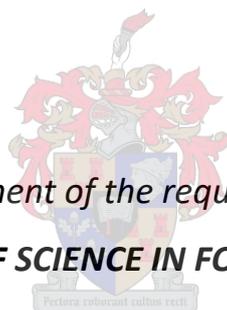


The meat quality of bontebok (*Damaliscus pygargus pygargus*)

by

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DECLARATION

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SUMMARY

The aim of this study was to establish baseline data for the South African meat industry on the meat quality of bontebok (*Damaliscus pygargus pygargus*) males and to investigate the influence of muscles (*Longissimus thoracis et lumborum*/LTL, *semimembranosus*/SM, *biceps femoris*/BF, *infraspinatus*/IS, *semitendinosus*/ST, *supraspinatus*/SS and the *psaos major*/fillet) on the meat quality of the former species. Carcass yields, overall meat quality (physical characteristics and chemical composition), optimum ageing period, microbial activity/safety and sensory attributes of bontebok meat were established and compared to its closely related sub-species, the blesbok (*Damaliscus pygargus phillipsi*), where applicable.

Two trials were conducted: one in March (n=12) and one in April (n=8). All seven above-mentioned muscles were quantified for carcass composition (April and March trial) and chemical characteristics (March trial), however, physical characteristics were determined for all seven muscles in the March trial but only for three muscles in the April trial (SM, BF and LTL). Ageing and microbial tests were performed on the LTL muscle from the April bontebok. Sensory attributes were compared using two muscles (BF and SM) from bontebok (n=7) and blesbok (n=7), both species harvested in April. The carcass yields of bontebok was similar to that of blesbok as established in literature. The dressing percentages (calculated from warm carcass weight) of bontebok were 50.4% (± 1.55) and 50.7% (± 3.07) for March and April, respectively. Furthermore, the muscle with the largest percentage of the cold carcass weight, was the LTL muscle in both March ($3.1 \pm 0.05\%$) and April ($2.8 \pm 0.22\%$), whereas the fillet was the smallest muscle, contributing least towards the cold carcass weight, in both March ($0.4 \pm 0.03\%$) and April ($0.4 \pm 0.04\%$). Additionally, the external offal (head, horns, skin and genitals) percentages were higher in March ($14.4 \pm 0.88\%$) and April ($15.1 \pm 0.77\%$) than internal offal percentages (stomach, organs and intestines) in March ($34.4 \pm 0.96\%$) and April ($31.7 \pm 3.49\%$).

All physical and chemical characteristics were influenced ($p \leq 0.05$) by muscles in the March trial and only ultimate pH (pH_u) and cooking loss percentage in the April trial ($p \leq 0.05$). The pH_u values found for bontebok harvested in March were high (5.84-6.21) and drip loss percentages were generally low (0.7%-0.9%). The fillet and forequarter muscles (IS and SS) had the highest pH_u values (>6.1) and the lowest Warner-Bratzler shear force values (more tender). The IS muscle had the lowest cooking loss percentage of 28.9% (± 2.57) ($p \leq 0.05$). Furthermore, the muscles that were most red were the forequarter muscles and fillet ($a^* = 12.96-13.73$) and the lightest muscle was the

ST ($L^* = 35.90$). From the three muscles analysed (SM, BF and LTL) in the April trial, the average pH_u values were lower than found for March bontebok with the BF muscle having a significantly higher pH_u (5.71) than the LTL muscle (5.50). The cooking loss percentage in the latter trial was found to be significantly higher in the SM muscle ($38.8 \pm 1.09\%$) than the BF ($35.3 \pm 1.48\%$) and LTL ($34.7 \pm 2.87\%$) muscles. The chemical composition of bontebok meat resulted in meat with extremely low average intramuscular fat (IMF) contents (0.8 g/100 g) with the fillet containing the highest IMF content (1.1 g/100 g). The two hindquarter (BF, SM) muscles and the LTL muscle had significantly higher protein (~ 23.0 g/100 g meat) and lower moisture (~ 75.5 g/100 g meat) contents than the other muscles analysed. The ash content was significantly lower in the forequarter muscles: IS (1.1 g/100 g meat); and SS (1.16 g/100 g meat) than the other muscles analysed. Although the differences between muscles were significant, they are also marginal and thus may not be of biological value in terms of human nutrition. Regardless, all bontebok meat had low IMF and high protein contents which could be preferred by modern-day consumers that regard a low-fat and high protein diet as “healthy”.

The LTL muscle of eight male bontebok was aged over eight separate time points (day 1, 2, 4, 6, 7, 8, 10, and 12). Bontebok meat tenderised rapidly and the optimum ageing time for bontebok LTL steaks was determined to be eight days at 4°C under vacuum packaging conditions. The Warner-Bratzler shear force (WBSF) decreased until an optimum tenderness for this ageing trial was reached on day 8 (57.2 N) after which it plateaued until day 12. The decrease in tenderness was associated with an improved meat colour and increase in cumulative purge loss over time. Furthermore, the microbial activity over time indicated that no significant effects were detected for total plate count (TPC) or *Escherichia coli*/coliforms between ageing time points and all counts (log CFU/g) were within specified safety limits. Additionally, all bontebok samples tested negative for the presence of *Salmonella*.

With the similarity in diets for bontebok and blesbok (both strict grazers), differences in terms of sensory attributes between the latter species were expected to be minor during a descriptive sensory analysis (DSA) where two muscles (SM and BF) of blesbok and bontebok were compared. No differences were found in flavour or aroma profiles between species or muscle type ($p \geq 0.05$), except gamey flavour that was slightly higher in blesbok than bontebok. Gamey flavour (~ 75) and aroma (~ 74) proved to be the largest contributors to overall flavour and aroma on a 100-point scale (0=none; 100= prominent). Certain textural attributes differed significantly between species and muscle type. The bontebok had a significantly higher WBSF, lower sensory tenderness, higher residue and lower mealiness compared to blesbok and the SM muscle proved to be superior compared to the BF muscle due to its significantly higher sensory tenderness and initial juiciness.

Regardless, bontebok meat compares favourably to blesbok meat and it is postulated that meat consumers would struggle to differentiate between blesbok and bontebok meat. Overall, bontebok meat proved to be safe and of good quality and could be utilised in the South African game meat industry. The meat was significantly influenced by muscle type and compared well to its closely related sub-species, the blesbok.

OPSOMMING

Die doel van hierdie studie was om basisdata vir die Suid-Afrikaanse vleisbedryf op die vleiskwaliteit van bontebok (*Damaliscus pygargus pygargus*) ramme vas te stel en om die invloed van spiere te ondersoek (*Longissimus thoracis et lumborum/LTL*, *semimembranosus/SM*, *biceps femoris/BF*, *infraspinatus/IS*, *semitendinosus/ST*, *supraspinatus/SS* en die *psoas major/filet*) op die vleiskwaliteit van die voormalige spesie. Karkasopbrengs, algehele vleiskwaliteit (fisiese eienskappe en chemiese samestelling), optimale verouderingstydperk, mikrobiële aktiwiteit /veiligheid en sensoriese eienskappe van bontebok vleis was bepaal en vergelyk met sy nouverwante sub-spesie, die blesbok (*Damaliscus pygargus Phillipi*), waar van toepassing.

Twee proewe is uitgevoer: een in Maart (n = 12) en een in April (n = 8). Al sewe bogenoemde spiere is gekwantifiseer vir karkas samestelling (April en Maart proef), chemiese eienskappe (Maart proef) en fisiese eienskappe in die Maart proef, maar net vir drie spiere in die April proef (SM, BF en LTL). Veroudering en mikrobiële toetse is uitgevoer op die drie spiere van die April bontebok. Sensoriese eienskappe is vergelyk tussen twee spiere (BF en SM) van bontebok (n = 7) en blesbok (n = 7) spesies, albei in April geoes. Die karkasopbrengs van bontebok was soortgelyk aan die van blesbok soos in literatuur vasgestel. Die afslagpersentasies (bereken vanaf warm karkasgewig) van bontebok was 50.4 % (± 1.55) en 50.7% (± 3.07) vir Maart en April proewe, onderskeidelik. Die spier met die grootste persentasie van die koue karkasgewig was die LTL-spier in beide Maart ($3.1 \pm 0.05\%$) en April ($2.8 \pm 0.22\%$), terwyl die filet die kleinste spier was en die minste bygedra het tot die koue karkas gewig, in beide Maart ($0.4 \pm 0.03\%$) en April ($0.4 \pm 0.04\%$). Boonop was die eksterne afval (kop, horings, vel en geslagsdele) persentasies hoër in Maart ($14.4 \pm 0.88\%$) en April ($15.1 \pm 0.77\%$) as interne afvalpersentasies (maag, organe en ingewande) in Maart ($34.4 \pm 0.96\%$) en April ($31.7 \pm 3.49\%$).

Al die fisiese en chemiese eienskappe is beïnvloed ($p \leq 0.05$) deur die spiere in die Maart proef en slegs die uiteindelijke pH (pH_u) en die kookverliespersentasie in die April proef ($p \leq 0.05$). Die pH_u waardes vir die Maart bontebok was hoog (5.84-6.21) en die persentasie drupverlies was oor die algemeen laag (0.7%-0.9%). Die filet- en voorlyfspiere (IS en SS) het die hoogste pH_u waardes (>6.1) en die laagste Warner-Bratzler skuifkragwaardes (meer sag). Die IS-spier het die laagste kookverlies persentasie gehad ($28.9 \pm 2.57\%$) ($p \leq 0.05$). Verder was die rooiste spiere die voorlyfspiere en filet ($a^* = 12.96-13.73$) en die ligste spier was die ST ($L^* = 35.90$). Van die drie spiere wat geanaliseer is (SM, BF en LTL) in die April-proef, was die gemiddelde pH_u waardes laer as gevind vir Maart bontebok, met die BF-spier met 'n hoër beduidende pH_u (5,71) as die LTL-spier (5.50). Die persentasie kookverlies in laasgenoemde proef was aansienlik hoër in die SM-spier ($38.8 \pm 1.09\%$) as

die BF ($35.3 \pm 1.48\%$) en LTL ($34.7 \pm 2.87\%$) spiere. Die chemiese samestelling van bontebokvleis het gelei tot vleis met 'n buitengewoon lae binnespier vet inhoud ($0.8 \text{ g}/100 \text{ g}$), met die filet wat die hoogste vet inhoud gehad het ($1.1 \text{ g}/100 \text{ g}$). Die twee agterkwart (BF, SM) spiere en die LTL spier het 'n aansienlike hoër proteïen ($\sim 23.0 \text{ g}/100 \text{ g}$ vleis) en 'n laer vog inhoud ($\sim 75.5 \text{ g}/100 \text{ g}$ vleis) gehad as die ander spiere wat geanaliseer is. Die as-inhoud was aansienlik laer in die voorlyfspiere: IS ($1.1 \text{ g}/100 \text{ g}$ vleis); en SS ($1.16 \text{ g}/100 \text{ g}$ vleis) as die ander spiere wat ontleed is. Alhoewel die verskille tussen spiere beduidend was, is hulle moontlik nie van biologiese waarde in terme van menslike voeding nie. Alle bontebokvleis het 'n lae vet en hoë proteïen inhoud gehad. Moderne verbruikers wat 'n lae-vet/hoë proteïen dieët as 'gesond' beskou, sal die bontebok vleis verkies bo ander kommersiële rooivleis.

Die LTL-spier van agt manlike bontebokke is verouder vir agt afsonderlike tydperke (dag 1, 2, 4, 6, 7, 8, 10 en 12). Bontebok vleis het vinnig versag oor tyd en die optimale verouderings tydperk vir bontebok LTL steaks was bepaal as agt dae by 4°C onder vakuüm verpakking. Die Warner-Bratzler- skuifkrag (WBSK) het afgeneem totdat 'n optimale sagtheid vir hierdie verouderingsproef op dag 8 (57.2 N) bereik is, waarna dit 'n plato bereik het tot dag 12. Die afname in sagtheid het gepaard gegaan met 'n verbetering in vleiskleur en 'n toename in kumulatiewe vog verlies met verloop van tyd, met aanvaarbare gewigsverliespersentasies tot op dag 10, waarna die gewigsverlies $>4\%$ was, wat gekenmerk is as onaanvaarbaar deur algemene verbruikers. Verder het die mikrobiële aktiwiteit met verloop van tyd geen noemenswaardige effekte waargeneem vir totale plaattelling (TPC) of *Escherichia coli*/coliforms tussen verouderingstydperke nie. Verder, het alle mikrobiële tellings ($\log \text{ CFU}/\text{g}$) binne spesifieke veiligheidsgrense geval. Alle bontebok vleis monsters het ook negatief getoets vir die teenwoordigheid van *Salmonella*.

Daar is 'n streng ooreenkoms tussen diëte van bontebok en blesbok (albei streng grasvreters), daarom sou die verskille ten opsigte van sensoriese eienskappe tussen laasgenoemde spesies na verwagting klein wees. Tydens 'n beskrywende sensoriese analise (BSA), waar twee spiere (SM en BF) van blesbok en bontebok vergelyk is, was daar geen verskille in aroma- of smaakprofiel tussen spesies of spiersoort ($p \geq 0.05$) gevind nie, behalwe 'n wildagtige geur wat effens hoër in blesbok as bontebok was. Wildagtige geur- (~ 75) en aroma (~ 74) was die grootste bydraer tot die algehele geur en aroma op 'n 100-punt skaal (0=geen; 100=prominent). Sekere tekstuurkenmerke het betekenisvol tussen spesies en spiersoort verskil. Die bontebok het 'n aansienlike hoër WBSK, 'n laer sensoriese sagtheid, 'n hoër residu en laer "mealiness" in vergelyking met blesbok gehad. Die SM-spier blyk beter te wees in vergelyking met die BF-spier vanweë die aansienlike hoër sensoriese sagtheid en aanvanklike sappigheid. Hoe dit ook al sy, bontebokvleis vergelyk goed met blesbokvleis en dit word gestel dat vleisverbruikers sou sukkel om tussen blesbok- en

bontebokvleis te onderskei. In die algemeen is bontebokvleis veilig om te eet en van goeie gehalte en kan in die Suid-Afrikaanse wildsvleisbedryf gebruik word. Die vleis is aansienlik beïnvloed deur spiersoort en vergelyk goed met sy nou verwante subspesie, die blesbok.

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LIST OF ABBREVIATIONS

ABBREVIATION	EXPANSION
°C	Degree Celsius
%	Percentage
GLM	General Linear Models
ANOVA	Analysis of Variance
BF	<i>Biceps femoris</i> muscle
CFU	Colony forming units
cm	Centimetre
DFD	Dark, firm and dry meat
g	Gram
GIT	Gastro-intestinal tract
ha	Hectare
IMF	Intramuscular fat
IS	<i>Infraspinatus</i> muscle
kg	Kilogram
kN	Kilo-Newton
LSMeans	Least Square means
LTL	<i>Longissimus thoracis et lumborum</i> muscle
m	Metre
min	Minute
ml	Millilitre
mm	Millimetre
mm/minute	Millimetre per minute
MUFA	Monounsaturated fatty acids
N	Newton
n	Number
PCA	Principal component analysis
pH _u	Ultimate pH
PUFA	Polyunsaturated fatty acid
s	Seconds
SEM	Standard Error of the Mean
SFA	Saturated fatty acids

SM	<i>Semimembranosus</i> muscle
SS	<i>Supraspinatus</i> muscle
ST	<i>Semitendinosus</i> muscle
TPC	Total plate count
v/v	Volume to volume ratio
WBSF	Warner-Bratzler shear force
WHC	Water-holding capacity
µm	Micrometre

NOTES

The language and style used in this thesis is in accordance with the requirements of the International Journal of Food Science and Technology. It is structured to form several research chapters and is prefaced by an introduction chapter, followed by a literature review chapter and culminating with a chapter containing the general discussion and recommendations.

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CHAPTER 1: GENERAL INTRODUCTION

Meat is a major component of the South African cuisine and for some South Africans a meal without meat is not considered a meal at all. The above-mentioned “meat” could consist of a variety of wild animals and domesticated species (Erasmus & Hoffman, 2017). The land available in South Africa (and globally) for traditional domestic livestock production has become limited and the prospects for expansion of these species are limited (Hoffman, 2008). The meat production from domestic species is further challenged by stock theft, desertification and overgrazing resulting from climate change (Otieno & Muchapondwa, 2016) and therefore it is important to investigate the use of alternative non-traditional meat sources such as game meat in order to provide meat protein for the growing population of South Africa (Cawthorn & Hoffman, 2014).

Game meat is derived from wild, free-ranging ungulates in South Africa. These species are classified as “organic” and a healthier alternative protein source (Erasmus & Hoffman, 2017; Wassenaar *et al.*, 2019) due to their low IMF and high protein content (Neethling *et al.*, 2018; Needham *et al.*, 2019a). Although literature links the consumption of red meat to cancer and cardiovascular diseases (Cross *et al.*, 2007; Wolk, 2017), a recent study contradicts the above-mentioned statement in which results obtained found no relationship between the consumption of red meat and cardiovascular diseases or cancer (Zeraatkar *et al.*, 2019).

Climate change is a concern in South Africa, especially after the extreme drought that heavily affected the Western Cape Province during 2018 (Masante *et al.*, 2018). Game species are known to be more resistant to diseases, better adapted to warmer climates and can go without water for a longer period of time when compared to domestic livestock (Pollock & Litt, 1969). Otieno and Muchapondwa (2016) used a multinomial choice model to predict that with a rise in temperature, most livestock farmers would start converting to wildlife ranching in the future. The potential of wildlife ranching in South Africa was recognised for the first time during the 1950s (Carruthers, 2008). The game industry was established and started increasing exponentially ever since South Africa’s government gave legal ownership of wildlife to landowners in 1991 which provided financial value to game species (Taylor *et al.*, 2016). Since then, the game industry has expanded into four main economic pillars; breeding (includes breeding endangered/scarce species and live sales), eco-tourism, hunting (trophy and biltong) and meat production (Van der Merwe *et al.*, 2004). Numerous species are farmed and utilised in the game industry; popular species such as springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus pygargus phillipsi*) and kudu (*Tragelaphus strepsiceros*) (Hoffman, 2007), and more rare species such as bontebok (*Damaliscus pygargus pygargus*) which also play a role in the above-mentioned industry.

The bontebok is a previously endangered, medium-sized antelope currently listed as “vulnerable” according to the International Union for Conservation of Nature and Natural resources (IUCN) red data list (Radloff *et al.*, 2016; Lloyd & David, 2017), and meat derived from this species is currently under-utilized in the South African game meat industry. Bontebok trophy hunting is one of the largest economical pillars contributing towards the conservation of these animals (Furstenburg, 2016). Trophy hunting is known to annually contribute more than USD 341 million to the South African economy, additionally contributing to the low-income communities in terms of skill development and job creation (Saayman *et al.*, 2018). Bontebok are mostly found on privately owned farms and protected in enclosed areas such as the Bontebok National Park (Watson *et al.*, 2011). On these private breeding farms, 25% of the non-breeding male bontebok need to be harvested annually (Furstenburg, 2016). The utilisation of excess bontebok meat (non-breeding males and meat from the trophy hunting industry) is currently unknown. The meat production potential of bontebok can only fully be understood once the meat quality traits of these species have been determined which would improve the marketability of the meat from this species.

Meat quality influences the way in which consumers choose and perceive meat (Kudrnáčová *et al.*, 2018) and refers to properties that enable meat to be suitable for storage, further processing and safe consumption (Andersen *et al.*, 2005). Meat quality is known to be affected by many ante- and post-mortem factors affecting the species such as nutrition, sex, muscle type, age, breed, handling/breeding management, temperature, pH and ageing (Kudrnáčová *et al.*, 2018). Game species are commonly found to result in meat with an ultimate pH (pH_u) >6 , thus resulting in DFD meat (dark, firm and dry) due to animals being stressed ante-mortem due to harvesting procedures (Adzitey & Nurul, 2011). A high ultimate pH_u affects many meat quality traits negatively, such as the microbial safety, colour, water holding capacity and sensory profile such as the juiciness and tenderness of meat (Rudman *et al.*, 2018; Shange *et al.*, 2018, 2019). Therefore, many consumers have a negative perception of game meat associated with a dark colour and tough texture (Wassenaar *et al.*, 2019). Additionally, consumers tend to see game meat only as a “by-product of trophy hunting”. Consumers need to be educated in terms of species-specific sensory profiles and the species need to be marketed accordingly. This would only be possible if research is conducted in terms of meat quality characteristics to predict the potential of each species. Game species have been found to compare favourably to domestic species in terms of taste (Needham *et al.*, 2019b), proximate composition (Rudman *et al.*, 2018) and tenderness (North & Hoffman, 2015).

Due to the bontebok being labelled as “previously endangered”, meat quality data of this species is currently absent in literature. Therefore, the overarching aim of this study is to establish baseline data for meat quality characteristics of bontebok meat by determining carcass composition,

physical characteristics (pH, colour, Warner-Bratzler shear force and moisture loss), chemical composition (moisture, protein, intramuscular fat and ash), optimum ageing period, microbial safety and sensory profile of the former species. Additionally, the significant meat quality differences between muscle type will be investigated and the bontebok and its closely related sub-species, the blesbok's sensory profiles will also be compared.

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CHAPTER 2: LITERATURE REVIEW

2.1 THE POTENTIAL OF BONTEBOK (*DAMALISCUS PYGARGUS PYGARGUS*) IN THE GAME MEAT INDUSTRY

2.1.1 Game meat industry in South Africa

2.1.1.1 History

In South Africa, soon after the first settlers of the Cape arrived in 1652, uncontrolled slaughter of wildlife species took place and started spreading at an extreme rate (Mossman & Mossman, 1976). Animal skins, ivory, and horns were of main concern for these “big game hunters” according to historical sales lists. Three species were exterminated, namely the Cape lion (*Panthera leo melanochaitus*), quagga (*Equus quagga*) and the blue buck (*Ozanna leucophaea*). The bontebok (*Damaliscus pygargus pygargus*), black wildebeest (*Connochaetes gnou*) and the mountain zebra (*Equus zebra*) were close to extinction (Mossman & Mossman, 1976).

By the beginning of the 20th century, numerous wildlife species in South Africa were depleted as a result of over-exploitation by humans and disease epidemics (Bond *et al.*, 2004). In an attempt to resolve the problem, colonial governments banned the use of game species on a commercial and subsistence level. However, this initiative had the opposite effect and resulted in farmers neglecting wildlife conservation. The possession of wildlife species became a burden to landowners due to the diseases they carried, in addition to the competition with domestic livestock for resources. The latter usually resulted in either the neglect or eradication of wildlife species. To make wildlife species even further undesirable, the government only provided subsidies, investment in research, disease control and infrastructure for domestic livestock species (Bond *et al.*, 2004).

During 1991, South Africa’s government gave legal ownership of wildlife to landowners who obtained a Certificate of Adequate Enclosure (CAE) according to The Game Theft Act (No 105 of 1991). The wildlife to be owned was classified as “game” and it included all species to be used for hunting or commercial purposes (which included all portions of the carcass). The CAE exempted landowners from conservation regulations and this enabled them to capture, keep, hunt and sell game species at any time of the year. The Game Theft Act provided financial value to the game industry (by the provision of loans, credit, etc.) and therefore a general increase in price of wildlife species could be seen and this continued to increase with time.

2.1.1.2 The industry defined

The game industry is believed to provide animals that are used consumptively, non-consumptively or both (Barnes, 1998) and can be divided into four main economic pillars: ecotourism; hunting;

breeding game or endangered/rare game species; and processed game meat products (Van der Merwe *et al.*, 2004). Taylor *et al.* (2016) surveyed 251 wildlife ranches during 2014 and analysed the number of animals as well as the total revenue generated in rand from live sales, trophy hunting, biltong hunting and game meat production. These values can be seen in Table 2.1.

Table 2.1 The total estimated turnover and animals sold/hunted on 251 wildlife ranches in South Africa in 2014

Different categories	Turnover (billion rand)	Turnover%	Animals	Animal%
Live sales	4.328	57.35	225 500	27.85
Trophy hunting	1.956	25.92	130 186	16.08
Biltong hunting	0.651	8.63	277 027	34.21
Game meat production (excludes biltong)	0.612	8.11	176 969	21.86

2.1.1.3 Climate change

Climate change is a concern in South Africa. The average temperatures have increased by at least 1.5 times (0.65°C) that which is estimated to be the historical global average per year (Ziervogel *et al.*, 2014). The latter increases are largely due to greenhouse gas effects (Stott *et al.*, 2000) and are estimated to continue with time. Climate change would cause a threat to South Africa's water resources, biodiversity, ecosystems, and food security (Ziervogel *et al.*, 2014). The Western Cape Province of South Africa has gone through a severe drought in 2018, whilst the rest of the country has had areas that have experienced even longer periods of drought. Ever since 2015, rainfall has been below average and during 2018 water was so limited that many inhabitants in the Western Cape were living under water restrictions and rationing (Masante *et al.*, 2018). Such adverse weather conditions have had detrimental effects on the agricultural industry.

The quality of meat can be affected by climate change in two ways, the first could be the direct effect of the weather on the animal's physical condition. Secondly, abattoir and farming practises can be changed to enable species to adapt to climate conditions, by changing to better adapted genotype species, changing the animal diet or changing living conditions in order to better prepare the animals for these increased weather conditions (Gregory, 2010). Cattle were introduced to Southern Africa after the fourteenth century in contrast to African antelope species that have been able to adapt to the African climate for a much longer time. Game animals are more resistant to certain diseases than cattle, can go without water for longer periods of time and generally,

require less operating and developing costs (Pollock & Litt, 1969). These factors highlight the fact that game species could possibly continue to replace cattle, particularly in South Africa as temperatures continue to increase.

The role of wildlife in the adaptation to climate change in South Africa was studied by using a sample of 1071 livestock and wildlife farms. The multinomial choice model predicted that with a change in temperature, most livestock farmers would start changing to wildlife ranching in the future. Climate models indicated that the Northern and Eastern Cape would be mostly affected (Otieno & Muchapondwa, 2016).

However, one of the main impediments to the development of the wildlife sector is the low prices currently paid for game meat. There are numerous reasons for this from harvesting in the field to a lack of knowledge around the quality of the game meat and the factors that influence it. However, there are systems in place for the commercial harvesting of wildlife in the field (Van Schalkwyk & Hoffman, 2016). Typically, night cropping, boma cropping or helicopter cropping is used. Nonetheless, there is a lack of information around the possible impact of these cropping methods on the meat quality and which methods are best suited for which species. Another core area where there is limited information is the annual cropping rate that could be maintained per specific population within a species. Nor is there sufficient knowledge of the effect that extrinsic (i.e. species, feed, ante-mortem stress, season) and intrinsic (i.e. age, sex, muscle type) factors might have on the meat quality of wildlife. One such species where there is no information available is the bontebok, whilst there is only limited information available on its sub-species, the blesbok.

2.1.2 *Damaliscus pygargus*

Damaliscus pygargus, formerly known as *Damaliscus dorcus*, is a medium sized antelope with two well-differentiated subspecies, the bontebok (*Damaliscus pygargus pygargus*) and the blesbok (*Damaliscus pygargus phillipsi*) (Lloyd & David, 2017).

2.1.2.1 Bontebok (Damaliscus pygargus pygargus) defined

Bontebok are medium sized antelope endemic to the Western Cape, South Africa (Radloff *et al.*, 2016). They are distinguished from the sub-species blesbok, by the white blaze that continues from the base of the horns to the nose. The colour of the fore part can be identified as rufous fawn darkening into a blackish colour near the rump, shoulders, flanks and tail-tuft, whereas the rump, under-parts, upper half of tail and most of hinder surface is identified as white. The horns are darker than those found in blesbok and lyre-shaped and present in both sexes. The white patch around the bontebok tail area is also distinctively different to the brown patch found in blesbok (Ward, 1903).

Bontebok are endemic to the Western Cape, but more specifically the East Coast Renosterveld in the Overberg region (near Cape Agulhas, the most Southern tip of Africa) where they were historically found, before being located to different areas in South Africa; areas mainly owned by private farmers. The bontebok species can be classified as selective grazers (Beukes, 1987), with preference to recently burnt veld and short grass. Thus, they avoid woody vegetation and prefer open areas with low shrubs (Beukes, 1987; Novellie, 1987). In the Bontebok National Park, Watson *et al.* (2011) found that mountain zebra are not as closely associated to burnt veld as bontebok are. During the dry season, bontebok tend to stay within 1.5 km from water as it is an essential habitat requirement (Luyt, 2005).

