least 12 days. The fatty acid profile of black wildebeest LTL and BF muscles can be arranged as from the highest % composition to the lowest as follows :SFA >MUFA >PUFA. The predominant fatty acids in black wildebeest LTL and BF muscles at day 26 were C14:0, C16:0,

C18:0, C24:0, C18:1*ω-9c*, C18:2*ω-6c*, C18:3*ω-3*, C20:3*ω-3*, C20:4*ω-6* and C22:6*ω-3* whilst ageing had a minimal effect on the fatty acid composition. Future studies on the sensory quality and consumer acceptability of DFD meat compared to normal pH meat are recommended.

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Chapter 6: General conclusions and recommendations

In the current study, harvesting year was found to have an influence on the carcass composition of black wildebeest. The black wildebeest that were harvested in March 2016 were harvested after a long drought period; there was thus limited feed and water available. This affected the live weights of the animals; the animals that were harvested in 2017 had slightly higher live weights than those harvested in 2016. In the first section of the current study (Chapter 3), it was observed that black wildebeest compared favourably with other game species in terms of meat production, thus the species could make a valuable contribution in combating food supply challenges.

Two major factors, animal sex and muscle type, that influence meat quality were investigated. Sex showed an influence on most of the carcass components; of particular importance were the animal weights where males (141.4 kg) were found to be significantly heavier than females (117.4 kg). Sex did not show a significant effect on the other measured physical attributes, level of lipid oxidation, proximate composition and microbiological meat quality attributes. A significant sex effect was observed on the % composition of total polyunsaturated fatty acids (PUFA) and polyunsaturated fatty acids to saturated fatty acids ratio (PUFA:SFA); male (35.0%) black wildebeest had higher levels of PUFA than females (28.9%) and males (0.8) consequently had a higher P:S ratio than females (0.6).

The effect of muscle type on the physicochemical attributes of black wildebeest meat was also investigated (Chapter 4). The *Longissimus thoracis et lumborum* (LTL) muscle is the most sought after muscle in the industry because of its large size and commercial value, however there are other muscles which are biologically different and have potential to be used for fresh meat and processed meat products. Muscle type showed a significant influence on the colour coordinates (CIE L*, CIE b*, Chroma, hue angle), cooking loss percentage, Warner-Bratzler shear force (WBSF) and the proximate composition (moisture, protein, fat, ash). The LTL muscle had an overall lighter appearance whereas the *Semitendinosus* (ST) muscle had a darker red appearance. The ST muscle had the highest cooking loss percentage followed the *Semimembranosus* (SM) and the *Infraspinatus* (IS) muscle had the lowest cooking loss percentage (showing a high water holding capacity). WBSF values obtained from all six muscles ranged from 3.9 to 6.5 kg/1.27cmϕ, when arranging the muscles from the most to the least tender the sequence is as follows; IS>SS>LTL>*Biceps femoris* (BF)>SM>ST. These values are comparable to tenderness values from beef and other game species of similar size. The *Supraspinatus* (SS) muscles had the highest moisture content and the LTL had the lowest moisture content. The LTL had the highest protein and fat content. The ST muscle had the

lowest ash content whereas the BF had the highest ash content. Black wildebeest meat contains less fat and has a higher protein content compared to beef, these attributes make the meat a healthier red meat alternative than domesticated red meat such as beef. The physicochemical chemical attributes of black wildebeest meat are similar to those of other game species, thus the species can be used for fresh meat production.

Although the ultimate pH values did not differ significantly between the muscles, the values were above 6.0 thus the meat showed susceptibility to being dark, firm and dry (DFD). DFD is a phenomenon that is a result of *ante-mortem* stress which depletes glycogen stores in the animal. Thus less lactic is produced *post-mortem* and the meat pH remains high. DFD is known to produce meat of inferior quality. A contrast analysis between DFD (pH >6) and normal (pH <6) meat was conducted on the LTL muscle (Chapter 4). As expected the DFD meat was found to have an overall darker colour than the normal meat. The DFD meat also had lower cooking loss percentage than the normal meat, thus showing that DFD meat has a higher water holding capacity than then normal meat due to its high pH. DFD meat is reported to have a higher tenderization rate than normal meat; this was observed in the current study; after 3 days *post-mortem* the DFD meat had lower WBSF values than the normal meat.

