Influence of ultimate muscle pH on the microbial quality of black wildebeest (*Connochaetes gnou*) meat.

by Thandeka Nedia Makasi

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Supervisor: Prof. L.C. Hoffman

Co-supervisor: Prof. P. Gouws

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Declaration

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Abstract

The microbial growth, colour stability and pH changes for black wildebeest (*Connochaetes gnou*) meat under chilled (4.2±0.8°C) vacuum storage were investigated. The investigation centred on the role of ultimate muscle pH on shelf life of the meat. Although bacterial growth was observed over time for both DFD (pH >6) and Normal (pH <6) meat, DFD meat exhibited higher growth rates for lactic acid bacteria (LAB), total viable counts (TVC) and total coliforms. This was attributed to the combination of high pH and possibly the depletion of glucose in the DFD muscles. On the other hand, the growth rate of total coliforms was less than what was observed for the other microorganisms tested. It was assumed that chilled vacuum storage in combination with the high levels of LAB inhibited the growth of total coliforms. Salmonella was not detected in any of the samples analysed. There were no changes in pH during the 12 days storage period for DFD meat whereas pH for Normal meat decreased towards the end of storage possibly due to lactic acid production by LAB. The colour changes were more noticeable in Normal meat (more browning) than in DFD meat after blooming for 30 min. The conclusion for this study was that DFD meat spoiled faster than Normal meat.

The meat was further subjected to preservation by oregano essential oil (1% $v \cdot v^{-1}$). In this case, there was an initial inhibition of TVC, LAB and total coliforms. Furthermore, the growth rates for TVC and LAB were lower ($p<0.05$) in the oregano oil treatment group than in the control. For total coliforms however, there was only an initial inhibition observed and no effect on the growth rate. Addition of oregano essential oil also resulted in a significant lowering of meat pH. This may have added to the microbial inhibition observed. Based on TVC values, addition of oregano essential oil extended the shelf-life of black wildebeest meat by 3 days. At the beginning of the study, the lipid oxidation (TBARS) values were above the threshold for detection. Also, the percentage of metmyoglobin had exceeded the levels at which browning becomes visible. Therefore, conclusions on the effects of oregano essential oil on the colour and lipid oxidations were not made in this study. However, oregano essential oil inhibited microbial growth and stabilised TBARS throughout the 9 day storage period. Therefore there is potential to use oregano essential oil as a preservative for black wildebeest meat, although more research is needed.

Opsomming

In hierdie studie word die mikrobiese groei, stabiliteit en pH kleur verandering ondersoek vir swartwildebeestevleis onder verkoelde (4.2 ± 0.8 ° C) vakuum berging. Die ondersoek is spesifiek gefokus op die rol van die eind-spier pH op die raklewe van die vleis. Alhoewel mikrobiese groei vir beide DFD (pH >6) en Normal (pH <6) vleis waarneembaar was met verloop van tyd, het die DFD vleis hoër groeitempo vir melksuurbakterieë (MSB) en totale lewensvatbare tellings (LVT) getoon. Dit was as gevolg van die kombinasie van hoë pH en die moontlikheid van die vermindering van die glukose in die DFD spiere. Aan die ander kant was dit waargeneem dat die groeikoers van die totale kolivormig bakterieë minder was, teenoor die ander mikro-organismes wat getoets was. Dit was aangeneem dat die verkoelde vakuum stoor die groei van die totale kolivormig bakterieë geïnhibeer het. Salmonella was nie opgespoor in enige van die geanaliseerde monsters nie. Daar was geen verandering in pH tydens die stoor tydperk vir DFD vleis nie, maar die pH vir normale vleis het tydens die einde van die stoor tydperk afgeneem. Die kleur verandering onder vakuum stoor was meer waarneembaar in die normale vleis as wat dit was in die DFD vleis. Die gevolgtrekking van hierdie studie was dat DFD vleis baie vinniger bederf teenoor normale vleis. Maar daar was variasie op die gewig van die oorspronklike mikrobiese lading en dit kon die bakteriese groeitempo van die normale vleis beïnvloed.