David (1975) studied the behaviour of a large group of bontebok (n = 250) for 15 months within the Bontebok National Park. The author established that the mating season for bontebok is strictly seasonal, occurring from January to March followed by an eight-month gestation period. The social structure was found to have territorial males all year round, very small female nursery hybrids and large bachelor herds consisting of up to 100 males of different ages.

2.1.1.2 A sub-species: Blesbok (*Damaliscus pygargus phillipsi*)

The blesbok is also a medium sized antelope selective in their eating habits and classified as selective grazers, specifically interested in short grass and recently burnt veld, similar to their bontebok sub-species. Blesbok males have similar social structure patterns to bontebok, however, their nursery herds are generally larger than bontebok and males do not maintain their territories during winter and spring. Furthermore, unlike the bontebok's mating season (January to March), the blesbok's mating season peaks in April (Dalton *et al.*, 2017). They are defined by their white forehead with a thin horizontal stripe above the eyes, dark brown body, lighter shade of brown on the saddle of the back and even lighter shade on the rump. Their front upper legs have a white patch and lower legs are off-white. Blesbok rams and ewes are similar in appearance, however, males have slightly larger horns than females and thicker necks (Dalton *et al.*, 2017). They roam the more even grassland of central, northern and eastern parts of South Africa and tolerate heat very well. They rely on their fast speed to escape predation and are poor jumpers much like their sub-species, the bontebok (Kohn, 2014) and opposed to species such as eland (*Taurotragus oryx*). Historically the bontebok and blesbok are separated geographically by a Karoo semi-desert area of ± 200 km, however, both species currently also occur in areas outside their natural distribution ranges, mostly on privately owned farms (Radloff *et al.*, 2016).

2.1.2.3 *Saved from extinction: the bontebok story*

The bontebok species were saved from extinction in the mid-19th century by families farming in the Overberg region. According to the International Union for Conservation of Nature and Natural resources (IUCN) Red list, the number of bontebok decreased from locally abundant to the verge of extinction partially as a result of overhunting by European settlers. Farmers did not distinguish between bontebok and blesbok and identified bontebok as a threat to the survival of their livestock due to the competition for farmland (Radloff *et al.*, 2016). However, research done by Furstenburg (2016) suggested that it was not European ancestors causing the near extinction, but a combination of the local Stone Age people and global climate change.

Furthermore, Furstenburg (2016) explains that the split between blesbok and bontebok occurred approximately 1.2 million years ago. The sub-species were separated by the coastal mountain range and wide stretch of Karoo- type vegetation which both species despise. The main pre-historic bontebok habitat was gradually replaced as the ice from the poles melted and water replaced this land. Consequently, the habitat of bontebok was adjusted towards fynbos, bush, forest and a warmer climate (Furstenburg, 2016).

The Bontebok National Park, which was originally situated in the Overberg region was proclaimed in 1931. During the time of the proclamation a total of 17 (and later 16) bontebok were found to have survived in the National Park (Radloff *et al.*, 2016). According to Furstenburg (2016), these 17 animals were not the only Bontebok alive, because the IUCN Red List neglected to count the bontebok on four privately owned farms. Ever since, bontebok numbers steadily increased as bontebok were translocated to various other privately-owned farms and nature reserves across South Africa (Table 2.2).

Table 2.2 Historic data on bontebok numbers (Furstenburg, 2016)

Year	Inside historic range		Outside historic range	Total population (only in South Africa)
	Protected parks	Private land		
1837	0	87	0	87
1900	0	330	0	330
1927	0	121	0	121
1931	17	50*	0	67*
1939	123	100*	5	228*
1960	72	60*	200	332*
1978	250*	200*	250*	700
1982	320	300*	400*	1 020*
1999	500*	800*	1 000*	2 300
2008	600*	900*	2 000	3 500
2015	901	1 302	4 959	7 162
2016	900*	1 400*	5 029	7 329*

* Extrapolated numbers

It is challenging to determine the current number of bontebok in South Africa due to the questionable amount of hybrid species present within bontebok and blesbok populations (Radloff *et al.*, 2016). Bontebok present in National parks and protected areas are known to be pure as most have been genetically tested, however, not all bontebok in privately owned farms have been proven as genetically pure (Radloff *et al.*, 2016).

Nonetheless, the main threats to bontebok populations are the hybridisation with blesbok, poor gene flow between sub-populations, low genetic diversity and a lack of available habitat within their natural range areas (Van der Walt *et al.*, 2013). Although the two sub-species differ in coat colour, behaviour and social structure, hybridisation readily occurs (Birss *et al.*, 2013). The blesbok/bontebok offspring are fertile (Furstenburg, 2016) and this threatens the genetic integrity of the less abundant bontebok (Birss *et al.*, 2013).

According to IUCN red list assessment, the bontebok is classified as “vulnerable”, which describes a high-risk zone for an animal to become extinct unless the surrounding environment improves (Radloff *et al.*, 2016). In 1989, Fabricius *et al.* (1989) developed a phenotype test for bontebok purity certificates, which was later no longer supported by CapeNature. CapeNature is a governmental organisation responsible to protect, maintain and sustain the public nature reserves and wilderness areas in the Western Cape, South Africa. A genetic test was developed in June 2009 by Dr D. Dalton from the research and scientific services which ensured the accurate genetic

prediction of blesbok and bontebok and is supported by many peer-reviewed scientific publications (Dalton *et al.*, 2011; Van Wyk *et al.*, 2013). The purity of bontebok was investigated by visiting various farms across South Africa, which was the first study to investigate the molecular analysis on pure blesbok, bontebok, hybrid species and a group of unknown species. Evidence of clear partitioning between the two subspecies were found. Out of the “unknown” group which consisted of 121 animals, 33% of this group was found to be hybrid species (Van Wyk *et al.*, 2013).

In response to the vulnerable state of pure bontebok, CapeNature implemented a strict policy to ensure the sustainable use and conservation of bontebok that tested pure within its indigenous range, the improvement and rehabilitation of the habitat available to bontebok and the prevention of sub-species hybridisation (Birss *et al.*, 2013). In June 2015, the bontebok advisory committee of wildlife ranching South Africa (WRSA) wrote their first national bontebok management plan and in March of 2016 it was approved. This protocol addresses DNA analysis and genetic purity, breeding and camp systems, monitoring population enhancement, nutrition and health and the general species management (Furstenburg, 2016).

2.1.2.4 *Bontebok in the game meat industry*

In South Africa, wildlife ranches have a role to play in the conservation of species. This is largely due to the natural areas of habitat and funding resources provided to support reintroduction programs for threatened species. The latter is especially important due to the lack of governmental funding available for conservation (Cousins *et al.*, 2008). In general, bontebok is an expensive animal to hunt. In the category of medium sized antelope, bontebok is considered to have a high trophy fee due to its scarcity. Foreign citizens are required to provide proof of a permit to take a trophy back to their country. On the South African side, the landowner providing the service to the client is obligated to apply for a Threatened or Protected species (“TOPS”) permit in the client’s name (<https://www.shakariconnection.com/bontebok-hunting.html>). As a result of the trophy hunting industry, there is an over-abundance of adult bontebok rams, but a small demand exists from tourist hunters (Furstenburg, 2016). The latter could be due to cost implications and the number of strict regulations regarding bontebok hunting.

The live auction sales of bontebok is a major contributor towards the economic value of this species in the South African game industry. The average live auction sale price of bontebok gradually increased from R1000 per animal in 1992 to R6625 in 2007, where after the price exponentially increased after 2012, reaching a peak in 2015 with an average price of R122 909 per animal (Table 2.3).

Table 2.3 The mean annual South African auction price of bontebok from 2012-2017

Year [#]	South African Rand per animal
2012	9 806
2013	29 853
2014	37 004
2015	122 909
2016	66 708
2017	45 286

[#]References: 2012-2015 (Furstenburg, 2016); 2016-2017 (<http://www.gamefarmnet.co.za/veiling.htm>)

Bontebok breeding on privately owned land should ideally maintain a ratio of 8-12 ewes: 1 ram, however, this depends on the population size. Private breeding typically results in 25% of the herd becoming non-breeding male animals. In order to balance and re-establish the social breeding structure, surplus males need to be harvested annually, which ensures the optimum enhancement of the sub-population (Furstenburg, 2016). Therefore, several male bontebok can be harvested annually although the utilisation of the meat derived from surplus bontebok rams is currently unknown. The meat quality of bontebok has not been reported in literature, however, this knowledge could ensure a more economical and sustainable manner to utilise the meat derived from the harvesting of bontebok. Baseline data for wild antelope species enables researchers to build on, compare and improve data available in literature to provide science-based information to the meat industry.

2.2 MEAT QUALITY

Meat quality traditionally refers to the properties that enable meat to be suitable for consumption, further processing and storage. This includes properties such as food safety, flavour, texture, colour, water-holding capacity, oxidative uniformity and stability, nutritional value and lipid composition (Andersen *et al.*, 2005). These physical, chemical, microbial and sensory properties greatly influence the way in which consumers choose and perceive meat (Kudrnáčová *et al.*, 2018). In the past few decades high quality standards have ensured that the term “meat quality” also consider conditions under which the meat is produced (Andersen *et al.*, 2005). This includes ante-mortem characteristics such as animal nutrition, age, sex, breed, muscle type, handling or breeding management, as well as post-mortem factors such as temperature, pH and ageing of the meat (Kudrnáčová *et al.*, 2018). Game species differ from domestic species in terms of ante-mortem factors and slaughter processes influencing their meat quality, as summarised in Table 2.4.

Table 2.4 The ante-mortem and slaughter processes influencing the meat quality of domestic and game animals [as reviewed by (Neethling *et al.*, 2016a)].

Factor	Controllable	Uncontrollable	Explanation
	Domestic species	Game species	
Species	Yes	Yes	Although there are many game species harvested, these are easily identifiable
Age	Yes	Random	Mature game species are selected for harvesting.
Gender	Yes	Species-specific	With some game species the males are easily recognisable e.g. horns (kudu, <i>Tragelaphus strepsiceros</i>), while with other game species this proves more difficult, particularly with night harvesting (black wildebeest, <i>Connochaetes gnou</i>).
Ante-mortem stress	Yes	Difficult	Influenced by terrain, species, mating season, day vs. night harvesting and harvesting method (rifle vs. helicopter).
Method of killing	Yes	Partly	The major objective is killing with head shot using a free bullet; however, this is not always possible due to the ante-mortem stress factors.
Abattoir processes	Yes	No	All 'dirty' processes are conducted in the field where normal interventions such as electrical stimulation cannot be applied.
Cooling	Yes	No	Difficult to apply a standard cooling regime due to field slaughter/dressing and the use of refrigerated trucks.
Processing	Yes	Partly	Difficult to apply a standard cooling regime due to field slaughter/dressing and the use of refrigerated trucks. When linked to commercial export, well defined standard operating procedures (SOPs) exist. Most game meat is exported as deboned, vacuum-packed, frozen muscles/muscle cuts. Packaging material is not standardised. However, for home consumption there are no guidelines.
Cold-chain management	Yes	Partly	When linked to commercial export, well defined SOPs exist. However, for home consumption there are no guidelines and frequently no refrigeration facilities.
Hygiene practises	Yes	Partly	When linked to commercial export, well defined SOPs exist. However, for home consumption there are no guidelines. Water availability is often limited.

2.2.1 Sex

Historically, male and female ungulates have different behavioural patterns. Females are known to look after and protect their young as part of their reproductive success, whereas the success of males is determined during a short rutting season where they fight and compete for females. This includes males patrolling territories, tending to females and fighting with other males to gain access to females (Mysterud *et al.*, 2004). Male ungulates are thus more active (especially during the rutting period) and this results in meat from females generally having higher intramuscular fat contents (IMF) than males (Neethling *et al.*, 2016a). Male impala (Van Zyl & Ferreira, 2004; Hoffman *et al.*, 2009b), springbok (Van Zyl & Ferreira, 2004; Hoffman *et al.*, 2007a; Neethling *et al.*, 2018) and blesbok (Van Zyl & Ferreira, 2004; Mzuvukile, 2018) had significantly lower fat contents than their female counterparts. However, it has been found that sex had no effect on the chemical composition of meat in blesbok (Hoffman *et al.*, 2008; Neethling, 2012), black wildebeest (Hoffman *et al.*, 2010a) or kudu (Mostert & Hoffman, 2007). Neethling *et al.* (2018) found sex to influence only certain chemical and sensory attributes of springbok meat, hence their study concluded that sex does not need to be considered when marketing springbok meat and that other factors such as farm location have a much larger influence.

Disadvantages of intact male species (not castrated) include: undesirable odours and flavours; aggressive behaviour; lower meat quality; undesirable colour and lower meat tenderness (Seideman & Cross, 1982). The sensory quality of springbok meat was affected by sex and rams had a significantly lower sweet taste than their female counterpart (Neethling *et al.*, 2018). Similarly, meat from male black wildebeest (Hoffman *et al.*, 2010a), springbok (Hoffman *et al.*, 2007b), impala and kudu (Hoffman *et al.*, 2009b) were significantly less tender than meat from females. Tenderness is regarded as a very important aspect to enhance the meat quality of species (Koochmaraie & Geesink, 2006). On the other hand, sex did not have a significant effect on physical characteristics of wild fallow deer (*Dama Dama*) (Cawthorn *et al.*, 2018), blesbok (Hoffman *et al.*, 2010c), kudu and impala (Hoffman *et al.*, 2009b).

2.2.2 Carcass yield and composition

The harvesting of game species in field conditions usually leaves a considerable amount of internal offal (liver, kidney, heart, intestine, etc.) that are mostly left in the field for scavenging animals. Game offal forms part of the diet of many African people. It is a low-cost source of protein and seems to be culturally accessible, affordable, acceptable and considered safe (McCrinkle *et al.*, 2013). The carcass composition, muscle- and offal weights, carcass weights and dressing percentage is important for the game meat industry to give an indication of the meat production potential of

specific species (Huntley, 1971; Fitzhenry, 2016). Male antelope are generally heavier (live weight and carcass weight) than their female counterpart as seen in impala (Van Zyl & Ferreira, 2004; Hoffman *et al.*, 2009b) and kudu (Hoffman *et al.*, 2009b). However, no differences in slaughter weight, hot carcass weight, cold carcass weight and dressing percentages between sexes were found in eland (Needham *et al.*, 2019), springbok and blesbok (Van Zyl & Ferreira, 2004). Certain antelope such as kudu (Hoffman *et al.*, 2009b) have males with much larger horns and body weights than their female counterparts, whereas in species such as blesbok and bontebok it is difficult to distinguish between male and female due to both sexes having similar sized horns (Kohn, 2014). Blesbok carcass weights have been reported between 25.83 kg (4 males; 4 females; Hoffman *et al.*, 2008) and 28 kg (10 males; 10 females; Neethling *et al.*, 2014a). Furthermore, the average dressing percentages of blesbok (28 males; 37 females) from four regions was found to be 52.1% (Hoffman *et al.*, 2008). The methodology used (hot or cold carcass) for the determination of the abovementioned carcass weight and dressing percentages were not specified in the respective articles. Adult male impala (n=11) and kudu (n=7) were found to have carcass weights of 37.89 kg and 142.69 kg and dressing percentages of 60.9% and 58.3%, respectively (Hoffman *et al.*, 2009b). The above-mentioned values highlight the differences in carcass composition and dressing percentages between species and gender and the importance to report species-specific information in literature.

2.2.3 Production region and nutrition/diet

There are many types of biomes and vegetation types throughout South Africa; each with its own specific wildlife species (Van der Merwe & Saayman, 2014). Game species could be classified as grazers, browsers or mixed feeders depending on their selective or generalist eating habits. Grazers only consume grass, whereas browsers prefer shrubs, flowers, leaves, fruits and stems (Shiple, 1997). Different seasons produce different climates and in return natural vegetation and the behaviour of the animals change. It is important to note that different African ungulate species have different dietary preferences, which in turn influences the animal's meat quality, such as certain attributes pertaining to their chemical and sensory characteristics. Blesbok, red hartebeest and blue wildebeest are classified as grazers (Twine, 2002; Kraaij, 2011; Van Heerden, 2018), springbok and impala are mixed feeders and kudu are browsers (Gagnon & Chew, 2000).

The sensory flavour, aroma, and texture profiles in addition to the chemical composition (moisture, protein, fatty acids) of springbok meat was influenced by region (Neethling *et al.*, 2018). Springbok are mixed feeders and they prefer shrubs during the dry season and grass during the wet season (Bigalke, 1972). The different farms in the study had different vegetation types and this influenced the sensory quality and fatty acids. It is important to note that inconsistencies in the sensory quality of springbok meat due to dietary differences could have a negative impact on the

consumer perception of springbok meat (Neethling *et al.*, 2018) and this emphasises the role that dietary intake may have on the meat quality of wild ungulates.

2.2.4 Muscle types

Skeletal muscles consist approximately of 75% water, 20% protein, 1-10% fat and 1% glycogen (Listrat *et al.*, 2016) and exhibit a wide variety of sizes, shapes, physiological functions and anatomical locations. Not only do skeletal muscles contain muscle fibres, but also adipose, connective, nervous and vascular tissues. The intramuscular fat, intramuscular connective tissue and muscle fibre types play a key role in the meat quality (Li *et al.*, 2007).

Muscles are composed of different muscle fibres (Taylor, 2004) and connective tissues that are present in different quantities, different muscle types and locations in a variety of animal species. The latter results in quality variation amongst muscles such as tenderness differences (Li *et al.*, 2007). Muscle fibre types is the key factor responsible for differences in protein composition (Zou *et al.*, 2018) and are generally characterised by their metabolic or contractile properties (Lefaucheur, 2010).

Three main fibre types in large skeletal muscles of mammalian species exist. These are classified as type I (oxidative slow twitch), type IIA (oxidative fast twitch) and type IIB (glycolytic very fast twitch). The myosin heavy chain (MHC) expressed determines these properties. Type I fibres express MHC I, type IIA express MHC IIa and type IIX express MHC IIx. However, there is a fourth isoform called MHC IIb (Bottinelli, 2001; Kohn *et al.*, 2005). This isoform is more localised to specialized muscles such as an eye and not often mentioned in studies concerning muscles converted to meat post-mortem in African antelope (Kohn *et al.*, 2005; Kohn, 2014). Type IIX fibres prefer Adenosine triphosphate (ATP) anaerobically produced from blood glucose and glycogen, whereas type I prefers ATP aerobically produced from fat and glycogen. Type IIA uses both glycolytic and oxidative pathways to produce ATP (Taylor, 2004). Based on the differences in muscle fibre types, skeletal muscles can therefore be classified into two types, namely red muscles (slow use muscle or red meat) or white muscle (fast use muscle or white meat) (Swatland, 1994). Muscle fibre types are determined at birth and would only increase with size as the animal grows. Thus, the change in muscle fibre type is dependent on the plane of nutrition and degree of exercise to which the animal is exposed (Cassens & Cooper, 1971). The effect of external stimuli from physical activity have been the factors known to influence the muscle fibre type of some African ungulates (blesbok, blue and black wildebeest, greater kudu and mountain reedbuck) (Kohn *et al.*, 2007; Kohn, 2014).

The characteristics of impala (*Aepyceros melampus*) skeletal muscles were analysed by Kohn *et al.* (2005). Four muscles [*semimembranosus* (SM), *longissimus thoracis et lumborum* (LTL), *psaos major* (fillet) and *deltoideus*) predominately made-up of type IIA MHC isoform and contained a

smaller percentage of type I MHC isoform. Type IIX was only present in one of the four muscles, thus emphasising the variability of muscle fibres between these four anatomical locations. Furthermore, Kohn (2014) investigated the *Vastus lateralis* muscles of blesbok, greater kudu (*Tragelaphus strepsiceros*) and mountain reedbuck (*Redunca fulvorufula*), where the author found that these three species expressed type I, IIA and IIX MHC isoforms with IIX fibre type being most abundant. Blesbok, kudu and mountain reedbuck seemed to display high glycolytic and oxidative properties, however, species differences were also found (Kohn, 2014). The muscle fibre type composition of the springbok LTL and BF muscles was determined by North and Hoffman (2015a). Springbok muscles consisted primarily of type IIX fibres (64 – 78%). This suggests a muscle dominated by glycolytic metabolic mechanisms which agrees with the sprinting and jumping ability of this species. Springbok are excellent jumpers compared to other species such as the bontebok and blesbok that are poor jumpers. This demonstrates that muscle location and species movement differences could affect the meat quality of animals.

The muscle structure of bovines has compared well to South African game species, however there are a few variations present for selective species. Muscles found in the back: the LTL and fillet. The hind limb consists of the *semitendinosus* (ST), *biceps femoris* (BF) and SM muscles. Furthermore, the forelimb muscles consist of the *infraspinatus* (IS) and *supraspinatus* (SS). These muscles are mostly used for commercial meat production purposes in the game meat industry.

The fillet runs along both sides of the spine, it is not a weight bearing muscle and contains less connective tissue and therefore is found to be very tender. The loin consists of two sections, the *longissimus lumborum* (LL) that represents the dorsal end and the *longissimus thoracis* (LT) that represents the cranial end. Together the latter sections form the LTL muscle. The LTL muscle stretches along the length of the vertebrae and maintains the stability and balance during moving in addition to assisting with the respiratory and neck movements. The muscle fibres are not uniform in the LTL and larger fibres are found to the posterior LL (bottom) than to the anterior/cranial LT (top) end (Swatland, 1994).

The hind limb muscles are usually the larger, more tender muscles as compared to the fore limb muscles. The BF is situated in the most lateral face of the posterior muscles (in the thigh). The ST and BF muscles assist in extending the hock. The BF is known to have a uniform tenderness, whereas the ST has a less desirable texture. The SM muscle is situated on the posterior face of the hind limb. The latter is a large muscle, positioned medial to the ST muscle. The IS and SS muscles are part of the fore limb. They are known as the “shoulder muscles” and serve to stabilise, move, extend and flex the shoulder. The IS muscle is a very strong shoulder joint ligament found ventral to the spine on the *scapula*, whereas the SS muscle is dorsal to the spine on the *scapula* (Swatland, 1994).

It is clear that muscles differ in anatomical location and function, thus these muscles have different metabolism activity levels. The latter will influence meat quality differences between muscles.

2.2.5 Physical meat attributes

In literature, when evaluating the physical meat quality of game species, the following attributes are typically tested for: Warner-Bratzler shear force (WBSF); ultimate pH (pH_u); colour; drip loss and cooking loss. These attributes often influence each other and in certain species (depending on the influence of ante-mortem and post-mortem factors) would also be linked to other changes in meat quality such as the sensory characteristics (Cawthorn *et al.*, 2018; Kudrnáčová *et al.*, 2018; Rudman *et al.*, 2018; Needham *et al.*, 2019).

The Warner-Bratzler shear force (WBSF) test is an instrumental method used to measure the tenderness of meat (lower WBSF values equal more tender meat) (Novaković & Tomašević, 2017) and has reportedly been the most important meat quality characteristic (Bhat *et al.*, 2018). Meat tenderness is determined by various factors such as sarcomere shortening during rigor mortis, the amount and solubility of connective tissue and post-mortem proteolysis of myofibrillar-associated proteins (Koochmaraie & Geesink, 2006). The post-mortem sarcomere length is determined by the degree of shortening occurring at rigor mortis which is determined by pre-rigor metabolism together with post-mortem temperature control. The pre-rigor muscle metabolism can vary and is significantly influenced by factors such as muscle fibre type, ante-mortem stress and genetics (as reviewed for wildlife by Neethling *et al.*, 2016a).

Furthermore, meat colour is also an important factor influencing the acceptability of meat by consumers. The meat colour of free-ranging wild ungulate species is generally classified as red in colour and known to be slightly darker than animals farmed in a feedlot (Neethling *et al.*, 2017). These species often have a higher pigmentation due to a higher level of exercise in wilder species (Hoffman & Mcmillin, 2009). The colour is primarily determined by the chemical state and amount of myoglobin present in meat and by the muscle tissue structure which is directly linked to the ultimate pH of meat (pH_u) (Neethling *et al.*, 2017; Shange *et al.*, 2019). These chemical states are present within raw meat in three myoglobin state: oxymyoglobin, deoxymyoglobin and metmyoglobin. The oxymyoglobin state is characterised by a desirable bright red colour, deoxymyoglobin by a red-purple colour and metmyoglobin by a brownish colour (Cornforth & Jayasingh, 2004). Myoglobin is a protein and is therefore susceptible to changes in environmental conditions as seen with all other proteins (Neethling *et al.*, 2017). Post-mortem changes in temperature and pH could alter the functionality and structure of the protein due to protein denaturation and these changes could affect the colour of meat (Kim *et al.*, 2014). The colour of meat could be evaluated instrumentally through reflectance spectrophotometry or visually by panellists as part of descriptive sensory

analysis and colour charts for meat. Spectrophotometers and colorimeters are instruments that assist in the description of meat colour, such as L* (lightness), a* (redness), b* (yellowness), hue angle and Chroma (saturation index) (American Meat Science Association, 2012). Neethling *et al.* (2016b) investigated the muscle specific colour-stability of blesbok muscles and found muscle variation; the IS muscles had a significantly higher colour stability, redness and chroma than the BF and LTL muscles.

One of the main indicators of physical meat quality is muscle pH and this indicator could affect the colour and tenderness of meat. The pH in live animals is between 6.8-7.3 and is lowered post-mortem due to the by-product (lactic acid) released during anaerobic glycolysis. The ultimate pH of meat at different times post-mortem (depending on the species) should be found between 5.4-5.8 (Lonergan *et al.*, 2010). Two well-known phenomena affecting meat quality is cold-and heat shortening. If meat goes into rigor mortis at a high pH and temperature below 10°C, cold shortening takes place (increased meat toughness). If temperatures exceed 25°C during rigor, it could also negatively influence toughness by causing heat shortening (Bhat *et al.*, 2018)

Moisture loss in meat is almost inevitable post-mortem. This occurs due to reduced space in myofibrils to retain water, the loss of adenosine triphosphate (ATP) and the drop in pH post-mortem (Huff-Lonergan & Lonergan, 2005). As the pH drops, the muscle proteins become less charged as the meat's pH drops and reaches their iso-electric point and the water previously bound to proteins is now released into intrafibrillar spaces. The released water is then redistributed to extracellular and sarcoplasmic spaces during muscle contraction and known as "exudate" (Leygonie *et al.*, 2012). The amount of exudate released influences the drip- or thaw loss. The water holding capacity of meat is not only important in terms of meat quality, but also influences product yield negatively which in turn has detrimental economic implications (Cheng & Sun, 2008).

The physical characteristics of game species can be influenced by a wide variety of intrinsic and extrinsic factors and differ significantly between game species as seen in Table 2.5.

Table 2.5 The physical characteristics of selected game species determined on raw meat

Animal species	Sample analysed	N	pH _u	DL%	CL%	WBSF (N)	L*	a*	b*	Chroma	Hue-angle	Reference
¹ Springbok	LTL	12	5.63	2.80	31.50	-	31.75	13.29	8.10	15.63	31.47	(Hoffman <i>et al.</i> , 2007b)
Blesbok	LTL	75	5.47	4.56	35.02	-	30.50	12.57	4.27	15.29	33.39	(Hoffman <i>et al.</i> , 2010c)
Black wildebeest	LTL	6	5.65	-	-	-	33.08	13.6	10.29	17.1	36.85	(Shange <i>et al.</i> , 2019)
Wild fallow deer	LTL, BF, SM, ST, IS, SS	12	5.48	1.42	33.13	41.8	31.23	14.1	10.25	17.47	35.9	(Cawthorn <i>et al.</i> , 2018)
Eland	LTL, BF, SM, ST, IS, SS	12	5.63	0.83	32.8	83.32	34.47	14.1	11.78	18.42	39.98	(Needham <i>et al.</i> , 2019)
² Impala	LTL	32	5.57	1.2	31.0	-	-	10.63	7.67	13.24	35.63	(Hoffman <i>et al.</i> , 2009b)
³ Kudu	LTL	35	5.63	1.4	31.5	-	-	11.39	8.49	14.32	36.67	(Hoffman <i>et al.</i> , 2009b)

^{1,2,3}Springbok, impala and kudu included adult and sub-adult.

Abbreviations: N= Number of species, DL%= drip loss percentage, CL%= cooking loss percentage, WBSF = Warner-Bratzler shear force, LTL= *M. longissimus thoracis et lumborum*, BF= *M. biceps femoris*, SM= *M. semimembranosus*, ST= *M. semitendinosus*, IS= *M. infraspinatus*, SS= *M. supraspinatus*.