Game meat is known for producing tough meat due to the increased level of physical activity in game species compared to domesticated species as well as the stressful conditions under which game species are mostly harvested. Ageing is performed in order to improve meat tenderness which is known as the main que for consumer's purchasing intent. In the current study (Chapter 5), changes in the physicochemical and microbiological attributes of LTL and BF muscles during ageing were investigated. The BF muscle was also chosen for its large size and thus potential to be used for the production of primal meat cuts or processed meat products; as found in literature larger muscles often show variation even within the muscle so it was appropriate to investigate the changes in this muscle. The muscles were aged for 26 days at 4°C under vacuum packaging with the aim of determining the ideal ageing period. Muscle type showed a significant influence on the weep loss percentage, level of lipid oxidation and the composition (%) of C18:2*ω-6c* fatty acid. The LTL muscle had a higher weep loss percentage than the BF. On the other hand, the BF muscle had a higher level of lipid oxidation than the LTL muscle. The composition of C18:2*ω-6c* was higher in the LTL (12.4%) muscle than in the BF (5.7%). Ageing time (days) showed a significant influence on all the measured physicochemical and microbiological attributes. Variation was observed in the pH, cooking loss percentage and WBSF values after day 12 of storage. The pH gradually decreased until day 12 where a slight increase was observed. The same variation was observed for cooking loss percentages and WBSF values. This may have occurred due to the different locations within the muscle from where the samples were obtained. It is important

to highlight that larger muscles are renowned for showing variation in their characteristics. WBSF values decreased until day 12 of storage where after it remained relatively stable. The level of lipid oxidation (TBARS value) also showed variation, this was because of repeated analysis on day 4 and day 8 ageing samples thus increasing exposure to oxygen which could explain the up and down pattern. It would be suggested that analysis be performed in the same manner for all samples in order to avoid variation in the results. The level of lipid oxidation is influenced by numerous factors which include anatomical location and myoglobin content; as a result, the two muscles in the current study differed in their extent of lipid oxidation. For future studies on ageing, it would be recommended that the time points be spread out in equal and/or shorter intervals (for example, 2 days increments); this will allow for better observation of the optimal tenderization period (where WBSF values reach a plateau state) as well as allow for modelling of the gradual increases in lipid oxidation and microbial counts. The microbial counts increased with longer ageing time but the counts were below the reported maximum tolerance limit of 7 log cfu/g. Off odours were noticed from day 18 of storage. From the physicochemical and microbiological results obtained the optimum tenderization period was found to be at least 12 days. The initial microbial counts in the current study were high; this may have been due to contamination during the slaughter process and handling of the carcasses. It is thus suggested that adherence to hygiene be practised and monitored during the slaughter process and *post-mortem* handling of the carcasses. Vacuum packaging maintained the microbial counts below the maximum tolerance limit, thus vacuum packaging would be recommended as the ideal packaging type in the distribution of black wildebeest meat in the industry.

This study was the first on the physicochemical and microbiological attributes of aged black wildebeest meat. The aforementioned attributes were investigated successfully in the study and the results obtained indicated that black wildebeest has potential to be utilised for fresh meat production and contribute towards the current food crisis. The results (mainly pH and surface colour) also showed the susceptibility of black wildebeest to produce DFD meat as a result of *ante-mortem* stress, DFD meat is reported to be of inferior quality and rapid spoilage compared to meat of normal pH. Future studies on the sensory analysis and consumer acceptability of DFD meat in comparison to normal meat are recommended. The animals can be sampled under different production systems (intensive or extensive), where the animals can adapt to being handled differently and thus might be less stressed during harvesting; this may help reduce the susceptibility to DFD. A sex effect may possibly be observed if the animals were harvested at different locations with different forages; these aspects also warrant further research.