Die vleis is verder behandel met oregano essensiële olie (1 % $v \cdot v^{-1}$) vir preservering . In hierdie geval, was daar 'n aanvanklike inhibisie van LVT, MSB en totale kolivormig bakterieë. Verder was die groeitempo vir LVT en MSB aansienlik laer ($p<0.05$) in die behandelings groep teenoor die in die kontrole . Vir die totale kolivormig bakterieë was daar egter net 'n aanvanklike inhibisie waargeneem en geen effek op die groeikoers nie. Die byvoeging van oregano essensiële olie het ook gelei tot 'n beduidende verlaging van die pH. Dit kon gelei het tot die mikrobiese inhibisie wat waar geneem was. Gebaseerd op die LVT, het die byvoeging van oregano essensiële olie gelei tot die verlenging van die swartwildebeeste vleis se raklewe met 3 dae. Aan die begin van hierdie studie was die lipied oksidasie (TBARS) waardes bo die drumpel van opsporing. Ook, die persentasie van metmyoglobin het die vlakke waarop verbruining sigbaar word, oorskry. Daar is potensiaal vir die gebruik van oregano essensiële olie as n middel vir die verlenging van swartwildebees vleis, maar nog navorsing is nodig.

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Chapter 1: Introduction

In South Africa, the game meat industry has experienced significant growth; Cloete & Rossouw (2014) remarked that the industry has evolved from just an alternative use for land to a 'multi-dimensional' industry. Saayman *et al*. (2011) reported that although trophy hunting remains one of the major drivers of game ranching, biltong hunting is also growing. Saayman *et al*. (2011) further noted an increase from R3.1 billion in 2005 to R4.1 billion in 2007 in biltong hunting revenue. Oberem (2011) reported that for South Africa to cope with population growth in the next 15 years, there is a need for doubling of food production. Apart from providing a partial solution to global food crisis (Heeb *et al*., 2011), game farming can also stimulate economic growth through job creation (Van der Merwe *et al*., 2004). Hoffman (2007) reported that the game farming industry continues to grow, with an expected annual increase of 2.5%. It can also be expected that as game ranching increases, the amount of game meat available on the market will most likely increase.

Based on these increases, progressive research has been done on game meat, specifically focusing on differences between physical quality attributes as a result of season, gender or species differnces (Hoffman & Wiklund, 2006; Hoffman *et al*., 2008; Hoffman *et al*., 2011; Neethling *et al*., 2014). Currently, there is considerable knowledge on different game meat quality attributes as compared to the past years although there is still need to increase knowledge on production systems, slaughter procedures and their effects on the quality of meat produced (Hoffman & Wiklund, 2006). In addition, the increase in the potential for use of game meat locally and also for export necessitates the need to broaden the research to also focus on microbial quality and safety. Van der Merwe *et al*. (2011) reported that generally, the health status of game meat is unknown and this could potentially damage the image thereof if any zoonotic diseases or food poisoning arise as a result of game meat consumption.

Modern consumers are increasingly aware of their environment and health and the niche for game meat marketing has been identified as organic (Hoffman *et al*., 2004); in the sense that there is little to no agricultural input and the meat is therefore produced responsibly. Also, the nutritional profile of game meat means that it can be marketed as healthier red meat (Hoffman, 2007), which can serve as an alternative to the traditional red meat commonly consumed.

Problem statement

As with most wild animals, stress experienced during the harvesting process may result in meat of a poorer quality (Hoffman & Wiklund, 2006). Although harvesting systems are being developed to

ensure optimum meat quality (van Schalkwyk *et al*., 2011), sometimes the process can be unpredictable such that the meat produced may also be compromised. As observed for black wildebeest (*Connochaetes gnou*) meat, in some cases Dark, Firm and Dry (DFD) meat is produced as a result of ante-mortem stress. In meat from other species such as beef, this occurrence has been shown to quicken spoilage (Priolo *et al*., 2001). Adzitey & Nurul, (2011) reported that DFD occurs when an animal is exposed to prolonged stress prior to slaughter. Also, consumers discriminate on this quality defect, due to the darker colour of the meat. While the appeal of the meat is an issue, the microbial quality as a result of ultimate pH is also important in determining shelf-life and possibly safety.