2.2.5.1 Ante-mortem stress

Physical meat quality is greatly influenced by ante- and post-mortem physical conditions imposed on carcasses (O'Halloran *et al.*, 1997). Previous research has found that harvesting conditions could negatively influence game animal species meat quality (Shange *et al.*, 2018). After the harvesting of game species, glycogen (used as energy source) is converted to lactic acid. The latter implies that an animal that experiences ante-mortem stress, would have less glycogen reserves ante-mortem, resulting in less lactic acid produced and higher pH_u values in meat post-mortem. Two of the worst quality effects facing the meat industry are dark, firm and dry (DFD) and pale, soft and exudative meat (PSE). These conditions could occur in all animals, depending on how they are treated pre-slaughter and could be influenced by species, breed, sex, pre- and post-slaughter handling of animals. DFD meat is known to have a $pH \geq 6$, 12 – 48 h post-mortem (Shange *et al.*, 2019). Different pH_u values of wild game meat have also been categorized in three categories as high-quality meat ($pH < 5.8$), intermediate DFD ($5.8 \leq pH < 6.2$) and DFD ($pH \geq 6.2$) meat (Viganò *et al.*, 2019). DFD classified meat comes from carcasses that have been stressed, diseased or injured before slaughter (Chambers & Grandin, 2001). In a mini-review by Adzitey and Nurul (2011), DFD meat was further explained as meat with higher water holding capacity (water bound more tightly), dark in colour (absorbs more light), high variation in tenderness and meat with a higher spoilage rate.

Hoffman *et al.* (2007b) evaluated the physical characteristics of springbok (*Antidorcas marsupialis*) meat from different production regions. One region resulted in animals believed to be stressed ante-mortem as the meat had higher pH_u values (6.3 ± 0.07) and the meat was classified as DFD. Furthermore, the meat had lower cooking and drip loss values, as well as high WBSF values (meat was tougher). In the latter study, the pH_u was positively correlated to a^* and chroma values, indicating increased redness and higher colour saturation. Meat from reindeer (*Rangifer tarandus*) carcasses (Renecker *et al.*, 2005) and black wildebeest (*Connochaetes gnou*) (Shange *et al.*, 2019) with high pH_u values (classified as DFD) also resulted in meat with a significantly darker colour.

Furthermore, the sensory attributes of animals stressed ante-mortem could also be significantly affected by post-mortem pH; meat from Alaskan reindeer that experienced ante-mortem stress was grainy with a rancid flavour, however, the juiciness and gamey flavour was still generally accepted by the sensory panel (Renecker *et al.*, 2005).

2.2.5.2 Capture myopathy

Capture myopathy, also known as capture stress, overstraining disease and white muscle disease, is a disease associated with the handling or capturing of wild animals. The disease is associated with severe rhabdomyolysis (process of muscle tissue breakdown), kidney failure and elevated body

temperatures (Breed *et al.*, 2019). This disease mainly affects the cardiac and skeletal muscles which are pale 24 h post-mortem. Other symptoms occasionally include a dark, swollen kidney and the possibility of myoglobin in urine, however, the latter is difficult to detect (Shepherd, 1999). The occurrence of capture myopathy occurs mainly in wildlife transportation and translocation operations and in many cases could kill animals due to severe exertion and stress (Minka & Ayo, 2009).

If animals are very stressed and exposed to extreme exertion prior to harvesting, they could be affected by this phenomenon and the quality of meat would be detrimentally affected (Minka & Ayo, 2009). Hormones such as adrenaline, nor-adrenaline and catecholamines are released. A high glucose content is needed in an animal's flight-or-flight strategy when it feels threatened (Romero & Butler, 2007). After the body metabolism is increased for long periods of time, the glucose concentrations can become exhausted. Glucose is then generated by the breakdown of glycogen or obtained from non-carbohydrate sources by the processes of glycogenolysis and gluconeogenesis, respectively. After carbohydrates and fat reserves have been mobilised, protein will become the next target for degradation. In response to increased corticosterone secretion, nucleic acids and proteins will be broken down (Menon *et al.*, 2014; Slimen *et al.*, 2016).

Rhabdomyolysis is therefore a syndrome of skeletal muscle breakdown and leads to muscle content leakage. The most important of these substances is muscle protein myoglobin which gives rise to the presence of myoglobin in the animal's urine. An uncontrolled rise in free calcium and the activation of the calcium-dependant proteases leads to the lysosomal digestion of muscle fibre content and the destruction of myofibrils (Warren *et al.*, 2002).

2.2.6 Chemical composition of meat

Meat is a great source of vitamins, minerals and protein (Bohrer, 2017). Additionally, it is specifically a great source of folic acid, iron, vitamins A, B12 and selenium. The latter micronutrients are not all present in plant-derived food sources or not as bioavailable, as in meat-derived sources (Biesalski & Nohr, 2009). The chemical composition of meat includes the proximate composition and other attributes such as fatty acids and cholesterol content (Gašperlin *et al.*, 2006). The proximate composition of meat typically describes the moisture, protein, fat and ash content of meat (Wahrmund-wyle *et al.*, 2000).

Red meat consumers worldwide have formed part of a trend to purchase meat with less fat. They are also conscious of environment-friendly aspects and would rather pay more for "organic" meat (Wassenaar *et al.*, 2019). Game meat is obtained from farm reared, non-domesticated or/and free ranging animals (Hoffman & Cawthorn, 2012). Game species are perceived to be a healthier alternative to red meat from domestic species due to their low fat and high protein content

(Rudman *et al.*, 2018; Needham *et al.*, 2019). The average fat content of game species is typically below 3% (Table 2.6). The proximate composition of game species is influenced by factors such as the species diet (Neethling *et al.*, 2018). As previously mentioned, game species can be classified as browsers, grazers or mixed feeders that all prefer and consume different feed intake. In addition to diet, season (Neethling *et al.*, 2014b), sex (Needham *et al.*, 2019), age (Hoffman *et al.*, 2007a), weather conditions (drought 2018) (Rudman *et al.*, 2018) and muscle type (Neethling *et al.*, 2014a; Needham *et al.*, 2019) have been proven to influence the proximate composition of game species. Due to a number of factors influencing the proximate composition of game meat, it is expected that the proximate composition should also differ amongst species as illustrated in Table 2.6.

Table 2.6 The proximate composition (g/100 g wet weight base) of male African game species determined in raw meat

Animal species	Sample analysed	N	Moisture (g/100 g)	Protein (g/ 100g)	IMF (g/ 100g)	Ash (g/ 100g)	Reference
Springbok	LTL	90	74.2	-	1.4	1.2	(Hoffman <i>et al.</i> , 2007a)
	LTL	9	65.8	30.8	2.6	1.4	(North and Hoffman, 2015a)
Blesbok	LTL, BF, SM, ST, IS, SS	18	74.5	21.9	2.8	1.3	(Neethling <i>et al.</i> , 2014b)
	LTL, BF, SM, ST, IS, SS	10	76.0	21.4	2.8	1.2	(Mzuvukile, 2018)
Kudu	LTL	7	75.7	22.8	1.5	1.2	(Hoffman <i>et al.</i> , 2009b),
	LTL	8	74.5	23.6	1.6	1.2	(Mostert and Hoffman, 2007)
Impala	LTL	11	75.0	22.6	2.1	1.2	(Hoffman <i>et al.</i> , 2009b),
Red hartebeest	LTL	13	75.0	23.3	0.6	1.2	(Hoffman <i>et al.</i> , 2010b)
Blue Wildebeest	LTL, BF, SM, ST, IS, SS	16	77.0	21.1	1.8	1.0	(Van Heerden, 2018)

1-Muscles from both male and female blesbok from Autumn 2010 group.

Abbreviations: Ref= Reference, IMF= intramuscular fat, LTL= *M. longissimus thoracis et lumborum*, BF= *M. biceps femoris*, SM= *M. semimembranosus*, ST= *M. semitendinosus*, IS= *M. infraspinatus*, SS= *M. supraspinatus*, RF= *M. rectus femoris*.

2.2.7 Ageing

There are two main types of ageing commonly used in the industry; dry- and wet-ageing. Wet ageing, the more popular method, is described as the process where sub-primal cuts are kept under refrigerated vacuumed conditions for a specified time period. There are advantages associated with this method, such as less trim- and weight loss, less space occupied, less costs, ease of handling and storage and higher shelf-life of meat without neglecting palatability (Brad Kim *et al.*, 2018) as opposed to dry-ageing.

Ageing is used to improve the sensory properties (appearance, aroma, flavour and texture attributes) of meat and more specifically post-mortem to improve the tenderness of meat. During post-mortem ageing, beef was found to develop desirable flavours such as brothy, beefy, sweet and browned-caramel, while off-flavours such as sour and bitter decreased (Spanier *et al.*, 1997). However, certain sensory characteristics could become less desirable with extended ageing periods due to auto-oxidation caused by mitochondria and myoglobin levels and thus enhancing the negative characteristics (Hoffman & Mcmillin, 2009). North and Hoffman (2015b) found aged LTL muscles of springbok to produce meat with a higher occurrence of metallic, liver-like, sour and off/manure taints which could be ascribed to lipid- and protein oxidation.

Tenderness is known to be one of the most important characteristics of meat (Bhat *et al.*, 2018). Miller *et al.* (2001) found that an increase in shear force of beef steak (tougher meat) significantly decreased the consumer's acceptability toward the meat, thus highlighting the importance of tenderness. However, it is important to note that in selected African countries the "higher value cuts" such as the *psoas major* (fillet) is not seen as the most expensive muscle of the carcass and tougher muscles/cuts are preferred (Hoffman, L.C. Personal communication. August, 2019).

The tenderness of meat is improved by ageing due to the disruption of muscle structures by the intracellular proteolytic system (Lonergan *et al.*, 2010). After the slaughtering process, meat converts to muscle while undergoing many biophysical and chemical changes. These changes include a shift from aerobic to anaerobic metabolism (gradually lowering the pH due to a higher quantity of lactic acid production), depletion of available energy and rise in ionic strength. All these changes could have a significant effect on the various muscle cell proteins, particularly the proteolytic enzyme system that is believed to be involved in the post-mortem ageing tenderisation system (Lonergan *et al.*, 2010).

The muscle to meat process is divided into three phases. The first being the pre-rigor phase where toughness is mainly caused by collagen content. The second phase is called the rigor phase

and, in this phase, muscle shortening further contributes to toughness and lastly the tenderization phase. In the latter phase the muscles undergo a series of changes that improve the tenderness significantly (Bhat *et al.*, 2018). Thus, these three phases (ageing being part of the last phase) are the main factors determining the tenderness of meat. The optimum tenderization takes place post-mortem, however various genetic and environmental factors also play a role in ensuring uniformity in the tenderness (Geesink *et al.*, 2000). The main factors influencing the tenderness during ageing as defined by Bhat *et al.* (2018) are pH, temperature, ageing time, fibre-type composition, proteolysis and sarcomere length. The importance of connective tissue, sarcomere length and proteolysis are dependent on muscle type. Muscles vary significantly in tenderness as seen in wild fallow deer (*Dama Dama*) with the lowest tenderness found in the LTL (31.3 N) and BF (36.0 N) and the highest in the IS (43.1 N) and SS muscles (52.7 N) (Cawthorn *et al.*, 2018).

As mentioned above, muscle fibre type composition differs in different animal species, as well as muscles and may influence the tenderness of meat during ageing. The rate of ageing has been reported to be slower in slow-twitch oxidative muscles (type I) and faster in fast-twitch glycolytic muscles (type IIA). Muscles containing a higher amount of oxidative fibres are more susceptible to cold shortening and thus an increased toughness (Bhat *et al.*, 2018). A large part of the tenderisation process is dependent on the ability of cytoskeletal and key myofibrillar fibres to be disrupted by proteolytic enzymes (Bhat *et al.*, 2018). The most extensively studied enzyme system involved in meat tenderization is the calpain system consisting of a large unusual group of cysteine proteases (proteolytic enzymes) that require calcium to function. Their optimum performance is at a neutral pH and their function can be inhibited by calpastatin (Bhat *et al.*, 2018). Calpains and cathepsins are enzymes that degrade proteins and are active under post-mortem muscle conditions. The latter phenomenon contributes to the concept of “ageing” and the tenderisation process that follows (Beltrán *et al.*, 1997). An investigation into the optimum ageing period of springbok meat found WBSF values to decline over time (day five of ageing, indicating optimum tenderness), while the purge- and cooking losses significantly increased over time ($p < 0.05$). The latter study also found cathepsins to increase during ageing and calpain and calpastatin activity to decrease significantly up to the fifth day of ageing, suggesting that these enzymes could be responsible for the initial tenderisation process (North *et al.*, 2015).

The stability of the proteolytic enzymes of the muscle cells is influenced by the ultimate pH of the meat (Bhat *et al.*, 2018) in addition to the storing temperature of the meat stored during ageing. If the temperature is below -2°C to -3°C (freeze point) the enzymes involved in the ageing process will stop and if the temperature is too high the microbial risk increases. Moreover, an ideal temperature between 0°C and 4°C is recommended by most studies (Bhat *et al.*, 2018). However,

Kilgannon *et al.* (2019) established the effect of different temperature-time combinations when ageing beef meat, analysing the microbiological count and sensory attributes of the meat. The results showed that different temperature-time combinations of meat ageing (for example 5°C for 8 days) could produce meat with the same quality attributes as meat aged under conventional means (1°C for 14 days). The most significant and rapid changes in meat occur within the first three to seven days post-mortem, however this could vary (Brad Kim *et al.*, 2018). In beef, the most substantial changes occur within the first week, where after the rate of tenderization declines with time (Koochmaraie & Geesink, 2006). The ageing time affects the tenderness of meat and is influenced by muscle type, fibre type, type of ageing (dry- and wet ageing), muscle size and is also found to be species and breed specific (Bhat *et al.*, 2018).

The United State Meat Animal Research Centre makes use of the LTL muscle for research purposes when evaluating tenderness. It is primarily used due to its significant economic value (Koochmaraie & Geesink, 2006). The LTL has been proven to generally have a higher sensory liking than other muscles as illustrated in beef (Legako *et al.*, 2015). The optimum ageing period (under vacuum) for springbok meat using the *Longissimus thoracis et lumborum* (LTL) and the *Biceps femoris* (BF) muscle was determined to be five days (North *et al.*, 2015), however, North and Hoffman (2015b) found the LTL from springbok to have a maximum ageing period of eight days. Blesbok LTL and BF muscles have an optimum ageing period of seven to ten days (Mzuvukile, 2018). For eland (*Tragelaphus oryx*) the optimum ageing period for the BF and LTL muscles are 17 and 21 days, respectively (Needham *et al.*, 2020). For blue wildebeest (*Connochaetes taurinus*) the LTL muscle should be aged nine days and BF muscle 14 days (Van Heerden, 2018).

2.2.8 Microbial activity

Meat is highly perishable in its fresh form. The type and number of microorganisms present in meat depends on the environment and sanitary conditions from which the meat originated, the extent to which the meat was handled or processed, and the conditions involved in handling, storage and distribution (Koutsoumanis & Sofos, 2004). The organisms that are responsible for spoilage could gain access to the living animal endogenously or through post-mortem contamination exogenously (Lawrie & Ledward, 2007).

During the harvesting and evisceration process of wild ungulates, the microbiological safety of the meat is influenced by the processing and dressing procedures. Contamination with microorganisms could take place from hide-to-muscle (soil adhering thereto) or from the gastrointestinal tract (if released during dressing operations) (Ramanzin *et al.*, 2010). Contamination could also be linked to aqueous sources (water used to wash instruments and carcass), airborne contamination, infected instruments (hooks, knives, saw) or cross-contamination from the people in

charge of the slaughtering procedures (Lawrie & Ledward, 2007). During storage the microbial activity could be influenced by the internal parameters of the meat (pH, water activity, etc.) as well as storage temperature. The harvesting and dressing procedures for ungulates vary and are not standardised, thus explaining the variability in microbial quality of different species. If standardised dressing and harvesting procedures could be implemented for all game species, the microbial activity would be reduced to a far greater extent (Ramanzin *et al.*, 2010).

The aim of meat hygiene is to ensure that meat is of good quality and safe for human consumption. The most significant fresh meat hazards that could cause disease in humans can be identified as *Campylobacter*, Shiga toxin producing *E. coli* (STEC) and *Salmonella*. These pathogenic bacteria, especially STEC, only requires a minimal number of bacteria to poison humans (Van Schalkwyk & Hoffman, 2016).

Indicator organisms represent larger groups of bacteria that are easy to measure and include certain pathogenic bacteria (Van Schalkwyk & Hoffman, 2016). The testing of pathogenic bacteria should be conducted in order to ensure that the meat is safe for human consumption. The quality of meat could be determined by testing for *Enterobacteriaceae* and total plate count (TPC) (Magwedere *et al.*, 2013). Aerobic colony count is a good overall indicator of bacterial contamination or overall quality of meat due to its ability to represent organisms that grow in aerobic conditions at mesophilic temperatures between 30 and 45 °C. The TPC and presence of pathogenic bacteria may however not always be related (Van Schalkwyk & Hoffman, 2016). *Enterobacteriaceae* is an indicator organism that is found in the intestines of animals and in the environment and could survive in both aerobic and anaerobic conditions (Jay, 2000). This group includes the major food-borne pathogens such as *Salmonella*, *E. coli* 0157 and *Yersinia*. *Enterobacteriaceae* do not ferment lactose, however some members collectively called coliforms (excluding *Salmonella*, *E. coli* 0157 and *Yersinia*) are able to produce gas and acid by rapidly (within 24-48 h) fermenting lactose (Food Standards Australia New Zealand, 2016). The presence of *Enterobacteriaceae* could indicate environmental and faecal contamination. The latter group of organisms is readily inactivated by products such as sanitisers and therefore could typically reflect the degree of environmental hygiene and could be used as a good indicator of good manufacturing practices (Van Schalkwyk & Hoffman, 2016). *E. coli* is a bacterium that lives in the intestines of human and animals and is shed in the faeces. There are six pathogenic *E. coli* strains of which STEC is the most commonly found strain. All mammals contain a variety of bacteria in their intestines; however, it is the hygiene procedures followed which determine whether the meat will be contaminated (Van Schalkwyk & Hoffman, 2016).

The microbial activity and safety of springbok meat for exporting purposes was tested in Namibia (Magwedere *et al.*, 2013). All samples tested negative for *Salmonella*, faecal bacterial

(*Clostridium perfringens*, coliforms and enterococci) and the *Escherichia coli* tested negative for STEC virulence genes. Hoffman and Dicks (2011) found game meat to be slightly more resistant to microbial spoilage (*Staphylococcus aureus*) than the meat from other domestic species such as pork, mutton and beef. However, further research is needed to support this statement as they for example, did not consider the meat's pH.

Animals often suffer from bruising caused by various physical blows. Bruises are bloody areas that could decompose which could promote bacterial growth (Chambers & Grandin, 2001). A higher pH in meat contributes to microbial growth and this generally leads to a product with a shorter shelf-life (Chambers & Grandin, 2001). Shange *et al.* (2019) analysed DFD (pH \geq 6) and normal meat samples (pH \leq 6) from black wildebeest (*Connochaetes gnou*) meat and noted a higher pH_u generally correlated to an increase in microbial activity. Furthermore, ageing is also known to contribute towards the increase of microbial activity. The activity of micro-organisms was tested in beef meat over an ageing period and the standard plate count, *Enterobacteriaceae* and *Lactic acid bacteria* numbers increased over time, whereas no significant effects were detected for *Escherichia coli*, *Listeria*, *Salmonella* or *Pseudomonas* (Kilgannon *et al.*, 2019).

In South Africa, the Department of Health (DoH) is responsible for the control of game meat once it leaves abattoirs and the Department of Environment, Forestry and Fisheries (DEFF) is responsible for the import and export of game species and for the slaughtering of game animals in approved abattoirs (Bekker *et al.*, 2011). DEFF has regulations in draft format regarding game meat slaughter procedures (Meat safety Act 2000; Act 40 of 2000). It has last been updated on 4 November 2016 and is still under review. Throughout the year a large quantity of game meat is available that has not been checked or approved according to safety criteria (Carruthers, 2008) and many meat inspectors do not have proper knowledge about game meat processing as they have with meat from domestic livestock (Van den Honert *et al.*, 2018). The evidence of most South African game meat species in terms of safety for human consumption is therefore very limited (Van der Merwe *et al.*, 2013).

According to the (DEFF, 2010) the microbial standards for exporting meat ("carcasses and meat cuts of wild cloven-hoofed game and wild solipeds") from South Africa is described in Table 2.7.

Table 2.7 The microbial limits of micro-organisms for game meat exporting purposes (DEFF, 2010)

Micro-organism	Limits		Method
	m ¹ (log value)	M ² (log value)	
Aerobic colony count	100 000 CFU/cm ² (5.0 log)	550 000 CFU/ cm ² (5.7 log)	ISO 4833
<i>Enterobacteriaceae</i>	100 CFU/cm ² (2.0 log)	316 CFU/cm ² (2.5 log)	ISO 21528-2
<i>E. coli</i>	50 CFU/cm ² (1.7 log)	500 CFU/cm ² (2.7 log)	ISO 16649-2
<i>Salmonella</i>	Absent/25g	Absent/25g	EN/ISO 6579

1= 'm' is a defined value separating a good result from a marginally acceptable (values between m and M are marginally acceptable);

2= 'M' is the maximum value for a marginal result (values greater than M are unacceptable).

The criteria for European Union Regulation (EC) No 1441 (2007) stipulate values slightly stricter than microbial limits specified according to DEFF for South Africa. The TPC should be between 3.5 and 5.0 log CFU/cm² (ISO 4833). Furthermore, *Enterobacteriaceae* should be between 1.5 and 2.5 (ISO 21528-2) and *E. coli* between 1.7 log CFU/cm² and 2.7 CFU/cm² (ISO 16649-1 or 2).

2.2.9 Sensory characteristics and consumer perception of meat

The sensory and/or eating quality of meat is determined by various factors. Firstly, the appearance (raw and cooked) and then the cooked attributes such as aroma, juiciness, flavour, taste and tenderness/texture (Neethling *et al.*, 2016a). Hoffman *et al.* (2007c) investigated the sensory characteristics of springbok as influenced by production region, age and sex. Although the latter attributes influenced the sensory characteristics of springbok in certain attributes, the physical attributes of meat overshadowed the sensory aspects; an increase in pH_u of meat, decreased the acceptance of tenderness and juiciness in the springbok meat.

Aroma can be classified as orthonasal and retronasal aroma (Neethling *et al.*, 2016a). Orthonasal aroma is known as the entering of odours through the external nares to the nasal cavity during sniffing. Retronasal aroma, however, is the odour sensation during food consumption when flavour molecules move from the mouth to nasal cavity (Roberts & Aeree, 1995). Therefore, flavour refers to a combination of retronasal aroma and taste (perceived on tongue) and aroma to orthonasal aroma (Neethling *et al.*, 2016a). The visual appearance of red meat is the most important factor determining the consumer's choice at point of purchase and more specifically the colour of meat (Moore *et al.*, 2003). Game meat is known for having a darker colour compared to red meat from domestic livestock (Ramanzin *et al.*, 2010). However, at the point of consumption other factors such as taste, mouthfeel and aromatic properties (determined by sensory receptors) influence the consumer's acceptability of the meat (Neethling *et al.*, 2016a).

Game meat is marketed as “organic”, free- ranging animals available as an alternative, healthier meat source (Erasmus & Hoffman, 2017; Wassenaar *et al.*, 2019). Game species are known to have very low intramuscular fat (IMF) with high protein and polyunsaturated fatty acids (PUFA) contents, hence their desirable polyunsaturated:saturated ratios (Hoffman & Wiklund, 2006). These properties allow game meat to be appealing to modern day health conscious consumers that believe a “low fat/high protein” diet is healthy. Game antelope meat is regarded as a unique product and each species has its own distinct flavour and “acquired taste” (Neethling *et al.*, 2016a). These provenance differences could be used as a marketing strategy to market the meat as species-specific and not generally as “game meat” (Hoffman & Wiklund, 2006; Hoffman *et al.*, 2009a).

Descriptive sensory analysis (DSA) is a sensory testing method distinguished from all other testing methods by profiling a product and evaluating at all perceived sensory attributes. The analysis is performed by a trained panel of judges (Murray *et al.*, 2001). A major strength of the DSA is the ability of the test to allow the determination of relationships between instrumental and DSA or consumer preference measurements by means of Pearson correlations. An increased “reproducibility” and greater consistency of sensory results could be expected if the sensory panel is trained for a longer period of time above the fact that they might have years of experience (Otremba *et al.*, 2000).

The sensory characteristics of impala and kudu meat was compared by the performance of a DSA. The two antelope occupied the same habitat; however, kudu are classified as browsers and impala as mixed feeders and therefore their dietary intake differed. The diet of ruminants is known to affect the fatty acid composition and sensory characteristics of meat. The impala had a more intense game aroma and flavour as compared to kudu and this could be ascribed to the higher fat content and differences in fatty acid composition between the species (Hoffman *et al.*, 2009a). Differences in fatty acid composition was also found in impala from two different regions (Hoffman *et al.*, 2005). Impala from one region had a more grass-based diet and in effect, the meat of these impala had an increased level of α -linolenic- and stearic fatty acids than the other group of impala.

Non-ruminant animals conserve polyunsaturated fatty acids (PUFA) in adipose tissue, whereas ruminants do so predominantly in muscle. The increase in meat fat content is associated with flavour increase. When ruminants are fattened, however, there is potentially an inverse relationship between eating quality and nutritional value. This could be explained by the low level of PUFA in ruminant neutral lipids (Wood *et al.*, 2008). As mentioned in section 2.2.7, ageing improves the sensory characteristics of meat, however it has been found that springbok steaks aged for too long a period resulted in undesirable sensory characteristics that can be ascribed to protein- and lipid oxidation (North & Hoffman, 2015b). The higher rating of sensory attributes such as liver, gamey,

metallic aroma and flavour, sour taste, mealiness, manure-like aroma, residue and liver or rubber-like texture have been associated with the dislike of meat from consumers (Wiklund *et al.*, 1996, Wiklund *et al.*, 2003; Rudman *et al.*, 2018). Sensory attributes scored higher ratings for juiciness, tenderness, sweet associated aroma and taste and beef-like flavour and aroma as they are all associated with positive consumer preferences (Oltra *et al.*, 2015).

Generally, South African game species are associated with a stronger flavour compared to meat from domestic animals such as pork, beef and lamb, however this is species dependant and could be influenced by various factors (Neethling *et al.*, 2016a). The sensory characteristics of blesbok meat was analysed by Hoffman *et al.* (2010d) and although the fat content of blesbok meat was very low (0.21% - 1.48%; Hoffman *et al.*, 2008), the sensory panel used the upper part of the sensory scale, thus indicating a tender and juicy product.

2.2.10 Conclusion

South African farmers are predicted to start moving away from farming with domestic species towards game due to the climate change and rise in temperature in which wild species are better adapted. The demand for red meat is increasing as the population increases and the need for an alternative meat source is more evident. A large amount of game species has been studied in terms of meat quality such as the springbok, impala and blesbok. The bontebok was previously endangered and currently classified as “vulnerable” and meat quality information regarding the former species is currently unknown. A number of bontebok are harvested annually as non-breeding males and the utilisation of the meat from these species in addition to meat from animals that are hunted for trophy hunting, is currently unknown. The meat quality attributes of species help to promote the correct utilisation of this meat, hence a greater economic turnover and motivation to increase the breeding and improve the conservation of bontebok in South Africa. There are several factors influencing the meat quality of game species of which muscle type is a large contributor.