Aims and objectives

Currently, there is limited research on the microbial quality of indigenous game species in South Africa and none focusing on black wildebeest meat. Therefore the main goal of the study was to increase the knowledge base on game meat, with particular focus on the microbial quality of black wildebeest meat. This will possibly initiate research on the microbial profiles of different game animals and the shelf-life of the meat thereof. The study was divided into two sections; the aim of the first study was to determine the microbial quality of black wildebeest (*Connochaetes gnou*) meat and how ultimate pH (ranging from 5.54 to 6.49) influenced the growth of indicator microorganisms. The colour stability was also measured to determine whether the meat was acceptable according to instrumental colour. As mentioned, there is potential to market game meat as organic; therefore another aim of the research was to investigate the effect of a natural preservative (oregano essential oil) on the shelf-life stability of black wildebeest meat. As the meat used had different ultimate muscle pH values, the study also determined the influence of ultimate muscle pH on the efficacy of the essential oil. The colour and lipid oxidation stability during storage was also measured. Furthermore, Hoffman & Dicks (2011) gave preliminary results indicating that game meat was somewhat resistant to microbial contamination when compared to meat from domesticated species. Therefore, more research on the microbial aspect of game meat will lead to ascertaining these findings and possibly the development of distinct spoilage limits for game meat.

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Chapter 2: Literature review on the meat quality of game meat: Focus on microbiology of meat.

2.1 Introduction

Meat is consumed in many communities and continues to be one of the most esteemed foods. Meal planning is typically centred around meat (Haley, 2001) especially in the impoverished communities where it is the highlight of the meal (Viljoen *et al*., 2005). Livestock rearing has been practiced on different scales in South Africa for a considerable period of time. While the majority of the land is used for extensive grazing for domesticated animals, a growing industry is that of game farming. Popularity of game ranches is increasing and this has been characterised by the conversion of cattle farms into game ranches, or a combination of both (Bengis *et al*., 2002; Cloete *et al*., 2007). Apart from the use of game ranches for meat production, they can also be used for other activities. Berry (1986) identified non-consumptive uses of game ranches which include wildlife photography, game viewing and bird viewing. Van der Merwe *et al*. (2004) further identified the main pillars of game tourism as farming of endangered species, hunting, ecotourism and the production of processed game meat products. In South Africa, the majority of game ranches are in Limpopo, followed by the Eastern and Northern Cape (Hoffman, 2007). The game industry is growing steadily in South Africa and there is need for more research on game meat in general.

In parts of Europe and Australia, game meat is defined as venison, which includes meat from domesticated animals such as the deer or kangaroo (Hoffman & Wiklund, 2006). However in Africa, game meat is used to refer to terrestrial animals which can be used for food, either commercially or for subsistence purposes (Hoffman & Cawthorn, 2012). In the African context, game meat can include meat from small animals (hare, rodents, guinea fowl, etc.), meat from larger animals (blesbok, springbok, nyala, wildebeest, zebra, impala, etc.) or meat from large birds (ostrich) (Hoffman & Cawthorn, 2012). While there is research which shows that purchase of game meat by consumers is limited as a result of poor education and finance (Hoffman *et al*., 2005), the industry has great potential. In the developing African countries, game meat has been used for human consumption for a number of years (Mostert & Hoffman, 2007). In societies which are plagued by malnutrition, game meat can be used as an alternative protein source (Hoffman & Cawthorn, 2013). It is surprising to note that tourists visiting South Africa were more knowledgeable on the eating quality and health benefits of game meat as compared to locals (Hoffman *et al*., 2005). However, the perceptions of local consumers can be changed through positive marketing and education. The modern consumer

considers health as one of the most important factors when purchasing meat (Hoffman *et al*., 2005), therefore, game meat has great potential of being marketed as healthier red meat.

Another important factor considered with any food is the issue of safety. Food safety is a priority to consumers and food producers worldwide and it is continually enforced (Aymerich *et al*., 2008). As marketing and consumption of game meat increases, information regarding its safety also needs to be available. Bacteria are a common occurrence in the environment, where they can either be advantageous or otherwise. Bacterial growth in meat is influenced by a number of factors, including water activity, acidity, redox potential, nutrition available and temperature (Zhou *et al*., 2010; Hamad, 2012). Most meat preservation techniques aim at the inhibition of microorganisms through altering the environment surrounding the meat. Although focus can be placed on controlling one factor, for example temperature, it is common practice to use hurdle technology (Aymerich *et al*., 2008; Zhou *et al*., 2010); this is when a number of factors are changed, and they become barriers which the microorganism should overcome if it is to survive the process. While this is useful in most preservation methods, some meat types are more prone to spoilage than others. This may be due to intrinsic factors, one of which will be the main objective of this study (ultimate muscle pH). Some wild animals, including black wildebeest and ostrich are prone to a phenomenon called DFD (dark, firm, dry) meat which occurs when the pH of meat post-mortem remains higher than 6 (Lawrie, 1998). DFD is a result of glycogen exhaustion in the muscle prior to slaughter, which results in insufficient lactic acid production post-mrtem (Lawrie, 1998). In wild animals, this is usually due to stress during the harvesting process (Hoffman & Laubscher, 2009). Insufficient acidification consequently affects colour, water holding capacity and microbial quality of the meat (Newton & Gill, 1978; Lawrie, 1998).

Although a number of factors are involved in defining meat quality, these will not be explored in detail in this review. Firstly, game meat will be discussed in the South African context. Thereafter, a comparison of the nutritional content of game meat to other meats will be conducted. After that, the methods of cropping of game meat will be discussed, in relation to their effects on game meat quality. Thereafter, the mechanism of post-mortem glycolysis will be discussed in relation to ultimate pH and its effect on microbial growth. Microbial quality of game meat will then be introduced, in relation to this research project, followed by a discussion on meat packaging and spoilage. Lastly, preservation of fresh meat will be explored, with focus on the use of natural preservatives.

2.2 Game meat in the South African context

In South Africa, the most common game meat consumed is springbok (*Antidorcas marsupialis*) followed by blesbok (*Damaliscus pygarus phillipsi*), kudu (*Tragelaphus strepsiceros*) and gemsbok (*Oryx gazella*) (Jansen van Rensburg, 1997). Consequently, research on game meat has been mostly focused on springbok. In research by Hoffman *et al*. (2005) on the consumer perception of game meat, they found that South African consumers lack knowledge on the quality and preparation methods of game meats. Furthermore, white consumers were more willing than the black and coloured consumers to try out different species of game meat and were willing to purchase game meat for its health benefits (Hoffman *et al*., 2005). Black and coloured consumers considered game meat as too expensive and would not buy it based on cost. The research was successful in identifying the target market for game meat as white and other races depending on education level; the higher the level the more likely that they would purchase game meat. Tourists were also identified as a viable market for game meat (Hoffman *et al*., 2003). However, Bekker *et al*. (2011) reported that some consumers perceived game meat as being inferior to other types of meat. This perception may be because of the lack of standard cuts or quality in the game meat industry (Hoffman *et al*., 2004). To address this, Wildlife Ranching South Africa (WRSA) recently launched a quality grading system to ensure consistent quality of game meat being marketed (The International Registration of the WRSA Game Meat Standard, 2015). Furthermore, to appeal to a broader market, game meat can be made into familiar processed products (Hoffman, 2007; van Schalkwyk *et al*., 2011a), or the other cuts can be sold at a cheaper price (Heeb *et al*., 2011).

Game meat in South Africa can be classified as an organic product (Hoffman *et al*., 2007). The animals are not normally provided with formulated feeds and are left to roam freely (Hoffman & Wiklund, 2006). Furthermore, antibiotics, fertilizers and growth promoters which are used in conventional livestock farming are not used in game ranching (Du Buisson, 2006; Hoffman & Wiklund, 2006).