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CHAPTER 3: THE CARCASS YIELDS AND PHYSIOCHEMICAL CHARACTERISTICS OF BONTEBOK (*DAMALISCUS PYGARGUS PYGARGUS*) MEAT

ABSTRACT

The study aimed to generate baseline data on the carcass yields, physical characteristics and chemical (proximate) composition of mature male bontebok ($n = 12$) meat from seven muscles: *Longissimus thoracis et lumborum* (LTL), *Semimembranosus* (SM), *Biceps femoris* (BF), *Semitendinosus* (ST), *Infraspinatus* (IS), *Supraspinatus* (SS) and the *Psoas major* (fillet) during March of 2018. In a second trial (April of 2018), the carcass yields, and physical characteristics were determined for mature male bontebok ($n = 8$) with the physical meat characteristics only quantified for three muscles (LTL, BF and SM). The dressing percentages of bontebok were 50.37% (± 1.55) and 50.68% (± 3.07) for March and April, respectively which compared well to the bontebok's closely related sub-species, the blesbok. Edible organs contributed substantially towards the internal offal yield to be used as edible by-products. All physical and chemical traits were influenced ($p \leq 0.05$) by muscles in the March trial and only ultimate pH (pH_u) and cooking loss percentage in the April trial ($p \leq 0.05$). The March trial had high pH_u values (5.84-6.21) with the forequarter muscles (IS, SS) and fillet having the highest pH_u and most tender meat. The ST muscle was the lightest muscle ($L^* = 35.90$) and the forequarter (IS, SS) and fillet muscles were most red ($a^* = 12.96-13.73$). Bontebok has a favourable chemical profile (g/100 g) with high protein (21.4-23.2) and low intramuscular fat (IMF) (0.7-1.1) content. The ST muscle had the highest moisture (76.94) and lowest protein (21.35) content, while the fillet had the highest IMF (1.1) content and ST the lowest (0.7) (g/100 g). In the April trial, the average pH_u (~ 5.60) was found to be within the normal range for meat, although it was significantly higher in the BF (5.71) than the LTL muscle (5.50). Additionally, the cooking loss percentage was significantly higher in the SM (38.8) than LTL (34.7) and BF (35.3) muscles. The bontebok shows potential to be utilised in the game meat industry and to be marketed as a lean, healthy red meat source.

Key words: Game meat, Bontebok, Carcass yield, Muscle profiling, Physical characteristics, Proximate composition

3.1 INTRODUCTION

A protein shortage in South Africa is becoming more challenging as the population size continues to increase. Domestic cattle farming, more specifically beef, is threatened by the change in climate and game farming is a possible solution to continue producing meat under harsher environmental

conditions (Otieno & Muchapondwa, 2016). Game farming refers to the management of wildlife on privately owned land for commercial purposes (Taylor *et al.*, 2016). The meat production potential of various wild species in South Africa has been recognised (Hoffman & Wiklund, 2006; Taylor *et al.*, 2016), especially that from popular game species such as blesbok (*Damaliscus pygargus phillipsi*), impala (*Aepyceros melampus*) and springbok (*Antidorcas marsupialis*) (Hoffman, 2007). However, other species such as the bontebok are less known for their meat production; mainly due to their scarcity and consequently their price, as these factors makes this species only affordable and more appealing to trophy hunters.

Bontebok are medium sized antelope that were previously endangered in South Africa and classified as “high value species” according to Taylor *et al.* (2016). The vulnerability of this species prevented excessive research in terms of the species meat quality. Meat quality, and more specifically carcass yields of game species help aid in future processing decisions and marketing strategies. Furthermore, these yields are important as game animals are increasingly being sold per kg carcass weight (Hoffman & Wiklund, 2006). Game offal is frequently left in the field for scavengers to consume, whereas many Africans consume offal as an affordable and nutritious meal (McCrindle *et al.*, 2013). The determination of carcass yields is important to predict the utilisation potential for game species and has not yet been established for bontebok.

When evaluating the physical meat quality characteristics of game meat, attributes typically tested for are ultimate pH (pH_u), moisture loss, tenderness and colour (Cawthorn *et al.*, 2018; Rudman *et al.*, 2018; Needham *et al.*, 2019). The physical characteristics of meat is perceived by consumers through visual and palatability attributes (Cawthorn *et al.*, 2018). One of the first visual quality parameters observed at the point of sale is colour which could indicate the myoglobin state (oxymyoglobin, metmyoglobin and deoxymyoglobin) present in meat (Cornforth & Jayasingh, 2004). Consumers often use colour as an indicator of freshness and acceptability (Neethling *et al.*, 2017) and game meat is generally perceived to be darker than domestic animals (Hoffman & Mcmillin, 2009; Shange *et al.*, 2019). The most important palatability attribute is tenderness; which contributes towards the consumers eating experience. The tenderness of meat can be influenced by factors such as harvesting conditions (ante-mortem stress), temperature, species, sex, muscle type (fibre types and collagen content) and degree of muscle fibre shortening post-mortem (Troy & Kerry, 2010; Bhat *et al.*, 2018).

The pH_u post-mortem is typically taken post rigor development (>24 h post-mortem) and is linked to the water-holding capacity (WHC), tenderness and colour of the meat. A high pH_u (>6 or >6.2) is known to produce DFD (dark, firm, dry) meat (Adzitey, 2011; Adzitey & Nurul, 2011; Shange *et al.*, 2018). Animals that are stressed during the harvesting process, are known to have DFD meat

(Shange *et al.*, 2018), a phenomenon that leads to the ante-mortem depletion of glycogen reserves in muscles, decreasing lactic acid production post-mortem and thus resulting in meat with a higher pH_u . This high pH_u leads to meat with a reduced meat quality, as meat with higher pH_u values are known to have an increased microbial count over time, reduced shelf life, much darker colour and undesired sensory attributes (Renecker *et al.*, 2005; Hoffman *et al.*, 2007a; Shange *et al.*, 2018).

The proximate composition (g/100 g) of meat consists of moisture, protein, intramuscular fat (IMF) and ash content (Hoffman, 2000; Hoffman *et al.*, 2007b). Male wild ungulate species are known to have lower IMF contents than females, especially in the mating season where IMF is lost due to physical activity such as rutting and competing for females (Van Zyl & Ferreira, 2004; Neethling *et al.*, 2018). Furthermore, the weather conditions and seasons (particularly a drought) could also contribute towards changes in the IMF content of game meat due to either a lack or an excess amount of nutrients (Rudman *et al.*, 2018).

The meat quality of bontebok meat has not been established. Therefore, the aim of this study was to determine baseline data for bontebok meat in terms of carcass yields, physical characteristics and proximate composition. This would enable bontebok meat to be utilised optimally, marketed correctly and create a platform for scientist to further investigate factors affecting the meat quality of this species.

3.2 MATERIALS AND METHODS

3.2.1 Animals and study location

This trial was approved by the Stellenbosch University's animal ethics committee (10NP_HOF02) and, as bontebok are CITES Appendix 2, the required permits were also obtained (CN4-30-3265). Bontebok were harvested according to the guidelines supplied by Van Schalkwyk and Hoffman (2016) in the Western Cape Province of South Africa. The harvesting procedures took place at Elandsberg farm, Wellington (33°28'49.2"S 19°03'31.1"E). Animals were found in a 4000 ha camp and shot during the day (late morning to late afternoon) with environmental temperatures of 30-36°C. The study area's location is in the Coastal Renosterveld of the Fynbos biome although the veld type has been classified as the Swartland Shale Renosterveld and Swartland Alluvium Fynbos. This area is known for its winter rainfall (250-600 mm) and hot dry summers. Renosterveld is known to have hard, shrubland vegetation adapted to survive hot summers (Walton, 2006).

In March of 2018, twelve mature male bontebok were randomly selected from the heard and harvested. An additional eight male bontebok were harvested during April of 2018, following the same procedure and location as described above.

3.2.2 Harvesting and dressing procedures

Bontebok from both groups (March and April) were shot in the head using a .308 calibre rifle by a professional marksman. A head shot is more advantageous due to a body shot negatively influencing the meat quality of certain muscles affected by the bullet (yield loss). The hunter shot from the back of a vehicle with the intention of minimising ante-mortem stress. The animals were exsanguinated by slitting the throat with a sterile knife and allowing the carcass to bleed within the first 3-6 min after the shot, where after the carcasses were hung from both Achilles tendons to promote bleeding. The time of the shot and remarks with regard to ante-mortem animal behaviour were noted. The carcasses were transported to the slaughter facilities, where further skinning and evisceration took place. The undressed (dead) carcass weight, warm carcass weight and individual internal and external organs (excess fat removed) were weighed. Carcasses were transported in a cooling truck to the meat processing laboratory at the Department of Animal Sciences, Stellenbosch University. Temperature probes were inserted into each carcass to monitor the post-mortem temperature decrease over time. The carcasses were stored and suspended from both Achilles tendons in a refrigerator at 4°C for 24 h to allow rigor mortis to develop uniformly on both sides of the carcass (left and right).

3.2.3 Sample preparation

After the 24 h cooling period, cold carcass weights were recorded. Temperature regulating probes were kept in the refrigerator to regulate the temperature. Thereafter, further meat processing took place to prepare samples for analysis to follow. Seven muscles from the left and right side of each carcass were removed and weighed. These muscles included the hind limb muscles: *Semitendinosus* (ST); *Biceps femoris* (BF); *Semimembranosus* (SM); shoulder muscles: *Supraspinatus* (SS); *Infraspinatus* (IS) and the *Longissimus thoracis et lumborum* (LTL) and *Psoas major* (fillet). The LTL muscle was removed from the cervical vertebra to the last lumbar vertebra. The epimysium layers were removed from the muscles before weighing and further processing.

3.2.4 Physical and chemical analysis

The physical analysis procedures were the same for both March and April bontebok samples. The only difference between the two trials was the muscles used from the twelve male bontebok of the March trial focused on all seven of the above-mentioned muscles, whereas the eight male bontebok from the April trial only focused on three muscles (LTL, SM and BF). The chemical analysis was performed on all seven muscles from the twelve bontebok harvested in March. For the physical analysis, a steak (± 1.5 cm thick) from the left middle-posterior (bottom) side of each muscle was removed by cutting perpendicular to the longitudinal axis of the selected muscle. For chemical

analysis, ± 200 g meat was taken from the right posterior (bottom) side of each muscle, vacuum sealed into plastic bags and frozen at -20°C until further analysis.

3.2.4.1 Acidity (pH)

The March trial used a Crison pH₂₅ meter and the April trial an edge[®] pH meter (Hanna instruments Inc., USA) to measure the pH ± 24 h post-mortem (pH_u). The measurements were taken from the cranial end of the left muscles. The pH meters were calibrated with pH standards of pH 4.0 and pH 7.0. Distilled water was used to clean the pH meter between measurements.

3.2.4.2 Surface colour

Steaks (± 2 cm) were left to bloom for ~ 30 min where after five colour readings were taken from different areas on the sample surface (in contact with atmospheric air) using a Colour-guide $45^{\circ}/0^{\circ}$ colorimeter (BYK-Gardner GmbH, Gerestried, Germany). These measurements were according to the CIELab colour system that measures L* (lightness), a* (green-red values) and b* (blue-yellow values) ordinates. The chroma and hue-angle values representing saturation/colour intensity and colour definition, respectively were calculated (Meat color measurement guidelines, 2012):

$$\text{Hue-angle } (^{\circ}) = \tan^{-1} \left(\frac{b^*}{a^*} \right)$$

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

3.2.4.3 Moisture loss

Drip- and cooking losses were determined as an indication of the water holding capacity of the meat.

For the drip loss, a steak was suspended in an inflated polyethylene bag without touching the sides of the bag for 24 h under refrigerated (4°C) conditions. The initial and final weights of the steak were recorded and the moisture loss expressed as a percentage of the initial weight of the sample (Honikel, 1998).

Cooking loss percentage was calculated by calculating the moisture loss during cooking as a percentage of the initial weight of the steak (± 2 cm) (Honikel, 1998). The initial weight was recorded before suspending the piece of steak in a sealed plastic bag, cooking for 1 h at 80°C (in a water bath), where after excess water was removed, and the steak was allowed to cool for 24 h at 4°C . After cooling, excess moisture was blotted dry by using absorbent paper and final weights were recorded.

3.2.4.4 Warner-Bratzler shear force (WBSF)

The steak obtained from the cooking-loss procedure was used to determine the tenderness of the meat by measuring the shear force (Newton) with a 3345 Instron Universal Testing Machine (Apollo Scientific cc, Alberta, Canada) fitted with a Warner-Bratzler blade. The Instron ran at a crosshead

speed of 200 mm/min with a load cell of 5 kN. The Warner-Bratzler blade was triangular (equilateral) with a thickness of 1.2 mm. Each muscle sample (1 cm X 1 cm X 2 cm) was sheared perpendicular to the longitudinal axis of the sample's muscle fibres. Six measurements per steak was used to determine the average WBSF (Honikel, 1998).

3.2.4.5 Chemical analyses

Samples frozen for chemical analysis were thawed overnight (± 18 h) at 4°C. The samples were then homogenized (standard time for each sample ± 15 s). The excess moisture from the thawed muscles was added back to the samples to ensure accurate moisture and chemical determinations. The proximate composition was analysed in duplicate on each sample and an average calculated; when the two readings differed by more than 5%, the analysis was repeated for those specific samples.

The AOAC official method 934.01 (AOAC International, 2002) was used to determine the moisture content (g/100 g) of each meat sample.

The crude protein was determined by using the LECO instrument and combustion method ("Dumas method") as described by the official method 992.15 (AOAC International, 2002). The samples were analysed in the Leco Nitrogen/Protein Analyser (Leco Fp-528, Leco Corporation) to determine the percentage nitrogen (%N) of each sample. The crude protein (g/100 g) was then determined by multiplying the %N of each sample by a conversion factor of 6.25.

The total lipid content (g/100 g) was determined and will be referred to as the total IMF in this study. The analyses used 5 g of meat to perform a simple extraction with a solvent (1:2 (v/v) mixture of chloroform/methanol) as described by Lee *et al.* (1996).

The ash content was determined by using the moisture free sample after determining moisture loss. This sample was placed into a furnace set at 500°C for a period of 6 h to determine the ash percentage according to the AOAC method 942.05 (AOAC International, 2002).

3.2.5 Statistical analysis

The experimental design was completely random factorial with twelve animals hunted in March and eight in April. Two bontebok were removed as outliers from the April dataset, due to the meat from these animals being classified as DFD with pH_u values >6 ; these will be discussed separately where applicable.

Descriptive statistics were used to analyse the carcass composition of bontebok meat. Values were reported as means and standard errors where March and April data were not compared. The physical meat quality data (pH_u , drip loss, cooking loss, WBSF and colour) and proximate composition (moisture, protein, IMF and ash) was analysed by performing a mixed model univariate analysis of variance (ANOVA) using SAS™ software (Statistical Analysis System, Version

9.4, SAS Institute Inc., Cary, NC, USA), with animals as random effect and muscle type as fixed effect. The data was tested for normality using the Shapiro-Wilk test and the statistical significance was calculated using a Fisher's t-test at a 5% level of significance to compare means. The values are reported as the LSMeans \pm the standard error of the mean (SEM).

3.3 RESULTS

Bontebok were harvested in two separate trials, March (n = 12) and April (n = 8). The carcass composition and physical characteristics were tested for bontebok from both March and April trials on all seven muscles, except April's physical analysis that was done on only three muscles (LTL, SM and BF). The proximate composition of March bontebok were also analysed.

3.3.1 Carcass composition

Although the respective undressed (dead) carcass weights ('live weights'; kg) of the April group were heavier than that of the March, the weights did not differ significantly (Table 3.1). As a result of their heavier undressed weights, the April group also had heavier carcass weights (warm) (kg), but similar dressing percentages (~50%). The external offal (head, skin, horns and genitals) percentages were higher in March (14.4 ± 0.88) and April (15.1 ± 0.77) than internal offal percentages (organs, stomach and intestines) in March (34.4 ± 0.96) and April (31.7 ± 3.49). The head was the largest contributor towards the external offal for March (7.3 ± 0.69) and April (7.3 ± 0.39), whereas the gastrointestinal tract (GIT) made out the largest part for the internal offal for both groups of bontebok (March 30.7 ± 1.04 ; April 27.6 ± 3.46 ; Table 3.1).

The cold carcass weights (kg), carcass weight loss percentages (%) during chilling and muscle weights (kg) as percentages of the cold carcass weights are indicated in Table 3.2. The mean cold carcass weights (kg) and moisture loss (%) for March and April were $30.52 \text{ kg} \pm 5.58$; $3.5 \pm 0.64\%$ and $35.39 \text{ kg} \pm 3.25$; $1.4 \pm 1.10\%$, respectively. The muscle with the largest percentage of the cold carcass weight, was the LTL muscle in both March ($3.1 \pm 0.05\%$) and April ($2.8 \pm 0.22\%$), whereas the fillet was the smallest muscle, contributing least towards the cold carcass weight, in both March ($0.4 \pm 0.03\%$) and April ($0.4 \pm 0.04\%$).

3.3.2 Physical characteristics and chemical composition

All physical and chemical parameters differed significantly between muscles for the March bontebok, whereas the only physical parameters that differed significantly for April bontebok was pH_u and cooking loss percentage (Table 3.3; Table 3.4, respectively).

The pH_u values of the March trial ranged between 5.84 and 6.21 and were much higher than found for April bontebok (5.50-5.71). The forequarter muscles (IS and SS) and fillet muscle had

significantly higher pH_u values (>6.10) and lower WBSF values than the other muscles (BF, SM, ST and LTL) (Table 3.3). The April trial indicated that between the LTL, BF and SM muscles, the BF had the highest pH_u (5.71) and LTL the lowest (5.50), with the SM (5.60) only differing numerically from the BF and LTL. Furthermore, the latter trial found the SM to have a significantly higher cooking loss (38.5%) than the LTL (34.7%) and BF (35.3%) (Table 3.4). In bontebok from the March trial a significantly lower cooking loss was found in the IS muscle (28.9%) and significantly higher values in the hindquarter: BF (35.6%); SM (35.8%); ST (36.2%) and forequarter SS (35.1%) muscles. The March bontebok further indicated a significantly higher drip loss percentage in the LTL (0.9%) and fillet (0.9%) muscles compared to the BF, SM, IS and SS muscles ($\sim 0.7\%$), however, only numerically differing from the ST (0.8%) muscle (Table 3.3).

The ST muscle had the highest L^* value (35.90) and was thus the lightest muscle ($p < 0.05$). The highest a^* values (most red muscles) were found to be the forequarter: IS (12.96); SS (13.73) and fillet (13.38) muscles. The ST, SS and fillet muscles had higher b^* values (10.89, 10.24 and 10.48, respectively) than the other muscles with b^* values ranging between 8.53 and 9.40. The highest chroma values were found in the LTL (14.29), BF (14.37) and SM (14.98) muscles whilst the ST muscle was found to have the highest hue-angle value (42.41°) ($p \leq 0.05$).

The two hindquarter (BF, SM) muscles and the LTL muscle had significantly lower moisture (~ 75.5 g/100 g meat) and higher protein contents (~ 23.0 g/100 g meat) (Table 3.3) than the other muscles. The IMF content of bontebok was generally low with an average of 0.8 g/100 g. The fillet muscle had the highest IMF content (1.1 g/100 g meat). The ash content was significantly lower in the forequarter muscles: IS (1.1 g/100 g meat) and SS (1.16 g/100 g meat) (Table 3.3).

3.3.3 Outliers removed

Two male bontebok (x and y) were removed from the April data set as “outliers” due to extremely high pH values >6 (6.88; 6.10 respectively) compared to the average pH_u of 5.60 for other bontebok in the April trial. The extremely high pH_u of bontebok x (6.88) resulted in an abnormally low WBSF value (20.88 N) compared to bontebok y (83.27 N) (Table 3.5). The cooking loss for bontebok x (24.6%) and y (30.6%) was numerically lower than for the April trial animals with a mean cooking loss of 33.5% (Table 3.4). The colour values of bontebok were numerically similar, except for bontebok x having a much lower L^* (20.96) value than bontebok y (24.69) and the bontebok from the April trial (32.75).

Table 3.1 Means (\pm standard error) of the carcass weights (warm) (kg) and dressing percentages (%) of mature male bontebok during two separate trials: March (n=12); April (n=8)

Carcass yields	Unit	March (n=12)	April (n=8)
Undressed carcass weight	kg	62.69 \pm 10.83	70.44 \pm 4.32
Carcass weight	kg	31.63 \pm 5.79	35.74 \pm 3.61
¹ Dressing percentage	%	50.4 \pm 1.55	50.7 \pm 3.07
External offal (% of undressed carcass weight)			
Head	kg	4.55 \pm 0.74	5.16 \pm 0.43
	%	7.3 \pm 0.69	7.3 \pm 0.39
Legs	kg	1.34 \pm 0.14	1.54 \pm 0.07
	%	2.2 \pm 0.20	2.2 \pm 0.11
Skin	kg	3.1 \pm 0.95	4.0 \pm 0.44
	%	4.9 \pm 0.78	5.6 \pm 0.46
Total external offal	kg	9.03 \pm 1.74	10.63 \pm 0.87
	%	14.4 \pm 0.88	15.1 \pm 0.77
Internal offal (% of undressed carcass weight)			
² GIT	kg	19.19 \pm 3.12	19.34 \pm 2.13
	%	30.7 \pm 1.04	27.6 \pm 3.46
Heart	kg	0.50 \pm 0.10	0.61 \pm 0.08
	%	0.8 \pm 0.10	0.9 \pm 0.08
Lungs	kg	0.85 \pm 0.17	1.03 \pm 0.16
	%	1.4 \pm 0.13	1.5 \pm 0.21
Liver	kg	0.67 \pm 0.12	0.78 \pm 0.08
	%	1.1 \pm 0.11	1.1 \pm 0.13
Kidneys	kg	0.13 \pm 0.02	0.15 \pm 0.02
	%	0.2 \pm 0.01	0.2 \pm 0.02
Spleen	kg	0.10 \pm 0.02	0.13 \pm 0.02
	%	0.7 \pm 0.01	0.2 \pm 0.03
Testicles (without skin)	kg	0.09 \pm 0.07	0.16 \pm 0.02
	%	0.1 \pm 0.08	0.2 \pm 0.04
Penis	kg	0.03 \pm 0.02	0.04 \pm 0.01
	%	0.0 \pm 0.02	0.1 \pm 0.02
Total internal offal	kg	21.56 \pm 3.55	22.24 \pm 2.15
	%	34.4 \pm 0.96	31.7 \pm 3.49

¹Dressing percentage= Calculated from the undressed carcass weight (warm carcass) ²GIT= Gastrointestinal tract

Table 3.2 Means (\pm standard error) of the cold carcass and muscle weights (kg) and percentages of mature male bontebok during two separate trials: March (n=12); April (n=8)

Carcass weight	Unit	March (n=12)	April (n=8)
Cold carcass weight	kg	30.52 \pm 5.58	35.39 \pm 3.25
Carcass weight loss % (24 h PM ¹)	%	3.5 \pm 0.64	1.4 \pm 1.10
Muscle weights (% of cold carcass weight)			
LTL	kg	0.94 \pm 0.17	1.00 \pm 0.15
	%	3.1 \pm 0.05	2.8 \pm 0.22
SM	kg	0.82 \pm 0.13	0.783 \pm 0.11
	%	2.7 \pm 0.13	2.2 \pm 0.15
BF	kg	0.64 \pm 0.13	0.623 \pm 0.09
	%	2.1 \pm 0.12	1.8 \pm 0.13
ST	kg	0.22 \pm 0.02	0.231 \pm 0.02
	%	0.7 \pm 0.05	0.7 \pm 0.04
IS	kg	0.26 \pm 0.02	0.262 \pm 0.06
	%	0.9 \pm 0.07	0.7 \pm 0.16
SS	kg	0.23 \pm 0.04	0.245 \pm 0.04
	%	0.8 \pm 0.04	0.7 \pm 0.07
Fillet	kg	0.14 \pm 0.03	0.155 \pm 0.02
	%	0.4 \pm 0.03	0.4 \pm 0.04

Abbreviations: LTL= *M. longissimus thoracis et lumborum*, SM= *M. semimembranosus*, BF= *M. biceps femoris*, ST= *M. semitendinosus*, IS= *M. infraspinatus*, SS= *M. supraspinatus*, Fillet= *Psoas Major*
¹24 h post-mortem

Table 3.3 The LS Mean (\pm standard error) for the physical characteristics (pH_u, moisture loss, shear force, colour) and proximate composition (moisture, protein, IMF, ash) of seven muscles from bontebok (n=12) harvested during March of 2018.

Parameter	Muscle type							p-value
	LTL	SM	BF	ST	IS	SS	Fillet	
pH _u	5.89 ^c \pm 0.19	5.89 ^c \pm 0.16	5.84 ^c \pm 0.18	5.87 ^c \pm 0.14	6.21 ^a \pm 0.32	6.15 ^a \pm 0.30	6.03 ^b \pm 0.31	<0.001
Drip loss (%)	0.9 ^{ab} \pm 0.33	0.7 ^c \pm 0.12	0.7 ^c \pm 0.16	0.8 ^{bc} \pm 0.19	0.7 ^c \pm 0.13	0.7 ^c \pm 0.13	0.9 ^a \pm 0.32	0.0031
Cooking loss (%)	31.9 ^b \pm 3.52	35.8 ^a \pm 3.02	35.6 ^a \pm 2.51	36.2 ^a \pm 2.69	28.9 ^c \pm 2.57	35.1 ^a \pm 3.25	30.8 ^b \pm 2.12	<0.001
WBSF (N)	74.68 ^b \pm 15.74	76.22 ^b \pm 12.68	86.05 ^a \pm 16.57	53.73 ^c \pm 13.90	40.12 ^d \pm 13.33	43.87 ^d \pm 11.23	31.30 ^e \pm 11.33	<0.001
<i>Colour:</i>								
L*	31.82 ^{cd} \pm 1.34	32.13 ^{bcd} \pm 1.66	32.70 ^{bc} \pm 1.85	35.90 ^a \pm 2.18	31.46 ^d \pm 2.12	32.33 ^{bcd} \pm 2.05	32.94 ^b \pm 1.66	<0.001
a*	11.46 ^c \pm 1.72	11.76 ^c \pm 1.69	11.28 ^c \pm 1.44	11.98 ^c \pm 1.46	12.96 ^b \pm 1.31	13.73 ^a \pm 1.14	13.38 ^{ab} \pm 0.84	<0.001
b*	8.53 ^c \pm 1.07	9.27 ^b \pm 1.24	8.89 ^{bc} \pm 1.24	10.89 ^a \pm 0.85	9.40 ^b \pm 1.36	10.24 ^a \pm 1.12	10.48 ^a \pm 0.78	<0.001
Chroma	14.29 ^d \pm 1.99	14.98 ^d \pm 2.04	14.37 ^d \pm 1.82	16.21 ^{bc} \pm 1.44	16.02 ^c \pm 1.75	17.14 ^a \pm 1.44	17.01 ^{ab} \pm 0.95	<0.001
Hue-angle	36.74 ^c \pm 1.53	38.29 ^b \pm 1.96	38.20 ^b \pm 2.35	42.41 ^a \pm 3.21	35.82 ^c \pm 2.62	36.69 ^c \pm 2.33	38.07 ^b \pm 2.16	<0.001
<i>Proximate composition (g/100 g meat):</i>								
Moisture	75.4 ^c \pm 0.79	75.5 ^c \pm 0.74	75.5 ^c \pm 0.50	76.3 ^b \pm 0.81	76.3 ^b \pm 0.76	76.9 ^a \pm 0.77	76.7 ^{ab} \pm 0.70	<0.001
Protein	23.2 ^a \pm 1.10	22.8 ^a \pm 1.04	23.0 ^a \pm 0.65	22.2 ^b \pm 0.95	22.1 ^{bc} \pm 0.97	21.4 ^d \pm 1.09	21.5 ^{cd} \pm 1.00	<0.001
IMF	0.8 ^{bc} \pm 0.70	0.8 ^{bc} \pm 0.72	0.8 ^{bc} \pm 0.83	0.7 ^c \pm 0.66	0.9 ^{bc} \pm 0.86	0.9 ^{ab} \pm 0.89	1.1 ^a \pm 0.97	0.0219
Ash	1.3 ^a \pm 0.09	1.3 ^a \pm 0.10	1.3 ^a \pm 0.11	1.3 ^a \pm 0.07	1.1 ^b \pm 0.08	1.2 ^b \pm 0.01	1.2 ^a \pm 0.06	<0.001

Abbreviations: LTL= *M. longissimus thoracis et lumborum*, SM= *M. semimembranosus*, BF= *M. biceps femoris*, ST= *M. semitendinosus*, IS= *M. infraspinatus*, SS= *M. supraspinatus*, WBSF= Warner-Bratzler shear force, IMF= Intramuscular fat. ^{a-e} Row means with different superscripts differ significantly at $p \leq 0.05$.