The game meat industry has been damaged by numerous misconceptions however, including over emphasising toughness and dryness. While Jansen van Rensburg (1997) reported that springbok meat was dry, it was explained as being from stressed animals which could possibly result in DFD (dark, firm, dry) meat (Hoffman, 2001). Ensuring that the animals are not stressed prior to shooting could result in lower toughness and improved quality. Another area where focus could be placed to improve the image of game meat is educating consumers on the cooking methods to ensure improved tenderness. Although the concerns with game meat are usually toughness, dryness and colour, some consumers have issues with the 'gamey' flavour of game meat. This has been described as a metallic, urine-like,

faecal, straw-like flavour and smell (Jansen van Rensburg, 2001; North & Hoffman, 2015). From experience, acceptance of this flavour is an acquired taste and may not be acceptable to those who are not familiar with it.

While game animals flourish with no or minimal human interference, this also means that they are prone to illnesses which can be passed on to humans (zoonotic diseases). Magwedere *et al*. (2013) gave an in-depth analysis on the problem of zoonosis as a result of wildlife. Among the most dangerous zoonotic diseases are Rift Valley fever, foot and mouth disease, influenza (Magwedere *et al*., 2013), and bovine tuberculosis (bTB) (Bekker *et al*., 2012). As registered game abattoirs in South Africa are limited and mostly used for meat intended for export (Bekker *et al*., 2012), there is risk of zoonotic diseases being passed undetected from uncontrolled slaughter facilities to the unsuspecting consumer. With bTB in particular, carcasses with the *Mycobacterium bovis* lesions could be consumed thus increasing the risk of contracting tuberculosis (Van der Merwe & Michel, 2010). This may be problematic in the poorer or rural communities where most of the game carcasses and meat is not inspected. Educating consumers on the possible risk of consuming organs of game animals was recommended by Van der Merwe & Michel (2010). Although Van der Merwe & Michel (2010) found that the processing measures they used in their study were able to successfully kill *Mycobacterium bovis* in game animal tissue, there is need for more research to elucidate the threat of zoonotic diseases from game meats. Magwedere *et al*. (2013) also discouraged the consumption of uncooked organs or blood which is practiced in some cultures as this increases the risk of contracting zoonotic diseases. The threat of exporting game meat (legally and illegally) and the spread of these diseases to other parts of the world is not known (Hoffman & Cawthorn, 2013). There is need for systems to be in place which will eliminate or reduce the risk of zoonotic diseases being spread from infected game to humans.

2.2.1 Nutritional content of game meat in comparison to other red meat

Meat is a major source of proteins, vitamins and minerals. Conventional red meat such as beef, sheep and pork often have relatively higher levels of fat when compared with most game species. This can be attributed to the fact that they receive food throughout the year, while for game animals, food is usually scarce during the winter months (Wiklund, 2001). The effect of season on the body composition of game animals has been studied by Hoffman *et al*. (2009a) in black wildebeest. Black wildebeest meat harvested in spring had a lower protein content than that harvested during winter (Hoffman *et al*., 2009a). However, in blesbok season had no influence on the proximate composition of the meat (Neethling *et al*., 2014), this was attributed to the fact that the specific animals evaluated were in a region that received rainfall throughout the year and also blesbok are specialised grazers; therefore their diet would not have been influenced strongly by plant species. Apart from season,

other factors including species, gender or type of muscle can have an effect on the nutritional content of meat. The nutritional composition of game meat in comparison to beef and sheep are shown in Table 2.1; although there may be differences in fat content of game species based on gender, season and animal type, the fat content is still significantly lower (between 1 and 3%) than that of domesticated red meat.

Table 2.1: Nutritional content of beef and selected game species.

Species	Moisture %	Protein %	Fat content %	References
Beef (*Bos taurus*)(LD)	73.84	19.78±2.538	14.28±0.335	Vermaak, (2006)
Black Wildebeest (*Connochaetes gnou*)(autumn)	74.32±0.38	24.3±0.33	0.97±0.09	Hoffman *et al*., (2009a)
Blue wildebeest (*Connochaetes taurinus*)	76.04±0.54	22.28±1.20	1.06±0.55	Hoffman *et al*., (2011)
Blesbok (*Damaliscus phillipsi*)(LD)	73.64±1.153	22.92±1.036	2.34±0.36	Du Buison, (2006)
Eland (*Taurotragus oryx*)	75.8	ND	2.4	Von La Chevallerie, (1972)
Gemsbok (*Oryx gazella*)	76.9	ND	1.9	Von La Chevallerie, (1972)
Impala (*Aepyceros melampus*)(LD autumn)	74.52±0.15	21.66±0.28	2.22±0.10	Mostert, (2007)
Kudu (*Tragelaphus strepsiceros*)	74.49±0.162	23.6±0.181	1.58±0.056	Mostert & Hoffman, (2007)
Red hartebeest (*Alcelaphus caama*)	74.93	23.19	0.51	Hoffman *et al*., (2011)
Springbok (*Antidorcas marsupialis*) (LD)	72.16±1.682	24.18±1.476	2.27±0.579	Neethling *et al*., (2014)
Warthog (*Phacochoerus africanus*)	74.04	22.14	1.69	Hoffman & Sales, (2007)

LD =*longissimus dorsi* muscle

More importantly, the types of fatty acids which are more prevalent in a meat sample (saturated or unsaturated) are more important indicators of the impact that meat will have on health and diet. Saturated fatty acids associated with red meat consumption have been shown to have a link with chronic diseases such as type 2 diabetes, hypertension, cardiovascular diseases and cancers (McAfee *et al*., 2010). Although this seems to be an accepted concept, in the United Kingdom when there was a decrease in red meat consumption, the incidence of colon cancer increased (McAfee *et al*., 2010). This gives the impression that the implication of red meat in some of these lifestyle diseases may have been overestimated.

The increase in diet related diseases to an epidemic level has led to the development of guidelines to alleviate the problem. Approximately 1 billion adults in the world can be classified as overweight and from that, 300 million are considered clinically obese (WHO, 2003). This has been attributed to a number of factors, with changes in diet due to modernisation as one of the lead causes (WHO, 2003). The World Health Organisation has stipulated that the amount of calories derived from fat be between 15 to 30%, of which only 10% of that amount can be from saturated fats (WHO, 2003). The guideline shows that more important than total fat intake in the diet is the composition of that fat. Of particular importance is the ratio of saturated (SFA) to poly-unsaturated (PUFA) fatty acids and the cholesterol content. It has been established that a low PUFA:SFA ratio is responsible for the adverse effects associated with fats. Game meat carries the advantage of having relatively high PUFA:SFA ratios when compared to beef (Viljoen, 1999). In general terms, the saturated fatty acid profiles of game meat and other red meats are similar because of similar contents of the dominant saturated fatty acids (palmitic acid; C16:0 and stearic acid; C18:0) (Aidoo & Haworth, 1995). However, because game meat comes from a more natural environment, the unsaturated fatty acids (UFA) they possess are significantly different from those found in domesticated red meat (Hoffman *et al*., 2008). Game meat naturally possesses low cholesterol levels (Higgs, 2000). Viljoen (1999) reported that springbok meat has a high content of arachidonic acid (C20:4), which has the effect of lowering blood cholesterol levels. Furthermore, springbok meat has low levels of palmitoleic acid (C16:1), a fatty acid known to increase blood cholesterol levels. Elliot, (1993) encouraged the consumption of venison as an alternative to red meat. In that study, it was found that the fat content of venison compared favourably with that of chicken. Furthermore, venison was found to have a lower energy content compared to beef, pork and lamb and also a high PUFA:SFA ratio.

Having mentioned that a low fat content is favourable in terms of health, it is also important to note that fat content and composition have an effect on the flavour of meat. Intramuscular fat also has an effect on the perceived juiciness of the meat. Also, intramuscular fat gives the impression of initial juiciness while moisture left in meat after cooking is responsible for the lingering effect of juiciness (Warriss, 2000).

Hoffman *et al*. (2009b) found a correlation between the content of myristic acid (C14:0) and arachidic acid (C20:1) on sensory aroma in impala meat. It can also be suggested that the type of dominant fatty acids and the composition will have an effect on the flavour and odour of the meat. Although there are differences in fatty acid profiles between different game species and also between season (Hoffman *et al*., 2008), the PUFA:SFA ratio of game meat in general is more favourable than that of domesticated red meat.