Table 3.4 The LSMean (\pm standard error) for the physical characteristics (pH_u , cooking loss, shear force, colour) of three muscles from bontebok harvested during April of 2018. The parameters in bold differ significantly ($p \leq 0.05$)

Parameter	Muscle type			p-value
	LTL	SM	BF	
pH_u	5.50 ^b \pm 0.06	5.60 ^{ab} \pm 0.25	5.71 ^a \pm 0.25	0.0458
Cooking loss %	34.7 ^b \pm 2.87	38.8 ^a \pm 1.09	35.3 ^b \pm 1.48	0.0078
WBSF (N)	108.67 \pm 20.03	103.44 \pm 20.80	103.76 \pm 23.67	0.7650
<i>Colour:</i>				
L*	23.97 \pm 2.64	24.60 \pm 2.91	26.80 \pm 1.64	0.1624
a*	14.93 \pm 1.35	13.49 \pm 2.50	14.16 \pm 2.53	0.4216
b*	8.52 \pm 0.77	8.10 \pm 2.03	8.61 \pm 2.23	0.8310
Chroma	17.19 \pm 1.52	15.77 \pm 3.05	16.59 \pm 3.27	0.5555
Hue-angle	29.71 \pm 1.13	30.79 \pm 3.72	30.92 \pm 3.07	0.7291

Abbreviations: LTL= *M. longissimus thoracis et lumborum*, SM= *M. semimembranosus*, BF= *M. biceps femoris*, ST= *M. semitendinosus*, IS= *M. infraspinatus*, SS= *M. supraspinatus*, WBSF= Warner-Bratzler shear force, IMF= Intramuscular fat.

^{a-e} Row means with different superscripts differ significantly at $p \leq 0.05$.

Table 3.5 The average physical characteristic data of three muscles (LTL, SM, BF) from two bontebok (bontebok x; bontebok y) with $pH_u \geq 6$

Parameter	Bontebok x	Bontebok y
pH_u	6.88	6.10
Cooking loss %	24.6	30.6
WBSF (N)	20.88	83.27
<i>Colour:</i>		
L*	20.96	24.69
a*	13.60	13.63
b*	7.18	7.47
Chroma	15.40	15.55
Hue-angle	27.52	28.35

Abbreviations: LTL= *M. longissimus thoracis et lumborum*, SM= *M. semimembranosus*, BF= *M. biceps femoris*

WBSF= Warner-Bratzler shear force

3.4 DISCUSSION

With no information currently available in literature on the meat production potential of bontebok species, the overarching aim of this study was to generate baseline data on the carcass yields, meat production potential, physical characteristics and proximate composition of bontebok meat. The March trial (Table 3.3) indicated that all physical and proximate meat quality attributes were

affected by muscle type. However, the pH_u and cooking loss percentages were the only physical attributes that were significantly affected by muscle type in the April trial (Table 3.4). The latter trial analysed only three muscles ($n = 8$), whereas the March trial analysed seven muscles ($n = 12$). Due to this lack of data, where appropriate, the results were compared to that of a close relative, the blesbok on which a number of studies have been conducted.

3.4.1 Carcass composition

The carcass composition, carcass- and offal weights and dressing percentages contribute towards the meat production potential of species (Huntley, 1971; Needham *et al.*, 2019) as game is sold per animal or per kg (Hoffman & Wiklund, 2006).

The mean undressed (dead) carcass weights of both March (62.7 kg) and April (70.4 kg) bontebok compared well to its closely related sub-species, the blesbok with an average undressed carcass weight of 67.4 kg in a study including a large group of animals (both male and females; $n=65$) (Hoffman *et al.*, 2008). Similarly, the dressing percentages of these blesbok (52.1%) compared well to bontebok in the current trial (~50%) and to eland (50.8%) (Needham *et al.*, 2019). However, bontebok dressing percentages were much lower than found for male kudu (58.3%) and impala (60.9%) (Hoffman *et al.*, 2009a). The above-mentioned dressing percentages could be affected by the type of carcass weight (whether warm or cold carcass weights were used) and were not specified by Hoffman *et al.* (2009) and Hoffman *et al.* (2008). A higher dressing percentage is advantageous due to the higher meat yield obtained and is important when considering the meat production potential of a species type. The current study harvested bontebok during March/April of 2018 in which South Africa had undergone a severe drought. The latter could have influenced the bontebok carcass weights and dressing percentages due to the high environmental temperatures and a general lack of nutrition associated with a drought (Masante *et al.*, 2018). During 2018, Rudman *et al.* (2018) harvested Warthog that displayed lower dressing percentages than other warthog in literature and this was attributed to the drought South Africa had experienced during that time. Researchers have suggested that wild ungulates have similar or slightly higher dressing percentages than domestic livestock, as seen when comparing bontebok (50.5%) to South African sheep such as Merino, South African mutton Merino and Dorper lambs with dressing percentages of 42.5%, 45.5% and 47.9%, respectively (Brand *et al.*, 2018). In the case of sheep, the skin (including wool) could be the major contributor towards the higher live weight and lower dress-out percentages (Cloete *et al.*, 2004). Furthermore, bontebok dressing percentages were slightly lower than South African cattle: Nguni, Bonsmara and Aberdeen Angus with dressing percentages of 52.1%, 56.9% and 53.7%, respectively (Muchenje *et al.*, 2008).

The hunting of game species leaves a considerable amount of offal which can be utilised; however, it is mostly left in the field for scavengers to consume. The diet of many Africans include the consumption of offal which is seen as being high in protein, and an easily accessible and affordable source of food (McCrinkle *et al.*, 2013). The edible organs of bontebok makes out a substantial amount of the internal offal percentage (Table 3.1) and therefore should be utilised to its full potential as an edible by-product if harvesting/hunting conditions are favourable for their utilization.

The largest muscle was the LTL and the smallest muscle was the fillet, along with the forequarter muscles (IS, SS) and the ST (Table 3.2). The LTL muscle is stretched along the length of the vertebrae and aids in maintaining balance and stability during movements. The former muscle is known to be one of the most valuable muscles in the livestock (beef) industry due to its leanness, tenderness and limited amount of connective tissue and collagen (Frandsen *et al.*, 2009). This muscle is popular in the production of biltong by hunters due to its elongated shape, however, nowadays the hindquarter muscles (BF, SM and ST) are amongst the more popular biltong muscles used commercially (Jones *et al.*, 2017). The forequarter muscles (IS, SS) were three to four times smaller than the largest muscle (LTL) and are smaller due to the different function these muscles have. The IS muscle acts as a shoulder ligament to stabilise, flex and move the shoulder, whereas the SS muscle assists in extending the shoulder and acts as a ligament in the shoulder joint (Frandsen *et al.*, 2009).

3.4.2 pH_u, moisture loss and tenderness

The pH of meat 24 h post-mortem should lie between 5.3 and 5.8 (Honikel, 2004) as found in various game species: blesbok (Hoffman *et al.*, 2010); impala; and kudu (Hoffman *et al.*, 2009b). The results obtained from the April trial had bontebok with pH_u values that fell within the former range (5.5-5.71) (Table 3.4), however, the average pH_u of the bontebok harvested in March was found to be 5.98. Four of the 12 bontebok had individual average pH_u values >6 and this is due to these animals running slowly for ± 20 min before harvesting occurred. Bontebok are known to run for long periods at a slow gallop (David, 1970). This presumptively caused a degree of ante-mortem stress, which in return increased the muscles' pH_u values. When removing these animals from the dataset, the average pH_u decreased to 5.86. The animals were harvested in March of 2018 when South Africa was in the midst of a severe drought (Masante *et al.*, 2018), which could have caused food scarcity and thus a lack of glycogen levels in meat ante-mortem causing a higher ultimate pH post-mortem. Also, bontebok have strict mating seasons (January to March). Male antelope are known to be more active during these rutting seasons where they fight and compete for females (Myserud *et al.*, 2004). Challenge rituals between bontebok males involve soil horning in the combat position on

their knees facing each other (David, 1970), thus putting strain on the forequarter muscles (IS, SS). These behavioural patterns would contribute further towards the depletion of the muscles' glycogen stores, thus increasing their pH_u and possibly explaining the higher pH_u values in March bontebok as compared to April bontebok (rutting season ends in March). The environmental temperatures were high on the day of harvest (30-36°C) which may also have contributed to a higher pH_u due to heat stress. Animals with a $pH_u >6$ (Adzitey, 2011) and in some cases >6.2 (Viganò *et al.*, 2019) are classified as DFD meat. Game species are often inclined to ante-mortem stress prior to harvesting and this results in higher ultimate pH values and meat with a decreased meat quality (Shange *et al.*, 2019).

Skeletal muscles have various sizes, shapes, anatomical locations and functions, which influences their meat quality due to differences in muscle fibre types between muscles (Kohn *et al.*, 2005, 2007; Cawthorn *et al.*, 2018; Needham *et al.*, 2019). The pH_u of March bontebok differed significantly amongst muscles with the hindquarter (BF and SM), LTL and ST muscles having a significantly lower pH_u than the forequarter muscles (IS, SS). Similar differences were found for muscles in wild fallow deer (*Dama dama*) (Cawthorn *et al.*, 2018). Eland (Needham *et al.*, 2019) muscles also displayed the highest pH_u values in the IS and SS muscles. The higher pH_u in the forequarter muscles indicate a lower glycogen level compared to the other muscles ante-mortem. According to Adzitey and Nurul (2011), redder muscles around the shoulder and neck area (such as IS and SS) with a higher pH_u (even up to 6.3) could be considered normal. This is due to the proportion of oxidative, red muscle fibres with low ante-mortem glycogen levels resulting in low amounts of lactic acid accumulating under anaerobic post-mortem conditions.

The muscle drip loss percentage describes the loss of water from inter-filament spaces and from the myofibrils themselves (Cheng & Sun, 2008; Cawthorn *et al.*, 2018). The March trial drip loss percentages were significantly higher in the fillet and LTL muscles (Table 3.3). Contrasting results have been noted, e.g. where the drip loss percentages were highest in the ST muscle in wild fallow deer (Cawthorn *et al.*, 2018). This study and most other studies regarding game species do not include the fillet muscle. The cooking loss percentage (%) of meat refers to the loss of soluble matter and water (Cheng & Sun, 2008). The IS displayed the lowest cooking loss in the March trial (Table 3.3). Similarly, the eland (Needham *et al.*, 2019) and wild fallow deer (Cawthorn *et al.*, 2018) IS muscles also had the lowest cooking loss. The three muscles analysed in the April trial also differed significantly between one another with the cooking losses being significantly higher in the SM muscle compared to the BF and LTL muscles (Table 3.4). The average bontebok cooking loss percentage was 36.3% which is numerically higher than the cooking loss of bontebok from the March trial which had an average cooking loss of 33.5%. This could be explained by the higher

average pH_u in the March trial which led to a higher WHC and thus lower cooking loss percentages (Lawrie & Ledward, 2007).

The pH of meat is a key factor linked to the WHC and is known to influence it (Bendall & Swatland, 1988; Cheng and Sun, 2008). The bontebok IS, SS and fillet muscles can possibly be classified as DFD/intermediate DFD (Adzitey, 2011; Viganò *et al.*, 2019) meat muscles due to their ultimate $pH > 6$. Higher pH_u values in DFD classified meat increases the WHC which decreases the drip- and cooking loss percentages by reducing intramuscular water shifts (Lawrie & Ledward, 2007). This is evident in the bontebok where the IS muscle had the highest pH_u and had one of the lowest drip- and cooking loss percentages (Table 3.3). When eland muscles were compared, Needham *et al.* (2019) found the IS muscle to have the highest pH_{36} (5.9) and was found to be darker, most tender and the muscle with the lowest cooking loss percentage. The average bontebok drip- and cooking loss percentages were 0.8% and 33.5%, respectively. The bontebok drip loss percentage is less than reported for wild ungulates in literature: blesbok was found to be 4.6% (Hoffman *et al.*, 2010); impala 1.2%; kudu 1.4% (Hoffman *et al.*, 2009a); springbok 2.8% (Hoffman *et al.*, 2007a) and wild fallow deer 1.4% (Cawthorn *et al.*, 2018). This could be explained by the generally higher average ultimate pH_u (5.98) of bontebok, which is borderline DFD classified meat (Viganò *et al.*, 2019). At pH_u values above the iso-electric point (5.4-5.5), proteins have a negative charge which increase their ability to hold water and therefore result in a lower drip loss percentage (Huff-Lonergan & Lonergan, 2005; Lawrie & Ledward, 2007). The cooking loss, however, compared well to certain wild ungulates in literature: blesbok was found to have 35% (Hoffman *et al.*, 2010), impala 31%, kudu 31.5% (Hoffman *et al.*, 2009a), springbok 31.5% (Hoffman *et al.*, 2007a) and wild fallow deer 33.1% (Cawthorn *et al.*, 2018) cooking loss when determined under similar experimental conditions. The drip and cooking loss help indicate the WHC of meat and is described as the ability of meat to retain added and inherent water. It is important for economic implications (the more water is lost, the less income is generated), however it also influences the product quality (especially visually for consumers), tenderness and juiciness (Cheng & Sun, 2008). A higher drip loss is often associated with a negative appearance and lower consumer acceptability (Troy & Kerry, 2010). A higher cooking loss is associated with a “dryer” and tougher meat due to the amount of moisture lost during cooking (Warriss, 2010). It could be anticipated that the bontebok fillet, LTL and IS would be the juicier muscles due to their significantly lower cooking loss percentages (Table 3.3), however, this could be counteracted by the fact that a high pH_u in meat promotes bacterial growth and undesired sensory traits, thus potentially lowering shelf stability and consumer preference (Lawrie & Ledward, 2007; Shange *et al.*, 2019).

The tenderness of meat is recognised as one of the most important eating quality aspects of meat and is influenced by various intrinsic and extrinsic factors such as pH_u , temperature, muscle anatomical location and fibre type, amount of IMF, sarcomere length and the proteolysis of meat (Maltin *et al.*, 2003; Koohmaraie & Geesink, 2006; Neethling *et al.*, 2016). The average bontebok shear force value was calculated as 58 N. The shear force value of other medium sized wild antelopes have been found to be: for blesbok 53.3 N (Mzuvukile, 2018) and 27.31 N (Neethling, 2012); wild fallow deer 41.8 N (Cawthorn *et al.*, 2018); and impala 23.2 N (Engels, 2019). Similarly, male eland meat was found to have a high average shear force value of 82.8 N and Needham *et al.* (2019) suggested ageing these meat cuts could improve the tenderness.

The bontebok shear force values were found to be significantly lower in the fillet and forequarter muscles (IS and SS) with a $pH_u > 6$ than in the other muscles with a $pH_u < 6$. The most tender muscle in the eland was also found to be the IS and the least tender muscle was the LTL (Needham *et al.*, 2019). Contrasting results were found in wild fallow deer, where the hindquarter muscles (BF, SM) and LTL had the lowest shear force values and were the most tender (Cawthorn *et al.*, 2018). The lower shear force values in the IS, SS and fillet muscles of bontebok could be explained by the depletion of glucose reserves of these muscles ante-mortem. Carbohydrates are broken down first, followed by fat and then proteins (Menon *et al.*, 2014; Slimen *et al.*, 2016). The glycogen reserves are predicted to have been very low ($pH_u > 6$ in these muscles) in addition to the low IMF (Table 3.3), which could have accelerated the process to reach the breakdown of protein for energy. Although a low shear force value and “tender meat” is appealing to consumers, this could be counteracted if the meat pH is above a certain threshold causing the meat quality to decrease. Reindeer with $pH_u > 6$ produced tender meat, however, the sensory panel found the rancidity and graininess of the meat to be unacceptable (Renecker *et al.*, 2005). Furthermore, Shange *et al.* (2019) found DFD meat to have decreased meat quality in terms of shelf-life storage and safety for consumption.

3.4.3 Colour

All colour parameters ($L^*a^*b^*$, chroma and hue-angle) differed significantly between bontebok muscles ($p < 0.01$) from the March trial (Table 3.3). Colour is one of the most important attributes for the consumer at the point of sale (Wassenaar *et al.*, 2019) and the red colour of meat is determined and influenced by the chemical state of myoglobin (Cornforth & Jayasingh, 2004).

The L^* value indicates the lightness of meat (0= black; 100= white) and was found to be significantly higher in the bontebok ST muscle, indicating a lighter appearance; the other muscles ranked from light to dark: fillet, BF, SS, SM, LTL, IS. The ST also displays the highest L^* value in wild fallow deer (Cawthorn *et al.*, 2018), eland (Needham *et al.*, 2019) and blesbok (Neethling, 2012). L^*

values >33 (Shange *et al.*, 2018) is believed to be acceptable and desirable by venison consumers. The eland ST muscle was similarly found to have the highest L* value and this phenomenon was ascribed to the high levels of type IIB glycolytic muscle fibres in this muscle, thus limiting the muscle's oxidative capacity and, hence the reduction in discoloration when exposed to oxygen (Needham *et al.*, 2019)

The a* value represents the redness of the meat (-60 to 0 = green; 0 to 60 = red) and was significantly higher in the bontebok forequarter (IS, SS) and fillet muscles than the hindquarter (SM, BF), LTL and ST muscles. The a* values in blesbok (Neethling, 2012) were also found to be significantly higher in the forequarter (IS, SS) muscles. The a* value is known to be positively correlated with the amount of myoglobin content present in muscles. It could therefore be postulated that the bontebok forequarter (IS, SS) and fillet muscles have a higher myoglobin content than the other muscles, however, this should be confirmed by the measurement of myoglobin content in Bontebok muscles. Furthermore, Shange *et al.* (2018) describes an a* value >13 to be acceptable by venison consumers, thus eliminating all muscles except the bontebok IS and fillet muscles that also had pH_u values >6. The latter could counteract these "acceptable" a* values due to a decrease in meat quality/safety. Bontebok meat colour does not compare well with retail beef colour. A total of 39 muscles were dissected from 142 beef carcasses to investigate the colour parameters of beef; the average L* and a* values were 41.06 and 29.57, respectively (Seggern *et al.*, 2005). The bontebok meat is thus much darker (L* = 32.75) and less red (a* = 12.36) than beef.

The structure of the muscle tissue also influences the colour and is directly linked to the ultimate pH of meat (Neethling *et al.*, 2017). Myoglobin is a protein and proteins are susceptible to changes in pH and temperature, which could lead to the denaturation of proteins, lower WHC and paler appearance (Solomon *et al.*, 1998). The opposite is true for meat with a high pH_u, where there is a higher WHC (Lawrie & Ledward, 2007), resulting in a more compact tissue that reflects less light. Furthermore, less oxygen diffuses into the muscle and deoxymyoglobin prevails in muscles. Thus, meat with a higher pH_u tends to result in a darker colour and meat with a lower pH in a lighter colour which possibly explains the darker colour found in bontebok meat.

The b* value represents the yellow-blue value of meat (-60 to 0 = blue; 0 – 60 = yellow) and is found to be the highest in the ST, SS and fillet (more yellow) bontebok muscles and lowest in the LTL muscle (less yellow) (Table 3.3). The b* value was also found to be significantly lower in the LTL (as well as BF) muscle of wild fallow deer (Cawthorn *et al.*, 2018)

The hue-angle and chroma values represent the colour definition (purity of colour) and colour intensity [dull (grey) to saturated (vivid/clear)], respectively and are determined by the a* and b* values (Meat color measurement guidelines, 2012). The chroma values were significantly lower in

the hindquarter (BF, SM) and LTL muscles than the forequarter (IS, SS), ST and fillet muscles. Thus, it could be concluded that the smaller bontebok muscles: ST, IS, SS and fillet had a more vivid or saturated colour and the larger muscles: BF, SM and LTL had a less saturated, dull (grey) colour. The hue-angle value is a good indicator of discoloration in meat. Larger values indicate more metmyoglobin formation and less redness (American Meat Science Association, 2012). The hue-angle values were the highest in the ST muscle and lowest in the forequarter muscles (SS, IS) and LTL. Thus, the forequarter muscles (IS, SS) and LTL display a higher red hue than the ST muscle.

3.4.4 Proximate composition

The proximate composition analyses were conducted on bontebok from the March trial and all parameters were influenced by muscle ($p \leq 0.05$), with concentrations (g/100 g meat) ranging within very small margins: moisture (75.4-76.9); protein (21.4-23.2); IMF (0.7-1.1); and ash (1.1-1.3) (Table 3.3). These values compare relatively well to values (g/100g meat) reported for male blesbok: moisture (74.5, 76.0, 74.4); protein (21.9, 21.40, 21.7); IMF (2.8, 2.8, 2.2); and ash (1.3, 1.2, 1.3) from three separate trials (Du Buisson, 2006; Neethling *et al.*, 2014; Mzuvukile, 2018), respectively. All three trials included the same muscles (except the fillet) tested for in this trial. The proximate composition of meat is influenced by nutrition which is influenced by diet, season and climate. Therefore, it is difficult to compare game species in this regard (Needham *et al.*, 2019). Blesbok and bontebok are closely related sub-species, both selective grazers (similar diets) and both medium sized antelopes (Radloff *et al.*, 2016), thus their proximate composition is expected to be similar, despite the influence of the above-mentioned factors. Despite the proximate composition of blesbok and bontebok being similar, it is important to note that the bontebok IMF is numerically lower than blesbok (Neethling *et al.*, 2014). A possible reason for the low IMF could be the climate in March of 2018 when the bontebok were harvested. These animals had a lack of nutrition and thus lower lipid energy reserves due to the severe drought in South Africa during 2018 (Masante *et al.*, 2018). Male warthogs were found to have low IMF contents (1.19 g/100 g) and this was similarly attributed to the drought these species were exposed to before harvesting (Rudman *et al.*, 2018). Furthermore, bontebok males were supposedly more active in this month due to the mating season that commences in this time of the year. The bontebok males are more active in the latter period due to their rutting behaviour which further contributes towards a reduced IMF content (Mysterud *et al.*, 2004). Male game species are known to have a lower IMF than females. This was seen in springbok (Van Zyl & Ferreira, 2004; Hoffman *et al.*, 2007b; Neethling *et al.*, 2018), impala (Van Zyl & Ferreira, 2004; Hoffman *et al.*, 2009b) and blesbok (Van Zyl & Ferreira, 2004). The bontebok's low IMF, high protein ratios could be advantageous in the marketing of bontebok meat due to the modern-day, health-conscious consumers who believe that high fat and protein diets (such as banting) is healthy.

As pertaining to specific analyses between the muscles, the smaller bontebok muscles (ST, SS, IS and fillet) had significantly higher moisture contents (although numerically small differences) than the larger muscles (BF, SM and LTL) (Table 3.3). This could be ascribed to higher pH_u values (above iso-electric point of 5.4-5.5) in the IS and SS muscles; the high pH_u increases the WHC (retains more moisture) of meat and thus a higher moisture content is expected (Huff-Lonergan & Lonergan, 2005; Lawrie & Ledward, 2007). Eland muscles also did not differ ($p < 0.05$) between each other in terms of moisture content (Needham *et al.*, 2019).

The protein content of the larger muscles (BF, SM and LTL) was significantly higher in bontebok; and the SS and fillet muscles significantly lower than the ST and IS muscles (Table 3.3). Similarly, Neethling *et al.* (2014) noted that the protein content of blesbok LTL muscles (Autumn group) were significantly higher than the other muscles and the SS had the lowest protein content. In the current study, a trend was seen where a higher protein content related to a lower moisture content. The LTL muscle had the highest protein and lowest moisture content and the SS muscle had the highest moisture and lowest protein content (Table 3.3). This could be explained by the binding of water to protein molecules when pH_u of meat is above its iso-electric point of 5.4-5.5 (the average pH_u of bontebok was far above this value (Table 3.3) (Keeton & Eddy, 2004).

The IMF content was found to be the highest in the fillet and lowest in the ST muscle (Table 3.3); the decreasing concentrations were: fillet, SS, IS, BF, SM, LTL, ST. Contrasting to these results, the ST was found to have the highest IMF content in blesbok from their Autumn harvesting group (Neethling *et al.*, 2014). Furthermore, the LTL of other game species in literature were found to have significantly lower IMF contents in the LTL muscle: eland (Needham *et al.*, 2019); and blesbok from the Autumn group (Neethling *et al.*, 2014). IMF is known to be indirectly correlated to moisture content, where a higher IMF content results in a lower moisture content in meat (Hoffman *et al.*, 2009a). However, this phenomenon did not hold true for bontebok, where the LTL had the second lowest IMF content and the lowest moisture content. The fillet had the highest IMF content and the second largest moisture content (Table 3.3). This is due to the extremely low IMF which nullifies the former phenomenon.

The ash that displays the quantity of inorganic and salty constituents, was found to be significantly lower in the forequarter muscles (IS, SS) than hindquarter (BF, SM, ST), LTL, and fillet muscles of bontebok meat. Similarly, the eland displayed ash values significantly (however very small numerical differences) lower in forequarter (IS, SS) muscles than hindquarter (BF, SM, ST) and LTL (Needham *et al.*, 2019), where blesbok showed lower ash values in IS, SS and LTL muscles (Neethling *et al.*, 2014).

3.4.5 Outliers

Two male bontebok (x and y) were removed from the April data set as “outliers” due to extremely high pH values (6.88; 6.10) compared to the average pH_u of 5.6 in the April trial. Meat from animals with pH_u values >6 or >6.2 are known to be classified as DFD (Shange *et al.*, 2019; Viganò *et al.*, 2019).

When comparing bontebok y to the bontebok group from Table 3.4, it clearly has a higher pH_u (6.1), slightly lower WBSF (83.3 N) and lower cooking loss percentage (30.6%) than bontebok from the April group with values: 5.6; 105.3 N; and 36.3%, respectively. Bontebok x showed interesting results with an extremely low shear force (20.9 N), cooking loss percentage (24.6%) and abnormally high pH_u (6.88) (Table 3.5). Both these animals were very stressed ante-mortem during harvesting, and it was observed that bontebok x was running for an extended period (± 60 min). The extreme tenderness of bontebok x could possibly be attributed to a phenomenon called capture myopathy or severe rhabdomyolysis which is found in animals that are exposed to severe exertion and stress. This is known to lead to a syndrome where skeletal muscles are broken down (Warren *et al.*, 2002; Minka & Ayo, 2009). Stressful situations and physical activity are known to lead to the depletion of glycogen stores in muscles which leads to higher ultimate pH values which affects meat shelf life, texture, colour and the water holding capacity of meat (Shange *et al.*, 2018, 2019). A group of springbok were found to have an average pH_u of 6.3 and classified as DFD; the springbok’s meat had higher shear force values (tougher) and lower cooking loss percentages (higher WHC) than meat obtained from the other springbok from regions with normal pH_u values (Hoffman *et al.*, 2007a) The higher toughness was attributed to a reduced sarcomere length which could explain the higher shear force in bontebok y from the April trial (Table 3.5). Devine *et al.* (1993) found mature lambs with a pH_u between 5.5 and 5.9 to have a positive correlation with shear force values (higher pH_u = increased toughness), however lambs with a pH_u above 6.3 produced meat with the lowest shear force (most tender). Renecker *et al.* (2005) also found reindeer with pH_u values between 5.8 and 6 to have increased toughness (as pH increases), however, above a pH of 6 the shear force (N) decreased. Similarly, bontebok x shear force decreased at a much higher pH_u.

Bontebok y had a slightly darker appearance with a L* value of 24.69 (Table 3.5) compared to the April group with an average L* of 25.1 (Table 3.4). Bontebok x was much darker with a L* value of 20.96 (Table 3.5). The a* and b* values were similar for bontebok x and y and are slightly lower than a* and b* for the group (less red and less yellow). The extent of protein denaturation and muscle structure (which is influenced by pH_u) influences light scattering from meat surfaces, which in return influences the L* value of meat. When the pH of meat is high and protein denaturation is decreased, light scattering decreases resulting in a darker meat (Neethling *et al.*, 2017). Thus, this

explains the lower L* values (darker) in both bontebok x and y. The DFD L* values were also significantly lower (darker) than the normal meat samples.

3.5 CONCLUSION

This study represents the first attempt to establish baseline data on the carcass yields, physical characteristics and proximate composition of several bontebok muscles. The bontebok's carcass yields compared favourably to its closely related sub-species, the blesbok. The internal offal made out a substantial amount of the bontebok's total offal percentage and shows potential to be utilised as an edible by-product, however, microbial activity and safety of organs would have to be investigated as they are known to have higher microbial contents. Bontebok meat was generally darker and tougher than other wild ungulates and this was attributed to a severe drought experienced in the Western Cape during the harvesting period and ante-mortem stress which influenced the meat quality. The undesired toughness of the meat could be improved through ageing to improve the tenderness before consumption. The proximate composition of the bontebok is ideal for the health-conscious consumer due to the extremely low fat and high protein content. As the physical and chemical meat quality traits differed significantly between muscles; these differences should help determine which cuts are suitable for prime cuts or for further processing. In this study, the generally higher pH_u of bontebok meat and the forequarter muscles resembling characteristics of DFD meat made it difficult to predict and compare muscles in terms of production potential. Two bontebok were classified as DFD with very dark meat colour, lower shear force values and higher WHC. Further investigation on a variety of bontebok muscles with minimal ante-mortem stress and pH_u values within meat's normal range is needed to further establish the baseline data of bontebok and its commercial potential.

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CHAPTER 4: THE MICROBIAL ACTIVITY AND OPTIMUM AGEING PERIOD OF BONTBOK (*DAMALISCUS PYGARGUS PYGARGUS*) MEAT

ABSTRACT

This study determined the optimum ageing period for vacuum-packed bontebok *Longissimus thoracis et lumborum* (LTL) muscle and the microbial activity over time. Muscles were obtained from eight mature male bontebok aged at 4°C for 12 days (day: 1, 2, 4, 6, 7, 8, 10, and 12 respectively). All physical characteristics [ultimate pH (pH_u), weep loss, tenderness and colour] differed significantly between the ageing days, except for the cooking loss percentage. The Warner-Bratzler shear force (WBSF) decreased until an optimum tenderness was reached on day 8 (57.2 N) after which it plateaued until day 12. The increase in tenderness was associated with an increase in cumulative purge loss over time. The colour improved with ageing resulting in optimum a* and L* values on days 6 and 7, respectively. The total plate count (TPC) and *Escherichia coli*/coliform microbial activity did not differ significantly between ageing days and were within specified safety limits. Additionally, *Salmonella* was not detected in any of the animals. The study found that bontebok LTL muscles should be aged for 8 days at 4°C under vacuum in order to achieve the optimum tenderness for this ageing trial.

Key words: Bontebok, ageing, tenderness, microbial activity

4.1 INTRODUCTION

The popularity of wildlife ranching is increasing as more farmers convert from domestic species farming to game species. The latter is mainly attributed to climate change and warmer temperatures in which wild species are generally better adapted (Otieno & Muchapondwa, 2016). The game industry is mainly divided into four sectors: breeding; hunting; ecotourism; and the production of meat (Van der Merwe *et al.*, 2004). Game meat is derived from free-roaming antelope with the most well-known and abundant species being springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus pygargus phillipsi*) and impala (*Aepyceros melampus*) (Hoffman, 2007). Other games species such as the previously endangered bontebok in South Africa is currently under-utilized in the game meat industry but highly valuable in the live sale and trophy hunting industries (Furstenburg, 2016).

Game meat has proven to show great potential for the red meat industry in South Africa (Neethling *et al.*, 2018), however, game meat has previously been perceived as “tough” (Needham *et al.*, 2019). The ageing of meat is a process known to improve the sensory properties of meat and more specifically the tenderness of meat (Brad Kim *et al.*, 2018). The process involves fresh meat cuts kept under vacuum at chilled temperatures (<4°C) in order to improve the tenderness of meat

by disrupting muscle structures with the intracellular proteolytic systems (Bhat *et al.*, 2018). The calpain system is believed to play a major role in the proteolysis of muscle cytoskeletal and myofibrillar proteins (Bhat *et al.*, 2018). Other factors such as intrinsic (e.g. sex, marbling, muscle type and age) and environmental factors (e.g. nutrition, slaughter process, ante-mortem stress and chilling conditions) could also influence the meat quality during the ageing process.

Meat is known to be highly perishable in its fresh form. The microbial load of meat is known to increase with ageing time (Kilgannon *et al.*, 2019; Shange *et al.*, 2019) and could be influenced during ageing by factors such as temperature, pH_u (thus, ante-mortem glycogen reserves) and exposure to oxygen. During the culling and evisceration of game species, the microbiological safety of the meat is influenced by dressing and processing procedures in which contamination could take place from the gastrointestinal tract (GIT) or from hide-to-muscle (Ramanzin *et al.*, 2010). The safety and quality of meat could be tested for by means of the Total Plate Count (TPC) or testing for *Enterobacteriaceae* which include *Salmonella* and *E. coli*. *Salmonella* is a pathogenic bacterium found in the faeces and/or GIT of warm-blooded animals; *E. coli* is a sub-group of the faecal coliform group and could also be found in faeces and the GIT tract of animals.

The aim of this study was thus to establish the optimum ageing period for vacuum packed LTL muscles in order to ensure optimum tenderness and microbial safety of bontebok meat.

4.2 MATERIALS AND METHODS

4.2.1 Harvesting location and procedures

The harvesting procedure involved the culling of eight mature male bontebok during April of 2018 on the farm Elandsberg, Wellington (Western Cape). All information regarding harvesting, evisceration techniques and carcass handling procedures up until 24 h post-mortem is described in Chapter 3.2: Material and methods.

4.2.2 Sample preparation

The left and right LTL muscles were removed from the eight carcasses 24 h post-mortem. The LTL muscle was removed from between the natural termination of the muscle at the cervical vertebra and the last lumbar vertebra. The ageing steaks were cut into eight ± 1.5 cm steaks (day 1 – day 12) and vacuum packed in a 70 μm poly-ethylene and nylon bag (moisture vapour transfer rate of 2.2 $\text{g}/\text{m}^2/24$ h/ 1 atm, O_2 permeability of 30 $\text{cm}^3/\text{m}^2/24$ h/1 atm and a CO_2 permeability of 105 $\text{cm}^3/\text{m}^2/24$ h/1 atm). All samples were vacuum packed (except for day 1 samples), labelled and stored at 4°C until further physical analysis. Day 1 represented the day the muscles were removed from the carcass 24 h post-mortem, therefore the physical analysis on day 1 took place before the other ageing day samples were vacuum packed. The ageing day steaks were randomly allocated

across the left and right LTL muscle and the allocation randomisation was determined in advance. At the end of each ageing time point, the steaks were removed from the vacuum bag and subjected to certain physical analysis techniques. Samples of 25 g was aseptically removed from each steak portion (eight ageing time points; eight animals) prior to physical analysis and immediately frozen at -20°C. Two samples of 25 g each were taken from day 1 and only one from day 2 to day 12 (Fig. 5.1).

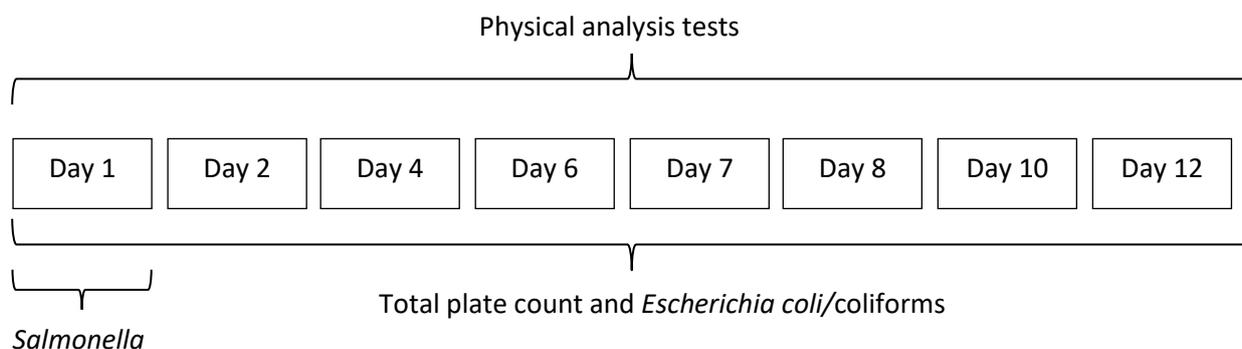


Figure 4.1 A summary of the experimental layout of the ageing trial days and the microbial activity tested for over the ageing time points.

4.2.3 Physical analyses

Physical meat quality tests were conducted on each ageing time point (day 2 – day 12; Fig. 4.1). The physical characteristics of bontebok steak from day 1 was reported in Chapter 3: “The physical and chemical meat quality of bontebok (*Damaliscus pygargus pygargus*); April trial”. The physical meat quality analyses for ageing steaks day 2 to day 12 (i.e. pH, colour, moisture loss, Warner-Bratzler shear force) were in accordance with Chapter 3: Materials and methods. The only difference being the pH measured in the middle of the ageing steak and not on the left cranial end of the LTL muscle as mentioned in Chapter 3. The other difference is “cumulative purge loss” that replaced “drip loss”. It is important to note that day 1 would therefore have no value for cumulative purge loss. Each ageing steak was weighed prior to vacuuming on day 1 to determine the initial mass. The ageing steaks were subsequently weighed at completion of the respective ageing time points to determine the moisture loss during ageing. The latter is known as the cumulative purge loss (or weep loss) and is expressed as a percentage of the initial mass of each ageing steak.

4.2.4 Microbial activity

4.2.4.1 Sample preparation

The eight ageing steaks were cut from both the left and right LTL muscles. A sample size of 25 g of meat was removed aseptically from each ageing steak, vacuum packed, labelled and frozen

immediately at -20°C until further analysis. Ageing steak day 1 had two 25 g samples removed. One for *Salmonella* and one for TPC and *E. coli*/coliforms.

The previously frozen samples (25 g) were thawed at 4°C overnight (~12h), after which they were individually aseptically suspended into 225 mL sterile peptone buffered water (Merck, South Africa) and digested in a stomacher bag (Lasec, 80-400 mL) for 60 s at room temperature (~21°C) (BagMixer 400CC, Interscience). A dilution series was made for each ageing day of each animal (8 ageing time points, 8 animals) and 1 mL of each dilution was plated aseptically to each labelled plate in duplicate. The test for *Salmonella* involved no dilution series, therefore day 1 of each animal was only tested for the absence/presence of *Salmonella* in a 25 g sample. This test was conducted in duplicate.

For the determination of TPC, Plate count agar (PCA; Merck, South Africa) was used, petri dishes were incubated at 30°C for 72 h and colonies were counted manually (ISO 4833, 2013). *E. coli*/coliforms tests used 3MTM petrifilms that were incubated for 24 h at 35±1°C. Colonies were counted manually and interpreted using the 3MTM petrifilm interpretation guide and the total coliform count was reported as the sum of red and blue colonies, representing coliforms and *E. coli*, respectively (AOAC 998.08; 991.14; 3MTM PetrifilmTM). The presence/absence of *Salmonella* was tested for by incubating the PBW at 37°C for 18 ± 2 h. The secondary enrichment was Rappaport-Vassiliadis Soya Peptone (RVS) broth (Oxoid) and incubated in individual test tubes (41.5 ± 1°C, 24 ± 3 h) after which Xylose Lysine Deoxycholate (XLD) plates were used to streak on. The XLD plates were incubated at 37 ± 1°C for 24 ± 3 h before interpretation of results (SANS 6579:2003; ISO 6579:2002).

4.2.5 Statistical analysis

The microbial content (TPC and *E.coli*/coliforms) and physical meat quality data (pH_u, drip loss, cooking loss, WBSF and colour) of each ageing steak was analysed with a mixed model univariate analysis of variance (ANOVA) using SAS software (Version 13.4, SAS Institute Inc., Cary, USA), with animals as random effect and ageing days as fixed effect. The data was tested for normality using the Shapiro-Wilk test. Least Squares Means (LSMeans) were calculated and compared using the p-value. Differences were considered significant when at a level of ≤5% (p ≤ 0.05). The values are reported as the Least square means ± the standard error of the mean (SEM). Where applicable, tables and graphs were generated from means data.

4.3 RESULTS

4.3.1 Physical characteristics over time

All physical meat quality characteristics were significantly different between ageing days (days 1 - 12), except cooking loss percentage ($p = 0.87$) (Table 4.1).

The pH_u generally increased over time from the lowest value on day 1 ($pH_u = 5.5$) up until the highest values on day 8 ($pH_u = 5.62$) and day 12 ($pH_u = 5.59$). The weep loss percentage steadily increased over time from the lowest value on day 2 (1.2%) until the highest value on day 12 (4.4%). The WBSF decreased over time with day 8, 10 and 12 having significantly lower shear force values (57.20 N, 66.64 N, 62.22 N, respectively) than day 1 (108.67 N), however, only differing numerically from day 7 (71.16 N).

The L^* value was significantly higher in days 7 and 10 than in days 1 to 6 with a big increase from day 6 to 7 (24.61 to 27.09). The a^* value steadily increased from day 1 (14.93) up until the highest value on day 6 (18.74), where after the a^* values decreased up until day 12 (15.84). A similar trend was seen for b^* values with values increasing from day 1 (8.52) up until the highest value on day 6 (11.24), however, day 6 was not significantly different from days 4, 7 and 8. Values decreased from days 6 to day 12 (9.85). Chroma values also increased from day 1 (17.19) until the highest value on day 6 (21.86) and decreased until day 12 (18.65). Day 6 was only numerically different from days 4, 7 and 8. The hue-angle values were significantly lower in days 1 and 2 than day 7.

4.3.2 Microbial activity over time

All of the eight bontebok tested negative for the presence of *Salmonella*, TPC and *E. coli*/coliform counts did not significantly differ over ageing time points (Table 4.1). These counts were within the specified limits allowed for game meat (EU Regulation 1441, 2007; DAFF, 2010; Van der Merwe *et al.*, 2011).

4.3.3 Outliers removed

Two bontebok (bontebok x; bontebok y) were removed due to these animals being classified as DFD (dark, firm and dry) meat with pH_u values of 6.1 and 6.88, respectively. The physical meat quality of these animals are depicted in Table 3.5, Chapter 3. Bontebok x had extremely low WBSF values over time compared to bontebok y and the bontebok from the current trial (Fig. 4.2). Furthermore, bontebok x and y had an increase in TPC with values outside the specified limits ($>10^5$ CFU/g) after days 8 and 10, respectively. The *E. coli*/coliform count were also out of specification ($>10^2$ CFU/g) in bontebok x on days 10 and 12 with log CFU/g values of 3.2 and 4.5, respectively.

Table 4.1 The Least square mean (\pm standard error of the mean) for the physical characteristics (pH_u, moisture loss, shear force, colour) and microbial activity (TPC, *E.coli*/coliforms) of the LTL muscle from bontebok harvested during April of 2018

Parameter	Ageing days								p-value
	1	2	4	6	7	8	10	12	
pH _u	5.50 ^c \pm 0.60	5.52 ^{bc} \pm 0.08	5.52 ^{bc} \pm 0.05	5.58 ^{ab} \pm 0.05	5.56 ^{abc} \pm 0.08	5.62 ^a \pm 0.14	5.57 ^{abc} \pm 0.08	5.59 ^a \pm 0.05	0.0135
WL (%)		1.2 ^d \pm 0.64	2.7 ^c \pm 1.98	2.9 ^c \pm 1.48	3.3 ^{bc} \pm 1.63	3.4 ^{bc} \pm 2.15	3.7 ^{ab} \pm 1.97	4.4 ^a \pm 2.09	< 0.001
CL (%)	34.7 ^a \pm 2.87	34.4 ^a \pm 2.05	34.5 ^a \pm 2.17	35.1 ^a \pm 1.10	33.7 ^a \pm 1.41	34.5 ^a \pm 1.60	34.2 ^a \pm 1.50	34.9 ^a \pm 1.76	0.8685
WBSF (N)	108.67 ^a \pm 20.03	94.49 ^{ab} \pm 20.33	91.95 ^b \pm 27.43	83.47 ^{bc} \pm 28.46	71.16 ^{cd} \pm 20.51	57.20 ^d \pm 12.37	66.64 ^d \pm 17.31	62.22 ^d \pm 16.42	< 0.001
<i>Colour:</i>									
L*	24.02 ^d \pm 2.58	25.00 ^{cd} \pm 1.06	25.22 ^{bcd} \pm 1.79	24.61 ^d \pm 2.29	27.09 ^a \pm 2.18	26.27 ^{ab} \pm 2.31	26.90 ^a \pm 1.05	25.96 ^{abc} \pm 1.43	< 0.001
a*	14.93 ^c \pm 1.35	15.05 ^c \pm 1.39	16.84 ^b \pm 3.66	18.74 ^a \pm 1.58	16.91 ^b \pm 1.92	16.82 ^b \pm 2.18	15.62 ^{bc} \pm 1.91	15.84 ^{bc} \pm 2.42	0.0012
b*	8.52 ^c \pm 0.77	8.51 ^c \pm 1.38	10.40 ^{ab} \pm 2.73	11.24 ^a \pm 1.53	11.06 ^{ab} \pm 1.75	10.63 ^{ab} \pm 2.01	9.82 ^{bc} \pm 1.93	9.85 ^{bc} \pm 1.80	< 0.001
Chroma	17.19 ^c \pm 1.52	17.30 ^c \pm 1.78	19.80 ^{ab} \pm 4.55	21.86 ^a \pm 2.07	20.22 ^{ab} \pm 2.50	19.90 ^{ab} \pm 2.90	18.47 ^{bc} \pm 2.59	18.65 ^{bc} \pm 3.00	0.0010
Hue-angle	29.71 ^c \pm 1.13	29.37 ^c \pm 2.70	31.52 ^{ab} \pm 1.14	30.87 ^{bc} \pm 1.95	33.07 ^a \pm 2.03	32.10 ^{ab} \pm 1.85	31.93 ^{ab} \pm 2.57	31.77 ^{ab} \pm 1.01	< 0.001
<i>Microbial activity (log CFU/g):</i>									
TPC	2.29 ^c \pm 1.22	2.86 ^{abc} \pm 0.58	3.26 ^{ab} \pm 0.63	3.30 ^a \pm 0.54	2.92 ^{abc} \pm 0.67	2.55 ^{bc} \pm 0.56	2.54 ^{bc} \pm 0.74	2.29 ^{abc} \pm 0.44	0.0963
<i>E. coli</i> / coliforms	1.23 ^{ab} \pm 1.60	0.44 ^{ab} \pm 0.60	1.95 ^a \pm 1.64	1.72 ^{ab} \pm 2.72	0.85 ^{ab} \pm 1.02	1.11 ^{ab} \pm 2.27	0.00 ^b \pm 0.00	0.57 ^b \pm 0.92	0.3302

Abbreviations: LTL= *M. longissimus thoracis et lumborum* WL%= Weep loss percentage, CL%= cooking loss percentage, WBSF= Warner-Bratzler shear force, TPC= Total plate count; CFU= colony forming units

^{abc} letters with different superscripts within rows differ significantly ($p \leq 0.05$).

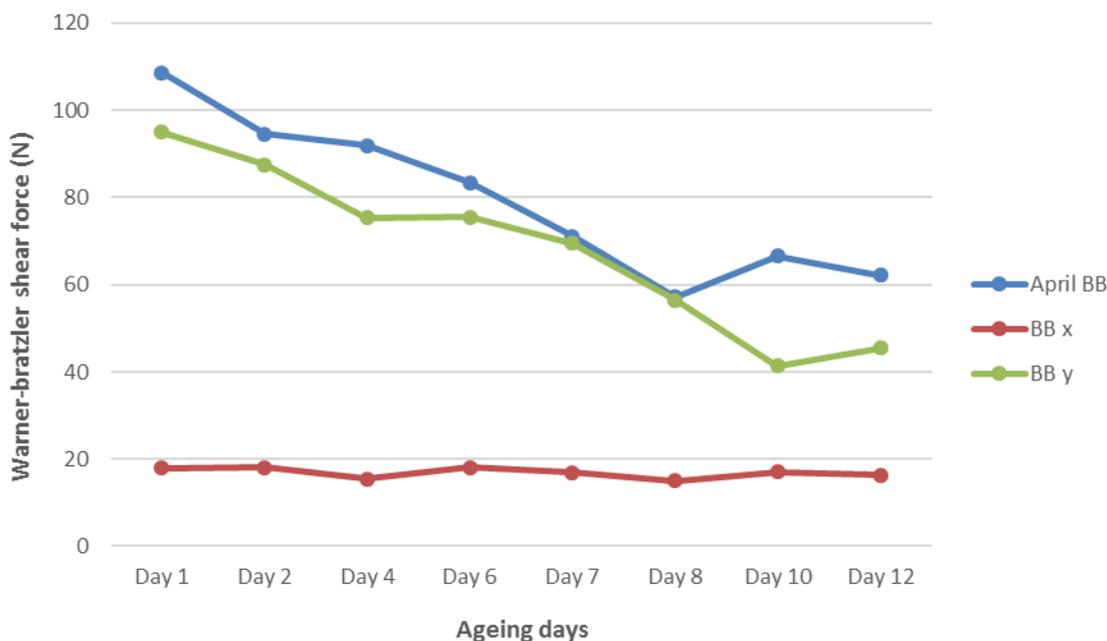


Figure 4.2 The Warner-Bratzler shear force (N) decline over ageing period for male bontebok, bontebok x (BB x) and bontebok y (BB y) *longissimus thoracis et lumborum* muscle.

4.4 DISCUSSION

4.4.1 Tenderness, pH and moisture loss over time

Ageing generally improves the tenderness of meat (Bhat *et al.*, 2018) and this is evident in bontebok samples where the tenderness of steaks decreased from day 1 (108.67 N) to the lowest shear force on day 8 (57.2 N), after which a plateau was observed up until day 12 (62.22 N). Thus, indicating bontebok meat needs to be aged at least 8 days at 4°C in order to obtain the optimum tenderness for this ageing trial. Scientific evidence suggests that the calpain system plays a major role in post-mortem ageing in which the proteolysis of muscle cytoskeletal and myofibrillar proteins occur (Bhat *et al.*, 2018). The collagen content in muscles is known to influence the tenderness of meat, however, it has been found that collagen content remains more or less constant throughout ageing, thus not being a major factor influencing ageing (Colle *et al.*, 2015; North *et al.*, 2015). The optimum ageing time period of springbok meat was established to be five days. The latter study found cathepsins to increase during ageing and calpain and calpastatin activity to decrease significantly up to the fifth day, suggesting that these enzymes could be responsible for the tenderisation of springbok meat (North *et al.*, 2015). Furthermore, a study including a trained sensory panel found that springbok meat should not be aged longer than eight days (North & Hoffman, 2015a). Additionally, the optimum ageing period for the LTL muscle of other game species were found to be

8 days for impala (Engels, 2019), 9 days for blue wildebeest (Van Heerden, 2018) and 21 days for eland (Needham *et al.*, 2020).

The Warner-Bratzler shear force (WBSF) decline for bontebok is 51.47 N from day 1 to 8, which is much higher than the 13.8 N after 5 days observed in springbok (North *et al.*, 2015). However, the springbok started with much lower initial shear force values (~40 N), possibly explaining the smaller drop in shear force (N) compared to bontebok in this study. A higher WBSF drop was found in eland due to a higher initial shear force of 94.5 N declining towards 57-67 N (Needham *et al.*, 2020). The WBSF of the bontebok LTL muscle on day 1 (108.67 N) is unusually high compared to species such as springbok with 23.26 N (North & Hoffman, 2015a) and 40.8 N (North *et al.*, 2015), wild fallow deer at 41.8 N (Cawthorn *et al.*, 2018) and blesbok at 53.3 N (Mzuvukile, 2018) and 27.31 N (Neethling, 2012). A sensory panel determined beef tenderness ratings as tough when WBSF values >52.68 N, intermediate at values 42.87-52.68 and tender at values <42.87 N (Destefanis *et al.*, 2008). The latter categorising bontebok meat as very tough on day one (108.7 N) and still tough at the optimum tenderness determined for this trial at day 8 (57.2 N). Although the bontebok meat in this trial could be deemed very tough using the above classification, the potential of ageing bontebok meat was very effective and highlighted in this study.

The pH_u of bontebok muscles aged in the current trial (5.5-5.62) was regarded as normal within the pH_u range specified for meat (Honikel, 2004). The pH_u is known to be influenced by animal behaviour and ante-mortem stress and could thus influence the tenderness of meat. Hoffman *et al.* (2007) found springbok from a farm to be classified as DFD meat ($pH > 6$) and the meat from these animals were tougher than animals from other regions with normal pH_u values. The extremely high shear force (N) and normal pH_u in bontebok meat during this ageing trial is therefore unusual. However, the pH_u is not the only factor that influences tenderness and Bhat *et al.* (2018) describes factors such as temperature, muscle fibre-type composition and sarcomere length to further contribute to changes in tenderness. Furthermore, the pH_u of bontebok was found to be significantly different between ageing days. The pH_u increased during the ageing period with a slight drop in pH_u after days 6 and 8 (Table 4.1). The pH_u of springbok (North *et al.*, 2015) and eland (Needham *et al.*, 2020) was also found to have a general increase in pH_u with a slight drop at two ageing time points; the pH_u increase during ageing is due to alkalization caused by basic products released when protein breakdown occurs (Florek *et al.*, 2007). The latter could explain why the optimum tenderness and highest pH_u (57.2 N; 5.62) were reached on day 8 (Table 4.1).

The loss of moisture in the muscle-to-meat process is inevitable due to the cross-links formed during rigor mortis, ultimately pushing water out of cells due to the stiffening of muscle fibres. The continued water migration can be observed during ageing in the form of drip/purge loss

which is affected by pH and the size of myofibrillar spaces (Huff-Lonergan & Lonergan, 2005). Moisture loss is an undesirable side-effect during ageing, resulting in packaging with excess fluid and mass loss (unfavourable economic implications). The appearance of the latter phenomenon is not desired by consumers and should be reduced (Cheng & Sun, 2008). The bontebok weep loss (or cumulative purge loss) significantly increased over the ageing period with the lowest value found on day 2 (1.16%) and the highest on day 12 (4.37%) (Table 4.1). The bontebok purge loss percentages were comparable and also increased with time as reported for springbok (North *et al.*, 2015) and eland (Needham *et al.*, 2020). A purge/weep loss above 4% is believed to have a negative impact on consumer perception of retail meat (Johnson, 1974; Colle *et al.*, 2015). All bontebok weep loss percentages were therefore acceptable according to the latter guidelines except for day 12 (Table 4.1).

Cooking loss is a result of thermal denaturation of proteins, such as actin and myosin (Pearce *et al.*, 2011). The cooking loss of bontebok meat did not differ significantly between ageing time points. Similarly, no significant differences were detected in springbok (North & Hoffman, 2015b). However, North *et al.* (2015) only found a significant increase in cooking loss of springbok after day 8 until day 21 and further specifies that cooking loss differences between ageing time points are found to be very contradicting between studies.

4.4.2 Colour over time

The colour of meat is determined primarily by the concentration and chemical state of myoglobin present in meat, where metmyoglobin is responsible for the brown discolouration unaccepted by consumers and oxymyoglobin is formed when meat is exposed to oxygen and forms a desired cherry-red colour (Cornforth & Jayasingh, 2004; Neethling *et al.*, 2017). The initial colour and colour stability of meat is influenced by post-mortem ageing according to Brad Kim *et al.* (2018) and is evident in bontebok where the $L^*a^*b^*$, chroma and hue-angle values of bloomed meat differed significantly between different ageing days 1 to 12 (Table 4.1). Brad Kim *et al.* (2018) further emphasized the complexity of ageing influencing meat colour and oxidative stability and how the exact mechanisms to explain the latter is not yet fully understood.

The overall trend was not easily recognisable; however, it could be seen that the L^* value in bontebok generally increased slightly from day 1 (24.02) to day 12 (25.96) with a slight drop after days 4, 7 and 10 (Table 4.1) thus, indicating that ageing generally improves the colour of bontebok meat (becomes lighter) within a certain time period. Blue wildebeest (Van Heerden, 2018), eland (Needham *et al.*, 2020) and impala (Engels, 2019) also had a general increase in L^* value with a slight drop on certain days. The bontebok L^* values were much lower, thus “darker” than other game meat specified in literature. The L^* value of 24.02 was established for bontebok meat on day 1 and

this can be classified as unacceptable (below 33) for consumers of venison meat as reported by Shange *et al.* (2019). The specific acceptability ranges in colour for game meat has not yet been established in literature. Other medium sized game meats have been found to have higher L^* values: blesbok 30.5 (Hoffman *et al.*, 2010); springbok 31.75 (Hoffman *et al.*, 2007); black wildebeest 34.3 (Shange *et al.*, 2018); and impala 32.76 (Hoffman & Laubscher, 2009). According to Neethling *et al.* (2017) many intrinsic (pH, species, sex, age, muscle source, lipid oxidation) and extrinsic (season, feed/diet, ante-mortem stress, storage temperature) factors could influence the colour of meat although most of these factors were held constant during the current bontebok trial. Environmental temperatures were above ambient temperature on the harvesting day and South Africa had just faced a severe drought (Masante *et al.*, 2018), which could have caused ante-mortem stress and a lack of nutrition, possibly resulting in a lower ante-mortem glycogen reserve in the muscles of bontebok from this study. A higher pH_u of meat is known to result in meat with a darker colour (Shange *et al.*, 2018). The dark colour and high shear force (N) of bontebok is known to be associated with DFD or intermediate DFD meat (meat with $pH_u > 6$) (Shange *et al.*, 2018; Viganò *et al.*, 2019). However, the pH_u of bontebok was normal and fell within the ideal range (5.5-5.65), which is unexpected due to factors such as tenderness and colour indicating otherwise.

The a^* and b^* values (redness and yellowness) of bontebok gradually increased with the highest values found on day 6 (18.74 and 15.84, respectively). Bontebok had a redder and more yellow colour (much higher a^* and b^* values) than other game species in literature: black wildebeest 12.5, 9.9 (Shange *et al.*, 2018); impala 11.4, 7.7 (Hoffman & Laubscher, 2009); springbok 13.3, 8.1 (Hoffman *et al.*, 2007); and blesbok 12.6, 8.4 (Hoffman *et al.*, 2010), respectively. Bontebok a^* and b^* values are closer to beef retail values (Seggern *et al.*, 2005) than the game species mentioned above and ageing was found to improve the former values. The a^* values of meat is linked to myoglobin content (redness) and moisture loss is often associated with the loss of the protein myoglobin (amongst other nutrients) (Colle *et al.*, 2015), thus possibly explaining the decrease in a^* value after day 6 (Table 4.1). The increase in b^* value is possibly associated with the oxidation of oxymyoglobin to metmyoglobin which is known as a more "brown" colour (Cornforth & Jayasingh, 2004). Both chroma (saturation index) and hue-angle (purity of colour) values for bontebok meat increased slightly over time with a maximum value found on days 6 and 7, respectively. A higher chroma and a^* value therefore indicating a higher saturated red colour as bontebok meat is aged. Hue-angle is a good indicator of the discoloration of meat and larger values indicate more metmyoglobin formation (American Meat Science Association, 2012). The hue-angle generally increased with ageing, however, the values fluctuated between ageing days. The a^* , b^* , chroma and

hue-angle values of blue wildebeest (Van Heerden, 2018), impala (Engels, 2019) and eland (Needham *et al.*, 2020) also fluctuated over an ageing period.

4.4.3 Microbial activity over time

Meat is known to be highly perishable in its fresh form (Koutsoumanis & Sofos, 2004). The type and amount of microorganism present in wild ungulates are known to be influenced by processing and dressing procedures, as well as contamination [hide-to-muscle or gastrointestinal tract (GIT)] (Ramanzin *et al.*, 2010). The spoilage organisms could therefore gain access endogenously ante-mortem or exogenously post-mortem (Gill, 2007).

All bontebok meat tested negative for the presence of *Salmonella*. According to International standards, the detection of *Salmonella* is not allowed in fresh meat products (EU Regulation 1441, 2007). This pathogenic bacterium is known to live in the intestinal tracts, shed by faeces and only a small amount is required to poison humans (Nielsen, 2004). The microbial safety of springbok was tested by Magwedere *et al.* (2013) for meat exporting purposes and all springbok also tested negative for *Salmonella*. Despite the rather small sample size, the latter findings in addition to the current study's findings may suggest that bontebok and springbok might not be a reservoir for *Salmonella*, however, further research is needed to prove the latter statement. If this bacterium was detected, it could indicate the presence of *Salmonella* in the GIT, thus indicating contamination and highlighting unhygienic slaughtering practises.

Indicator organisms include a larger group of bacteria that are easy to measure and could include pathogenic bacteria (Van Schalkwyk & Hoffman, 2016). The TPC has the ability to represent organisms that grow at mesophilic temperatures (30 - 45°C) in aerobic conditions, therefore it is a good indicator of overall quality or bacterial contamination of meat (Van Schalkwyk & Hoffman, 2016). The TPC of bontebok did not differ significantly over ageing days (Table 4.1). The TPC of beef were found to increase as ageing time increased (Colle *et al.*, 2015; Kilgannon *et al.*, 2019). Similarly, the TPC of previously frozen (Shange *et al.*, 2019) and fresh (Shange *et al.*, 2018) black wildebeest samples increased with ageing. According to Van der Merwe *et al.* (2011), the standards for APC is $\leq 10^5$ CFU/g (5 log CFU/g), however, levels as high as $\leq 10^6$ CFU/g (6 log CFU/g) are acceptable for the global red meat market. The South African standards specify 5.0-5.7 log CFU/g (DAFF, 2010) and EU regulations between 3.5-5.0 log CFU/g (EU regulation 1441, 2007). The bontebok APC levels are all below these standards for the current ageing trial with the highest numerical value found on day 6 as 3.3 log CFU/g (Table 4.1).

Coliforms are bacteria found in the environment and in faeces of warm-blooded animals (Schaffner & Smith, 2004). *E. coli* is a subgroup of the faecal coliform group. There are different types of *E. coli* of which most are harmless and mostly found in the intestines of animals. Pathogenic

strains (such as Shiga toxin-producing *E. coli*) have been detected and could cause serious health implications when ingested in small quantities. The *E. coli*/coliform counts (log CFU/g) in bontebok meat did not differ significantly over time (Table 4.1). Similarly, *E. coli* was also not found to differ significantly with ageing time in black wildebeest (Shange *et al.*, 2018). According to Van der Merwe *et al.* (2011), the legal maximum *E. coli* count is 10^2 CFU/g (2 log CFU/g) for fresh game meat. However, the European Union and South African standards stipulate the minimum and maximum acceptable respective limits (log CFU/g) of *E. coli* should be 1.7 and 2.7 (EU Regulation 1441/2007; DAFF, 2010). All bontebok meat samples were below the acceptable limits specified (Table 4.1).

4.4.4 Outliers removed

Two male bontebok (x and y) were removed from the April data set as “outliers” due to extremely high pH values (6.88 and 6.10, respectively) compared to the average pH_u of 5.6 in the current trial. Meat from animals with high ultimate pH values > 6 or > 6.2 are classified as DFD (Shange *et al.*, 2018; Viganò *et al.*, 2019). The outliers’ physical characteristics of day 1 was discussed in Chapter 3.4.5: Discussion, outliers removed.

All bontebok in this trial had a steady decrease in WBSF over time, except for bontebok x as seen in Fig. 4.2. As discussed in Chapter 3, bontebok x had been severely stressed during harvesting. This animal had been running >40 min, thus possibly depleting glycogen reserves and resulting in a high post-mortem pH_u. Bontebok x possibly had ‘capture myopathy’, where carbohydrates and fat reserves are depleted, resulting in the breakdown of proteins, including skeletal muscles (Warren *et al.*, 2002; Minka & Ayo, 2009). The muscles of bontebok x were therefore found to be very tender on day 1 (20.88 N) compared to the other bontebok with an average WBSF of 108.67 N on day 1. Due to the low starting shear force (20.88 N) and a possible indication that these proteins were possibly degraded to the point that no more tenderisation could take place, no decline was noted during the ageing period for bontebok x. Meat with a low WBSF is known to produce tender meat desired by consumers, however, a sensory analysis would have to be conducted in order to establish the taste and aroma profile of the meat. Renecker *et al.* (2005) found reindeer with very tender meat due to extremely high pH_u values to be counteracted by the “off” flavours and “mushy” texture described by trained sensory panel members.

The microbial load increased with time in meat derived from bontebok (x and y) and were not within the limits specified by Van der Merwe *et al.* (2011). The *E. coli*/coliform counts were also not within limits specified by the latter. Meat with a higher pH_u is known to have increased microbial activity. The TPC was seen to increase faster in DFD than in normal meat samples during the ageing of black wildebeest (Shange *et al.*, 2018, 2019).

4.5 CONCLUSION

This study represents the first attempt to establish the optimum ageing period for bontebok meat, as well as the microbial activity over time of the aged LTL muscle. Bontebok meat was proven to tenderise rapidly during ageing with the optimum shear force for this trial reached by day 8, thus highlighting that bontebok LTL muscles should be aged for at least 8 days at 4°C under vacuum storage. The latter ageing time was also associated with favourable weep loss percentage and microbial activity, however, day 8 produced meat that could be regarded as tough and dark by consumers. Two bontebok were classified as DFD ($\text{pH}_u > 6$) due to ante-mortem stress and the data from these animals were consequently removed as outliers. The TPC and *E. coli*/coliforms counts did not differ significantly over time in the normal meat samples, but the DFD meat samples (outliers) had microbial counts exceeding the acceptable limits. The Western Cape had undergone a severe drought during the harvesting season of bontebok in this study and therefore a lack of nutrition was considered to influence the meat quality. There is large amount of intrinsic and environmental factors known to affect the meat quality of game species, therefore it is important that this study is repeated with a larger group of bontebok in a better harvesting season (no drought) with various bontebok muscles.

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CHAPTER 5: INFLUENCE OF TWO MUSCLE TYPES (*SEMIMEMBRANOSUS* AND *BICEPS FEMORIS*) ON THE SENSORY ANALYSIS OF BONTBOK (*DAMALISCUS PYGARGUS PYGARGUS*) AND BLESBOK (*DAMALISCUS PYGARGUS PHILLIPSI*)

ABSTRACT

The sensory attributes of the *semimembranosus* (SM) and *biceps femoris* (BF) muscles were compared between blesbok (n = 7) and bontebok (n = 7). A descriptive sensory analysis (DSA) determined the sensory characteristics of the meat using 10 trained panel members. Additionally, physical characteristics [cooking loss percentage and Warner-Bratzler shear force (WBSF)] and proximate composition (moisture, protein, intramuscular fat and ash content) were determined. Only gamey flavour differed significantly between species, which was slightly higher in blesbok than bontebok. The lack in differences could be attributed to both species consuming similar diets (both strict grazers). Textural differences were affected by species and muscle type to a greater extent compared to aroma and flavour differences. Sensory tenderness had a strong negative correlation to WBSF ($r=-0.84$; $p<0.05$) and residue ($r=-0.92$; $p<0.05$) and a strong positive correlation to mealiness ($r=0.82$; $r<0.05$). The bontebok had a significantly higher WBSF, lower tenderness, higher residue and lower mealiness compared to blesbok. Tenderness is generally one of the most important sensory attributes of meat, and it can be concluded that the SM muscle proved to be superior compared to the BF muscle due to its significantly higher tenderness and initial juiciness. Furthermore, baseline data was established for the sensory profile of bontebok meat which could assist in the marketing process. Bontebok meat compares favourably to blesbok meat and can be marketed as a healthy red meat source high in protein and low in fat, however, the meat could be perceived as tough. Nonetheless, it is postulated that meat consumers would struggle to differentiate between bontebok and blesbok meat.

5.1 INTRODUCTION

Bontebok (*Damaliscus pygargus pygargus*) and blesbok (*Damaliscus pygargus phillipsi*) are closely related sub-species similar in size, behaviour and dietary regimes (strict grazers) (Radloff *et al.*, 2016; Dalton *et al.*, 2017). However, blesbok are abundantly found, whereas bontebok were previously close to extinction and currently listed as “vulnerable” according to the IUCN red data list (Lloyd & David, 2017). The game industry in South Africa consists mainly of breeding, hunting, eco-tourism and the meat production sector (Van der Merwe *et al.*, 2004; Saayman *et al.*, 2018). Most hunting

practices (such as trophy hunting) produce carcasses that are available for human consumption, are currently underutilised and show potential for meat production purposes.

The production of game meat is crucial to enable the growth of the wildlife industry in South Africa (Taylor *et al.*, 2016). Game meat forms a large part of the South African consumers diet (Erasmus & Hoffman, 2017). Furthermore, game meat is known to be derived from wild, free-ranging ungulate species found in Southern Africa. These species do not receive antibiotics or hormone treatments and the meat is therefore known as “organic” in addition to being classified as a healthier alternative source of protein for the red meat industry (Erasmus & Hoffman, 2017; Wassenaar *et al.*, 2019). According to a wide spectrum of literature, red meat contains naturally occurring chemicals that are associated with cancer and cardiovascular diseases (Cross *et al.*, 2007; Wolk, 2017). A recent study found contrasting results indicating no relationship between a higher intake of red meat and the incidence of cancer or cardiovascular diseases (Zeraatkar *et al.*, 2019), highlighting the uncertainty of the above-mentioned statement. Thus, red meat shows the potential to provide high quality protein that may aid in establishing food security in developing countries and additionally aid in preventing a high occurrence of malnutrition in a large population of individuals.

Several factors influence the choices made by game meat consumers, such as health benefits, price, safety, availability and sensory characteristics. Wassenaar *et al.* (2019) investigated the above-mentioned factors and the outcome of the study highlighted the importance of sensory properties as it was found to override all other properties influencing meat consumers’ choices. The most important factor determining the consumers’ choice of red meat at the point of sale is visual appearance (Ramanzin *et al.*, 2010), however, at the point of consumption factors such as aromatic properties, taste and mouthfeel influence the consumers’ acceptability of the meat (Neethling *et al.*, 2016). The aroma and flavour of meat is known to be influenced by the fatty acid profile of the meat, which is influenced by the animal’s diet (Neethling *et al.*, 2016, 2018). Animals can be predominately browsers, grazers or mixed feeders which determines the dietary intake of game species (Shipley, 1997).

Due to blesbok and bontebok being sub-species, a question was posed whether there is a difference in the sensory profile between the two species. If bontebok has a distinguished flavour, aroma and texture profile it could aid in the marketing of the meat from this species in a niche market. Therefore, the aim of this study was to investigate the sensory differences between blesbok and bontebok meat (including differences between muscle type), as well as obtaining baseline data for the sensory attributes of bontebok meat as it has not yet been established in literature.

5.2 MATERIALS AND METHODS

5.2.1 Harvesting location and procedures

A total of seven mature male bontebok were harvested at the Elandsberg farm, Wellington, in April of 2018. The culling and evisceration procedures were explained in Chapter 3.

An additional seven mature male blesbok (*Damaliscus pygargus phillipsi*) were harvested at Brakkekuil farm (34°17'38.44"S; 20°47'41.89"E), in April of 2018. This farm is near Witsand, Western Cape in South Africa. The animals were maintained in a fenced ~108 ha camp. The area is classified as Coastal Renosterveld and receives 300-500 mm of yearly rainfall, although a higher precipitation is expected during February to March and September to November. The animals were all shot during the day with a .308 rifle from the back of a vehicle by a skilled marksman thereby ensuring minimal ante-mortem stress. Annual harvesting procedures take place in this camp to reduce the population size and ensure a sustainable annual yield by ensuring a positive population growth. The harvesting, culling and evisceration procedures were in accordance with guidelines provided by Van Schalkwyk and Hoffman (2016) and was described in further detail in Chapter 3.

5.2.2 Muscle sampling

The bontebok (n= 7) and blesbok (n= 7) carcasses were transported to Stellenbosch University meat laboratory and refrigerated at 4°C for 24 h. After 24 h post-mortem, rigor mortis had taken place and the muscles (BF and SM) were removed from the carcasses, together with the outer epimysium layer. For chemical analysis, ±200 g meat was taken from the right posterior (bottom) side of each muscle, vacuum sealed into plastic bags and frozen at -20°C until further analysis. All chemical analysis was done in accordance to Chapter 3, Materials and methods (3.2.4.5). The remaining muscles were weighed, vacuum packed and aged at 4°C for seven days. The latter ageing of bontebok and blesbok meat was aimed at increasing the palatability of the meat from these species. (Dransfield, 1994; North *et al.*, 2015). The aged meat was frozen at -20°C until the respective training and testing phase of the DSA.

5.2.3 Physical analysis

5.2.3.1 Cooking loss

The weight of each raw piece of meat (whole muscles) was recorded. After the cooking procedure (see 5.2.4.1), the meat was left to cool for 10 min, lightly dabbed with an absorbent paper towel to remove excess moisture and weighed. The cooking loss was determined as the difference between initial and final mass and expressed as a cooking loss percentage (AMSA, 2015).

5.2.3.2 Warner-Bratzler shear force (WBSF)

The cooked meat samples were used to determine the WBSF of the BF and SM muscles of each individual bontebok and blesbok. Six samples (taken from different areas across the two muscles) were used to calculate an average shear force (N) for each muscle using the 3345 Instron Universal Testing Machine (Apollo Scientific cc, Alberta, Canada). Further details regarding the method can be found in Chapter 3.

5.2.4 Sensory analysis

5.2.4.1 Sample preparation

Each muscle (BF and SM) of the bontebok and blesbok was thawed at 4°C for 36 h. Thereafter, each muscle was cooked in a Hobart oven, each in its own individual oven roasting bag. A thermocouple probe was inserted to the geometric centre of each muscle and attached to a handheld digital temperature monitor (Hanna Instruments, South Africa). Additionally, the roasting bags were closed with a twist tie around the probe and placed on a metal grid covered in aluminium foil on an oven tray. The oven was pre-heated to 160°C and the muscles were cooked until an internal temperature of 72°C was reached. Each muscle was left to cool for 10 min. The samples were then cut into 1cm³ cubes, where after they were individually wrapped in aluminium foil and placed into glass ramekins coded with 3-digit random numbers. Two muscles (BF and SM) from two species (blesbok and bontebok) were analysed, thus four meat cubes were placed in each ramekin, representing each individual treatment.

5.2.4.2 Descriptive sensory analysis

Descriptive sensory analysis (DSA) is a sensory method performed by a trained panel of judges to profile a product and evaluate all of its perceived sensory attributes (Murray *et al.*, 2001). The DSA was performed at the sensory laboratory of the Food Science Department, Stellenbosch University. A pre-existing panel consisting of 10 judges that have experience in the sensory analysis of game meat were used to analyse the meat samples in this study and were trained according to guidelines supplied by Lawless and Heyman (2010). These experienced sensory judges were exposed to six training sessions over a three-day period. During the training sessions, the judges were each given four meat cubes (1 cm³) from each of the six reference samples (Table 5.1). The aim of the training session was to create a list of sensory attributes (aroma, flavour, texture) associated with bontebok and blesbok meat and was achieved by using reference samples in order to establish a baseline to train the panel members. The panellists were trained according to the guidelines for sensory analysis of meat by the American Meat Science Association (AMSA, 2015) guidelines and the method for the descriptive sensory analysis as described in Lawless and Heyman (2010).

After training, the judges decided on 20 sensory attributes to be used in the final testing session. Eight blind-testing sessions of ± 45 min each were conducted in which each panel member received four meat cubes per treatment (two muscle types from two species) and were asked to score sensory attributes on a 100-point line scale (Table 5.2). Each panellist were seated in an individual tasting booth in a light-controlled room with controlled temperatures set at 21°C. Water and unsalted crackers were served as palate cleansers. Compusense® five software (Compusense, Guelph, Canada) was used to capture data.

5.2.5 Statistical analyses

The experimental design was a completely random factorial with 14 animals harvested, seven bontebok and seven blesbok. Two muscles (BF and SM) from the latter two species served as main factors. Two bontebok were removed as outliers from the dataset, due to the meat from these animals being classified as dark, firm and dry (DFD) with pH_u values >6 .

Prior to analysis, a Shapiro-Wilk test was used to test for deviation from normality and the statistical significance was calculated using a Fisher's t-test at a 5% level of significance to compare means. A two-way mixed model analysis of variance (ANOVA) was used to analyse sensory attributes. The random effect was judges and fixed effects were species and muscle type. Other variables (physical and proximate) were analysed using a two-way mixed model ANOVA with animals as random effect and species as well as muscle type as fixed effect. The ANOVAs were calculated using the General Linear Models (GLM) procedure of the SAS™ software (Statistical Analysis System, Version 9.4, SAS Institute Inc., Cary, NC, USA). In addition to univariate ANOVAs, the data was also subjected to other analysing techniques such as a principal component analysis (PCA). Pearson correlations coefficients (r) were also determined using XLStat (Version 2019, New York, USA). A probability level of 5% was implemented for the interactions, main effects and correlations. Values are reported as the LSMeans \pm standard error.

Table 5.1 The reference standards used during training of the descriptive sensory analysis for bontebok and blesbok meat

Reference standard	Attributes represented	Internal cooking temperature	Scale
Beef fillet	Beef-like aroma, flavour	72°C	0=none, 100=prominent
	Tenderness		0=extremely tough; 100=extremely tender
	Sustained juiciness		0=dry, 100=extremely juicy
	Mealiness		0=none,
	Residue		100=abundant
Beef (Ox) liver	Liver-like aroma, flavour	Pan-fried	0=none, 100=prominent
	Tenderness		0=extremely tough; 100=extremely tender
	Sustained juiciness		0=dry, 100=extremely juicy
	Mealiness		0=none,
	Residue		100=abundant
Brown meat edges	Sweet-associated aroma, flavour	72°C	0=none, 100=prominent
Zebra meat (tough)	Beef-like aroma, flavour	75°C	0=none, 100=prominent
	Tenderness		0=extremely tough; 100=extremely tender
	Sustained juiciness		0=dry, 100=extremely juicy
Bontebok meat (aged 7 days)	Gamey aroma, flavour	72°C	0=none, 100=prominent
Blesbok meat (aged 7 days)	Gamey aroma, flavour	72°C	0=none, 100=prominent

Table 5.2 The scale and definition of sensory attributes (aroma, flavour and texture) used in the descriptive sensory analysis

Sensory attribute	Description of attribute	Scale
<i>Aroma</i>		
Overall aroma intensity	Intensity of aroma in the first few sniffs	0=none, 100=prominent
Gamey aroma	Aroma associated with meat from wild animal species	0=none, 100=prominent
Beef-like aroma	Aroma associated with cooked beef fillet	0=none, 100=prominent
Liver-like aroma	Aroma associated with pan-fried beef liver	0=none, 100=prominent
Metallic aroma	Aroma associated with raw meat/blood-like	0=none, 100=prominent
Sweet-associated aroma	Aroma associated with Maillard reaction on meat surface (browning)	0=none, 100=prominent
Sour-associated aroma	Aroma associated with vacuum-packed, game/wild aged meat	0=none, 100=prominent
<i>Flavour</i>		
Gamey flavour	Flavour associated with the meat from a wild animal species	0=none, 100=prominent
Beef-like flavour	Flavour associated with cooked beef fillet	0=none, 100=prominent
Liver-like flavour	Flavour associated with that of pan-fried beef liver	0=none, 100=prominent
Metallic flavour	Flavour associated with raw meat/blood-like	0=none, 100=prominent
Sour-associated flavour	Taste associated with vacuum packed, game/wild aged meat	0=none, 100=prominent
Sweet-associated flavour	Taste associated with Maillard reaction on meat surface - browning	0=none, 100=prominent
Salty taste	Taste associated with sodium ions	0=none, 100=prominent

Table 5.2 *continued*

<i>Texture</i>		
Initial juiciness	Amount of fluid extruded on surface of meat sample when pressed between the thumb and forefinger (pressed perpendicular to fibres)	0=dry, 100=extremely juicy
Sustained juiciness	Amount of moisture perceived during mastication (after 5 chews)	
Mealiness	Disintegration of muscle fibre where mealy disintegrates into very small particles (perception within first few chews)	0=none, 100=abundant
Liver-like texture	Texture like that of pan-fried beef liver (spongy/pasty)	0=none, 100=prominent
Residue	Residual tissue remaining after mastication (after 10 chews)	0=none, 100=abundant
Tenderness	Impression of tenderness after mastication	0=extremely tough, 100=extremely tender

5.3 RESULTS

Two animals were removed from the dataset due to these animals having a $pH_u > 6$, thus being classified as DFD meat. No significant interactions were noted between the species and muscle type interactions for any meat quality traits except for three sensory attributes: overall aroma intensity; beef-like aroma; and metallic flavour (Table 5.4). The bontebok SM muscle had a higher beef-like aroma (43.18 ± 6.51) than the bontebok BF muscle (39.41 ± 6.83) and blesbok SM muscle (39.99 ± 6.39) was numerically smaller than the blesbok BF muscle (41.06 ± 7.48) on a 100-point scale (0=none; 100=prominent). Although interactions between overall aroma intensity and metallic flavour were observed ($p=0.046$ and $p=0.033$, respectively), the differences between the Least Square Means (LSMeans) of these interactions are negligible and will not be discussed further.

No significant differences were found for the proximate composition between species or muscle type (Table 5.3), except for the moisture content between muscles: SM ($74.7 \text{ g}/100 \text{ g}$); BF ($75.6 \text{ g}/100 \text{ g}$). Again, these differences between the muscle types are negligible. It is important to note the extremely low IMF content of both blesbok and bontebok meat ($0.5 \pm 0.13 \text{ g}/100 \text{ g}$; $0.4 \pm 0.08 \text{ g}/100 \text{ g}$, respectively).

No significant differences for aroma and flavour attributes were found between species and muscles except for gamey flavour that was slightly higher in blesbok (76.31 ± 6.45) than bontebok (73.44 ± 5.52) meat. Tenderness had a strong negative correlation with WBSF ($r=-0.84$; $p<0.05$) and residue ($r=-0.92$; $p<0.05$) and a strong positive correlation with mealiness ($r=0.82$; $r<0.05$). Bontebok meat had a significantly higher WBSF ($63.29 \pm 24.77 \text{ N}$), lower tenderness (49.63 ± 12.70), higher residue (21.86 ± 12.54) and lower mealiness (3.81 ± 6.45) as compared to blesbok (39.96 ± 14.53 ; 58.59 ± 11.47 ; 9.68 ± 10.24 and 11.09 ± 8.69 , respectively). In addition to Pearson correlations calculated for sensory attributes, several sensory attributes were associated with one another (and grouped together) (Fig. 5.1).

For muscle type, the initial juiciness was scored higher in the SM (54.75 ± 11.96) than the BF (50.33 ± 11.00) muscle and cooking loss percentage was found to be lower in the SM ($28.18 \pm 5.24\%$) than the BF ($33.63 \pm 5.15\%$) muscle. The BF had a significantly higher WBSF ($55.14 \pm 23.31 \text{ N}$), lower tenderness (52.03 ± 12.79), higher residue (18.05 ± 13.32) and lower mealiness (6.42 ± 7.59) as compared to the SM muscle ($45.81 \pm 21.58 \text{ N}$, 54.75 ± 11.96 , 11.46 ± 11.26 and 9.69 ± 9.26 , respectively).

Table 5.3 The Least Square Mean (\pm standard error) for meat quality parameters of two muscles (BF, SM) and two species (blesbok, bontebok).

Parameter	¹ Species		p-value	² Muscles		p-value
	Blesbok	Bontebok		BF	SM	
<i>Physical characteristics:</i>						
Cooking loss (%)	31.3 \pm 6.76	30.4 \pm 4.40	0.7036	33.6 \pm 5.15	28.2 \pm 5.24	0.0426
WBSF (N)	39.96 \pm 14.53	63.29 \pm 24.77	0.0140	55.14 \pm 23.31	45.81 \pm 21.58	0.0305
<i>Proximate composition (g/100 g):</i>						
Moisture	75.0 \pm 0.76	75.3 \pm 1.16	0.2754	75.6 \pm 0.76	74.7 \pm 1.00	0.0059
Protein	21.3 \pm 0.95	21.8 \pm 1.16	0.1735	21.5 \pm 1.05	21.6 \pm 1.15	0.7665
IMF	0.5 \pm 0.13	0.4 \pm 0.08	0.1091	0.4 \pm 0.10	0.4 \pm 0.12	0.4055
Ash	1.2 \pm 0.12	1.2 \pm 0.08	0.4697	1.2 \pm 0.08	1.2 \pm 0.12	0.1786
<i>Sensory attributes (100-point scale):</i>						
Overall aroma intensity	75.51 \pm 5.62	74.92 \pm 5.35	0.5728	75.06 \pm 75.47	75.47 \pm 5.13	0.8055
Gamey aroma	74.01 \pm 6.41	73.08 \pm 7.01	0.3365	73.48 \pm 6.49	73.77 \pm 6.87	0.9357
Beef-like aroma	40.53 \pm 6.95	41.30 \pm 6.90	0.5170	40.38 \pm 7.23	41.32 \pm 6.61	0.1573
Metallic flavour	15.11 \pm 6.79	15.96 \pm 6.17	0.3216	15.30 \pm 6.86	15.63 \pm 6.23	0.8758
Sweet-associated aroma	15.26 \pm 7.89	16.26 \pm 7.23	0.3477	14.96 \pm 7.60	16.39 \pm 7.61	0.1098
Sour associated aroma	9.78 \pm 7.14	10.31 \pm 6.11	0.4984	10.41 \pm 6.82	9.59 \pm 6.63	0.2578
Gamey flavour	76.31 \pm 6.45	73.44 \pm 5.52	0.0069	74.83 \pm 6.13	75.40 \pm 6.34	0.4365
Beef-like flavour	41.49 \pm 8.53	43.74 \pm 6.71	0.1385	42.41 \pm 7.64	42.43 \pm 8.16	0.9032
Metallic flavour	19.11 \pm 7.03	17.08 \pm 6.99	0.0738	17.83 \pm 7.11	18.70 \pm 7.03	0.1900
Sweet-associated taste	14.99 \pm 7.10	16.33 \pm 6.80	0.3017	15.69 \pm 7.33	15.41 \pm 6.67	0.9063
Sour associated taste	12.52 \pm 5.63	11.58 \pm 6.95	0.3483	11.73 \pm 6.16	12.53 \pm 6.27	0.1973
Initial juiciness	51.43 \pm 11.11	54.10 \pm 12.32	0.1436	50.33 \pm 11.00	54.75 \pm 11.96	0.0084
Tenderness	58.59 \pm 11.47	49.63 \pm 12.70	0.0006	52.03 \pm 12.79	57.68 \pm 12.14	0.0028
Sustained juiciness	47.51 \pm 9.68	45.87 \pm 8.95	0.2480	45.61 \pm 9.09	48.04 \pm 9.58	0.0540
Mealiness	11.09 \pm 8.69	3.81 \pm 6.45	0.0000	6.42 \pm 7.59	9.69 \pm 9.26	0.0060
Residue	9.68 \pm 10.24	21.86 \pm 12.54	0.0000	18.05 \pm 13.32	11.46 \pm 11.26	0.0006

Abbreviations: BF= *biceps femoris* SM= *semimembranosus*, WBSF= Warner-Bratzler shear force, IMF= Intramuscular fat.

Parameters in **bold** differ (species interaction; muscle interaction) significantly ($p \leq 0.05$).

1. Species mean values includes the average for both muscles (BF and SM)
2. Muscles mean values includes the average for both bontebok and blesbok

Table 5.4 The Least Square Mean (\pm standard error) for the sensory attributes of two muscles (BF, SM) derived from two species (blesbok, bontebok) (species*muscle interaction).

Parameter	Blesbok		Bontebok		p-value
	BF	SM	BF	SM	
Overall aroma intensity	74.73 ^a \pm 6.24	76.29 ^a \pm 4.85	75.52 ^a \pm 5.35	74.32 ^a \pm 5.33	0.0462
Beef-like aroma	41.06 ^{ab} \pm 7.48	39.99 ^b \pm 6.39	39.41 ^b \pm 6.83	43.18 ^a \pm 6.51	0.0050
Metallic flavour	19.41 ^a \pm 6.78	18.81 ^a \pm 7.32	15.61 ^a \pm 7.04	18.54 ^a \pm 6.68	0.0328

Abbreviations: BF= *biceps femoris* SM= *semimembranosus*

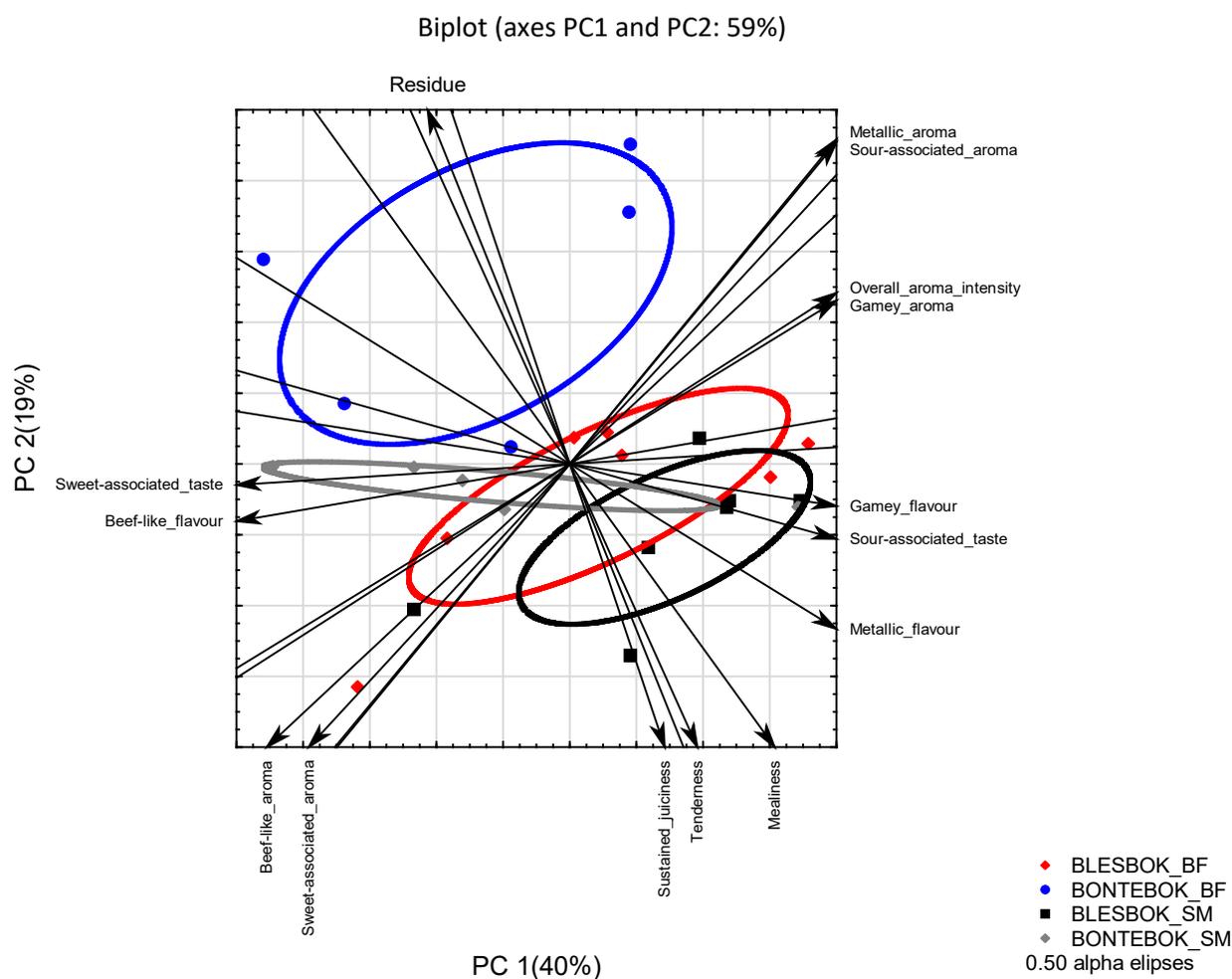


Figure 5.1 Principal component analysis (PCA) bi-plot indicating the means of sensory attributes observed for two species (blesbok, bontebok) and two muscle types (SM, BF)

5.4 DISCUSSION

The aim of the study was to establish the differences in sensory attributes, selected physical characteristics and the proximate composition of bontebok and blesbok sub-species and two muscle types: *biceps femoris* (BF); and *semimembranosus* (SM). The information could aid in the marketing and appropriate utilisation of meat derived from these species and muscle types. Furthermore, this study could also aid in establishing baseline data for bontebok sensory attributes.

The game industry in South Africa runs at a much smaller scale than the industry responsible for meat derived from domestic species (Hoffman, 2007; Erasmus & Hoffman, 2017). The culling of game species is often restricted to seasonal hunting periods and meat is often transported far distances, therefore, resulting in meat often being deboned, vacuumed packed and frozen until further usage (Van Schalkwyk & Hoffman, 2016). The current study simulated these conditions, by freezing and thawing muscles before the sensory analysis of the meat. Furthermore, blesbok and

bontebok muscles from the current study were aged for seven days before freezing as previous studies have suggested ageing to improve tenderness of meat (Lonergan *et al.*, 2010; Needham *et al.*, 2019a,b). The tenderising process is attributed to the intracellular proteolytic system disrupting the muscle structures (Lonergan *et al.*, 2010). Ageing is known to improve desired meat attributes such as tenderness and juiciness. However, undesirable sensory attributes could also increase during prolonged ageing periods in game species such as springbok, where metallic, gamey and liver-like flavours significantly increased during the ageing period (North & Hoffman, 2015). Microbial activity is also known to increase during ageing (Shange *et al.*, 2019), thus emphasising the importance of knowing the optimum ageing period of meat from different species. The ageing time for blesbok has been established as seven days (Mzuvukile, 2018) and according to Chapter 4, bontebok meat should be aged for eight days. Therefore, both species' meat was aged for seven days before the sensory analysis trial. The effect of freezing and thawing on the physical characteristics of meat has been studied, concluding in moisture loss to be the only re-occurring factor influenced by the former preserving method (Leygonie *et al.*, 2012). The effect of freezing on moisture loss is influenced by factors such as storage time as seen in frozen beef where slices of *longissimus thoracis et lumborum* (LTL) muscle resulted in a reduction of water holding capacity only after day 90 of freezing (Vieira *et al.*, 2009). A study involving roe deer (*Capreolus capreolus* L.) indicated that the meat can be frozen up to 10 months in order to sustain high meat quality (Daszkiewicz *et al.*, 2018). The bontebok and blesbok muscles were frozen for less than 90 days before usage and it is argued that although the freezing process could have influenced the quality of the meat from these species, this would not have been in any significant manner and, would have been the same for both species. Further research on the freezing of bontebok and blesbok meat would have to be conducted in order to support the above-mentioned statement.

The proximate composition of meat is known to be influenced by the dietary patterns of the animal, additionally having an effect on the sensory characteristics of the meat (Erasmus *et al.*, 2016; Neethling *et al.*, 2018). No significant differences were found between species or muscles for proximate composition, except for moisture content (Table 5.3) found to be higher in the BF than the SM muscles. The differences between the Least Square Means (LSMeans) values of the BF and SM muscles are not considered biologically significant, even though the p-value suggests otherwise ($p=0.006$). Moisture content in game has been reported to be 73.4-76.9 (g/100 g) in species such as springbok (Hoffman *et al.*, 2007), blesbok (Neethling *et al.*, 2014) and eland (Needham *et al.*, 2019a). Bontebok and blesbok are closely related sub-species, both grazers and harvested in the Western Cape. Therefore, the similarity in proximate composition between these species were expected.

It is important to note that the IMF content (g/ 100 g) of both bontebok and blesbok muscles were severely low as compared to BF and SM muscles from literature: blesbok (2.6-2.7) (Neethling *et al.*, 2014); and eland BF (1.6-1.8) (Needham *et al.*, 2019a). These low IMF values could be attributed to the drought South Africa had undergone during 2018, thus resulting in a lack of nutrition and lower fat reserves in the muscles of these animals. The month of April also falls within the rutting season of blesbok males and is right after the bontebok's rutting season during which they are more active when competing for females and feed less which could enhance the decrease in fat reserves for both species (Mysterud *et al.*, 2004; Furstenburg, 2016).

The sensory attributes of meat can essentially be divided into aroma, flavour and texture attributes. In the current study, meat aroma refers to orthosonal aroma as experienced through the nasal cavity and external nares. The flavour of meat refers to retronasal aroma which is experienced when consuming meat (Roberts & Aeree, 1995; Neethling *et al.*, 2016). No sensory attributes were affected by significant differences of the LSMeans between species and muscle type interaction, except for beef-like aroma. The beef-like aroma in the bontebok SM muscle was significantly higher than found in the bontebok BF muscle, whereas the beef-like aroma in the blesbok BF was numerically higher than in blesbok SM muscle (Table 5.4). A higher beef-like aroma in meat is preferred and desired by most consumers and is thus seen as a familiar and positive sensory attribute (Oltra *et al.*, 2015).

No aroma and flavour attributes were significantly affected by species, except gamey flavour that was slightly higher in blesbok than bontebok. The dietary intake of animals is known to influence their fatty acid profile and thus flavour of meat (Neethling *et al.*, 2016) as seen when comparing sensory profiles of kudu compared to impala where differences in sensory profiles were attributed to differences in diet (Hoffman *et al.*, 2009). Although the bontebok and blesbok were from two different regions, both in the Western Cape, they grazed on similar vegetation types. Bontebok and blesbok are both grazers consuming mainly short grass species and this could explain the lack of differences in aroma and flavour attributes, more specifically the small difference in gamey flavour between the two species. The sensory attributes of male blesbok from three regions were also found not to differ significantly between regions in terms of gamey flavour (Hoffman *et al.*, 2010). When converted to a 100-point scale, the average score for these blesbok were found to be 76.13 which is very similar to blesbok from the current trial (76.31) (Table 5.3). Gamey aroma was by far the largest contributor to overall aroma intensity (Table 5.3, Figure 5.1) and this has previously been found in other game species such as springbok meat (North & Hoffman, 2015). In contrast, beef-like aroma was found to be the largest contributor towards overall aroma in eland meat (Needham *et al.*, 2019b). It is important to note the complexity when accurately comparing DSA

studies due to differences in methodologies, sample collection and statistical analysis methods in addition to human assessors used (Neethling *et al.*, 2016). Furthermore, gamey aroma had a moderate positive correlation to metallic aroma ($r= 0.51$; $p<0.05$) just as gamey flavour has a strong positive correlation to metallic flavour ($r= 0.78$; $p<0.05$), which is in agreement to previous studies (Neethling *et al.*, 2016; Needham *et al.*, 2019b). Furthermore, gamey flavour also had a strong positive correlation to the sour taste ($r= 0.75$; $p<0.05$) which is known to be a negatively associated sensory attribute for consumers (Colle *et al.*, 2015). Blesbok and bontebok meat had a strong gamey aroma and flavour (Table 5.3) which could be disliked by red meat consumers; however, the flavour might be preferred by consumers who regularly consumer game meat. In literature, no robust study has been conducted on the diverse South African demographic groups in terms of game meat consumption. This information is needed to fully understand the utilisation and preference of game meat consumers' in terms of flavour, aroma and texture profiles. Furthermore, it has been suggested that the fatty acid profile of reindeer is influenced by diet. A higher natural grazing diet is linked to a "wild" attribute (which represents "gamey" in the current study) (Wiklund *et al.*, 2003). This was also supported by Maughan *et al.* (2012) where cattle that were fed on grass instead of grain had a higher gamey flavour. Blesbok and bontebok are both strictly grazers, possibly explaining their high gamey flavour. However, further research on the fatty acid profile of bontebok would be needed to support the above-mentioned statement.

Similar to the lack of differences in aroma and flavour attributes between species, no significant differences in the former attributes were detected between muscle type (BF and SM). Muscles differ in anatomical location, thus in function and muscle fibre type (Taylor, 2004). Different muscle fibre types utilise different main energy sources and it is expected that they will have different fatty acid profiles, consequently differences in flavour (Wood *et al.*, 2003; Li *et al.*, 2007). Both the BF and SM are hindquarter muscles, similar in function which is to aid in walking and running when threatened (Swatland, 1994). The similarity in function between the latter muscles could explain the lack of difference in terms of aroma and flavour attributes.

Texture attributes were greatly affected in both species and muscle type (Table 5.3). As expected, WBSF was a reliable instrumental prediction of sensory tenderness as perceived by a trained sensory panel. A strong negative correlation was found between WBSF and tenderness ($r= -0.84$; $p<0.05$), which is in agreement with other studies (Neethling *et al.*, 2018; Needham *et al.*, 2019b). North and Hoffman (2015) also found a negative correlation for WBSF and tenderness in springbok, however, an unexpected "weak" negative correlation was observed ($r=-0.4$). For species, the bontebok had a significantly higher WBSF and lower sensory tenderness than blesbok meat. According to Destefanis *et al.* (2008) consumers would classify blesbok as tender (<42.87 N) and

bontebok as tough (>52.68 N). Tenderness is known to be one of the most important characteristics of meat (Bhat *et al.*, 2018) and a higher tenderness is associated with positive consumer preferences (Oltra *et al.*, 2015). Further research is required in order to establish whether the WBSF of bontebok is generally tougher than blesbok (repeat sensory analyses on bontebok, larger group of bontebok, investigate muscle fibre typing, collagen content, etc.) or whether the bontebok from this trial is not a true reflection of its WBSF values. This could be due to the 2018 drought that could have influenced the sensory and physical characteristics of bontebok meat to a larger extent than blesbok. Alternatively, the tougher bontebok could be due to the higher level of ante-mortem stress experienced (see Chapter 3), an argument further supported by two of the bontebok being classified as DFD. Also, blesbok are known to tolerate heat well as they originate from warmer areas such as the flatter grassland of central, northern and eastern parts of South Africa, whereas the bontebok originates from the East Coast Renosterveld in the Overberg region (Furstenburg, 2016). Furthermore, the blesbok harvested for the current trial were harvested from an area close to the coast, thus it can be expected that this area has lower average daily temperatures than the area in which bontebok were harvested (warmer in-land area). Monthly average weather predictions confirm the above-mentioned statement (<https://en.climate-data.org/africa/south-africa/western-cape/wellington-23411/>; <https://en.climate-data.org/africa/south-africa/western-cape/witsand-27417/>).

For muscle type, the SM muscle had a significantly lower WBSF and higher tenderness than the BF muscle (Table 5.3), thus indicating the SM (independent of species) has a better tenderness profile than the BF muscle. The differences between the LSMeans were biologically small due to the SM and BF muscles both forming part of the hindquarter muscles with similar functions. The small differences in WBSF and tenderness between muscle type could be attributed to various factors such as differences in muscle fibre type, collagen content or pH_u as previously established to influence WBSF/tenderness of meat (Kohn, 2014; Bhat *et al.*, 2018; Rudman *et al.*, 2018; Needham *et al.*, 2019a).

Although other texture attributes such as residue and mealiness differed significantly between species and muscle type, it is important to note they were rated on the lower end of the 100-point scale. Residue in this study referred to the remaining residual tissue in the mouth after the first 10 chews, whereas mealiness was referred to as the remains of disintegrated muscle fibres left on the tongue after the first few chews (Table 5.2). In the current study, residue had a strong negative correlation to tenderness ($r=-0.92$; $p<0.05$) and mealiness ($r=-0.83$; $p<0.05$). The negative associations between the latter sensory attributes are clearly represented in the PCA biplot (Fig. 5.1). The same inverse relationship between tenderness and mealiness, was found for springbok ($r=$

0.59 and -0.63, respectively); Neethling *et al.* (2018) expected and observed an inverse relationship between tenderness and residue of springbok due to muscle fibres not disintegrating upon mastication because of a higher connective tissue content. Furthermore, the perception of tenderness was explained to be influenced by a lower IMF content of springbok. Bontebok and blesbok meat had extremely low IMF contents (Table 5.3) as compared to other game species in literature, which could possibly have influenced the perception of tenderness as observed by the sensory panel. The juiciness of meat is known to be related to the IMF of meat. This was not the case for the current study in which IMF content had an extremely weak positive correlation to initial juiciness ($r=0.01$) and sustained juiciness ($r=0.05$). This could also be attributed to the low IMF found in blesbok and bontebok meat (Table 5.3). Bontebok had a significantly higher residue and lower mealiness compared to blesbok, whereas the SM muscle had a significantly higher mealiness and lower residue than the BF muscle. Both the latter attributes are unfavourable to consumers. In terms of mealiness, the bontebok and BF muscle type had higher sensory scores than the blesbok and SM, respectively. For residue, the opposite is true.

A low-to-moderate negative correlation was found between the cooking loss percentage (%) and initial juiciness ($r=-0.40$; $p=0.05$), whereas the negative correlation between cooking loss % and sustained juiciness was not significant ($r=-0.35$; $p=0.07$). Higher cooking loss % (more moisture loss during cooking) is expected to result in a lower juiciness as observed by the sensory panel that analysed previously aged and frozen springbok steaks (North & Hoffman, 2015). As mentioned previously, the low IMF content of blesbok and bontebok from the current trial could have influenced the tenderness and juiciness perception of the sensory panel. Significant differences between muscle type (BF, SM) was found for cooking loss % and initial juiciness. The BF muscle had a significantly higher cooking loss % and a lower initial juiciness than the SM muscle. A higher rating for juiciness is known to positively influence consumer preference (Oltra *et al.*, 2015), thus further contributing to the SM being superior to the BF muscle.

Two outliers were removed from the dataset due to the animals having a $pH_u > 6$ and classified as DFD meat. The data from these animals could have skewed the data as it has been found in the past that DFD meat influences sensory and physical meat quality parameters (Devine *et al.*, 1993; Renecker *et al.*, 2005; Hoffman *et al.*, 2007). These animals were stressed before harvesting occurred and further information regarding the physical characteristics of these animals can be found in Chapter 3. In the current study, the trained panel picked up “off-flavours”, a very “sour” taste and a “mushy” texture from one of the outliers with an exceptionally high pH_u . According to Shange *et al.* (2019), once glycogen is depleted (high pH_u), free amino acids are used as

nutrient source, resulting in off-flavours and odours that are caused by the release of by-products such as hydrogen sulphide and ammonia.

5.5 CONCLUSION

This study investigated the differences in sensory attributes between muscles (BF, SM) and species (blesbok, bontebok). The only aroma and flavour attribute that differed significantly between the former species, was gamey flavour which was found to be slightly higher in blesbok than bontebok. This was attributed to the similarity in diets of the latter species (both specialized grazers). Gamey flavour and aroma were scored relatively high and was the largest contributor towards overall aroma for blesbok and bontebok. A high gamey flavour is known as an undesired meat characteristic for the general meat consumer; however, game meat consumers' might prefer the higher gamey sensory attributes if the meat is marketed correctly. Furthermore, the textural attributes that differed significantly between species were tenderness, mealiness and residue. Tenderness was positively correlated to mealiness and negatively correlated to residue. The bontebok had a significantly lower tenderness (higher WBSF) than blesbok and could be perceived as "tough" by consumers, as compared to blesbok meat that could be classified as "tender"; although it is not clear whether this was a sub-species difference or caused by differences in ante-mortem stress. Although a smaller difference was observed between LSMeans for the physical and sensory attributes between the different muscles (BF and SM), it was still noted that the SM muscle could prove to be superior due to its higher tenderness and initial juiciness based on previous studies. Bontebok and blesbok could both be marketed as a healthier alternative to red meat from domestic species, due to their low IMF and high protein contents. Furthermore, the general meat consumer would not necessarily be able to distinguish between meat from the two species.

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CHAPTER 6: GENERAL CONCLUSIONS AND RECOMMENDATIONS

This study is the first attempt to determine baseline data for meat quality characteristics of bontebok meat, to compare muscle types and to compare the meat quality traits of bontebok to its closely related sub-species, the blesbok, where applicable. Two trials were conducted: one in March (n=12); and one in April (n=8). Mature male bontebok from March represented bontebok used in Chapter 3 (carcass composition, physical and chemical characteristics) and April mature male bontebok in Chapter 3 (only carcass composition and physical characteristics), Chapter 4 (ageing/microbial activity) and Chapter 5 (sensory attributes). All indications are that bontebok can provide consumers with a safe, healthy meat of good quality, thereby contributing towards economic stability and food security in South Africa. Although bontebok meat quality compared favourably to the more commonly consumed blesbok and showed potential to be utilised in the game meat industry, meat from the former species was classified as “tough” and darker than other game species, which could negatively influence consumer acceptance. However, this could be due to the drought in the Western Cape during 2018, ante-mortem stress and active males during the mating season and therefore, this study should be repeated in more favourable weather conditions (particularly referring to the absence of a drought) with a higher number of bontebok used in each trial. True to their nature, the bontebok would run excessively when confronted with the harvesting team and this ante-mortem stress caused numerous physical meat quality traits associated with ante-mortem stress and dark, firm and dry (DFD) meat. Furthermore, muscle type was found to be a large contributor towards significant differences in physiochemical characteristics, indicating that muscle type influences meat quality and muscles should be marketed correctly for optimum utilisation of meat by consumers.

The carcass composition of bontebok is similar to blesbok and could not be compared to other bontebok due to the absence of information in literature regarding the yields and meat quality of the former species. However, lower carcass yields could be expected due to the drought and consequently the lack of nutrition, as well as active males losing weight during the mating season. Edible offal made out a substantial amount of the bontebok internal offal percentage and shows potential to be utilised as a low-cost edible high protein by-product. Before the organs can be distributed for commercial use, future research should investigate the microbial activity and safety of organs as they are known to have higher microbial contents.

All physical and chemical characteristics tested were significantly affected by muscle type. However, the chemical composition differences between muscles were marginal and would not be expected to influence consumer acceptability. The average pH_u values of bontebok meat was much higher (>5.8) than reported for “normal” meat in literature; this was attributed to the

aforementioned ante-mortem stress. The forequarter muscles (IS and SS) and fillet had significantly higher $pH_u > 6$ and lower WBSF values than the hindquarter and LTL muscles. The physical characteristics help aid in the prediction of which muscles could be used for further processing and which should be used as fresh meat cuts. However, the fluctuation in meat quality due to high pH_u values in the current study makes it difficult to predict the abovementioned statement although the fillet and LTL muscles are known to be meat cuts of higher tenderness and commercial value, respectively and forequarter muscles are generally known to be tougher. The bontebok's chemical composition displayed high protein and extremely low intramuscular fat (IMF) contents which can be favoured by "health-conscious" consumers who believe that low fat and high protein diets is healthy. However, extremely low IMF contents could pose the risk of negatively influencing the tenderness, juiciness and flavour perceived by consumers or a trained panel as seen in a descriptive sensory analysis that investigated the differences in aroma, flavour and texture profiles between two muscle types (BF and SM) of the two species (bontebok and blesbok). Although blesbok and bontebok did not differ substantially in terms of sensory attributes, the blesbok was rated superior in certain texture attributes compared to bontebok. Blesbok had a significantly higher sensory tenderness (lower WBSF) than bontebok. Furthermore, the SM muscle was more tender than the BF. These muscles are both hindquarter muscles, similar in function, thus future research should investigate other muscles such as the LTL and forequarter muscles (SS and IS) to compare sensory profiles if sample size allows it (as forequarter muscles are small). Furthermore, no significant differences for flavour and aroma profiles were found between muscle type or species, except gamey flavour that was the highest contributor towards overall flavour and was found to be slightly higher in blesbok than bontebok. The lack of differences is attributed to the bontebok and blesbok both being strict grazers, thus consuming similar diets (as both species also came from similar biomes in the Western Cape), resulting in similar aroma and flavour profiles. Bontebok harvested under better harvesting conditions (less ante-mortem stress) could result in better texture profiles such as higher sensory tenderness (thus lower WBSF) and juiciness and should be investigated. Night culling could be an option and warrants further research and evaluation as to its efficiency.

The ageing of bontebok meat significantly reduced the WBSF (higher sensory tenderness); the optimum ageing period was established to be eight days, when bontebok LTL muscle steaks were vacuum sealed and stored at 4°C. Although bontebok meat proved to rapidly tenderise over time, the meat could still be perceived as tough and dark by general consumers. Colour generally improved over time in addition to the weep loss percentages that were all within acceptable limits during the ageing trial, except at day 12 where a weep loss of >4% was found. Total plate count and *E. coli*/coliform counts did not differ significantly between ageing days and were all within

specifications as supplied by international standards throughout the entire ageing trial. In addition, all samples tested negative for *Salmonella*. Bontebok meat could be classified as safe for consumption up until day 12 under these experimental conditions, thus future research could repeat the ageing trial by extending the ageing period of bontebok meat in order to establish whether the tenderness of bontebok meat could be improved to acceptable limits or not. Better harvesting conditions (such as lower ante-mortem stress), could further aid in improving the colour, moisture loss and sensory attributes (specifically the texture attributes) during the ageing period.

Two bontebok animals from the April trial with pH_u values >6 were classified as DFD and removed from the dataset. One of the outliers had an extremely high pH_u that resulted in very tender meat (postulated as ante-mortem muscle breakdown), high water holding capacity, dark colour, unacceptable microbial activity and undesired sensory profile (“off”-odours, sour taste and a “mushy” texture).

The current study is the first attempt to quantify the baseline data for meat quality traits of bontebok. The latter species generally compared favourably to blesbok and other game species specified in literature in terms of carcass composition, sensory profile and proximate composition, however, the bontebok produced a tougher and darker meat. The platform for further research is extensive and a wide spectrum of factors could be tested such as the influence of sex, age, region and season. Furthermore, muscle fibre typing, fatty acid profile and collagen content of different bontebok muscles should be tested to further explain results obtained. Due to the limited availability of bontebok (currently listed as “vulnerable”), further research would be challenging, especially when investigating properties such as sex, where females are not harvested as regularly as males. The current study is very valuable in order to improve the marketability of bontebok meat due to bontebok meat currently being underutilised in the game meat industry. However, extensive future research on bontebok is debatable due to the lack of differences between bontebok and its closely related popular sub-species, the blesbok which occurs abundantly in South Africa. Furthermore, the blesbok is better adapted to warmer climates (environmental temperatures are increasing) and has a much better tenderness profile than bontebok.