Furthermore, referring to Table 2.1, the protein content of game meat is higher than that of beef, and has been the observed trend in literature. A possible explanation for the seemingly higher protein content in game meat could be the lower lipid content in the muscles of game animals (Kritzinger *et al.,* 2003).

While there are differences in the fat content and composition between game meat and other red meat, there are also similarities in some components of nutrition. Apart from the major nutrients that meat provides, it is also a source of vitamins and minerals. The vitamin content of game meat and other red meat is comparable. This may be because most of these meat animals' diet consists of grass and leaves which are a major source of vitamins. Game meat has a higher iron content when compared with chicken and beef (Anon., 2006). This is however dependant on species and muscle. Meat in general is commended for the high bioavailability of iron (Hurrell & Egli, 2010).

The nutritional value of game meat can thus be considered to be superior to that of conventional red meat, mainly because of the leanness and higher protein content. Van Zyl & Ferreira (2002) found that blesbok meat can provide up to 81.8% of the essential amino acids required by humans whereas springbok can provide 72.9%. Hoffman *et al*. (2005) found that consumers ranked 'low fat content' as the most important factor influencing purchasing decision of game meat. Following that, colour and freshness were also considered to be important. Modern day consumers are more aware of their health and environment friendly production methods and game meat provides a healthier alternative for the conscious consumer.

Conditions ante-mortem has been shown to have a direct effect on meat quality. The harvesting methods generally used in South African game meat will be discussed in relation to the resulting meat quality.

2.2.2 Game meat harvesting/cropping

Although consumers expect game meat to be truly free-roaming in the wild, this is not the case in European countries where the animals are frequently partly domesticated and sometimes even fed in feedlots. This applies to deer, wild boar and elk which are referred to as venison (Hoffman & Wiklund, 2006). In Africa however, the majority of game farms still retain their natural environment, where the animals can roam around freely in large areas which are fenced off. However, it is a challenge to ensure a practical harvesting methodology applicable to these wild animals that also meets the consumers demand (perception) as pertaining to animal welfare and safe meat.

Harvesting techniques which aim at maintaining meat quality have been developed (van Schalkwyk *et al*., 2011b). Previously, animals which graze in plains have been shot through the shoulder, aiming at

damaging the heart and lungs (Hoffman, 2007). This however leads to losses in meat quality due to the level of stress suffered by the animal. Furthermore, a significant portion of the carcass is lost due to wound damage. Nowadays, where the animals are mostly harvested and used for export purposes, shots are in the head or neck, leading to minimal losses in quality or meat (Hoffman, 2003). Shots to the shoulder or through the stomach are not as fatal as head shots and the animal can spend some time before dying (Hoffman, 2007). The level of stress experienced adversely affects quality. Also, the neck is considered to be of a lower economic value than the shoulder (Hoffman, 2007); so it makes economic sense to shoot the neck instead of the shoulder. It has also been established that hunting at night minimises stress to the animals and results in better quality meat (Hoffman, 2007; Kritzinger *et al*., 2003; Hoffman & Laubscher, 2009; Hoffman & Laubscher, 2010). At night, the marksman uses a truck to navigate the veld and a flashlight for better night vision and blinding of the animals. When they spot animals of interest, they stop at a distance and shoot. This can be done in a short space of time depending on a number of factors such as weather, visibility, terrain and temperament of the animals (Hoffman, 2007). Success rate of shooting and killing depends on the aforementioned factors. For example, it is more difficult to shoot animals in a vegetation dense area than it is when it is clearer such as typically found in grasslands and the Karroo. However, the quality of marksman used in most harvesting teams is such that they rarely miss and this also minimises stress to the animals (Hoffman & Wiklund, 2006). The animal is then tagged for identification and any abnormal occurrences during the process are noted.

After the animals have been shot, they are exsanguinated using sterile knives and loaded at the back of the vehicle to facilitate bleeding. They are then taken to the field abattoir where they are eviscerated and the head and feet are removed. Prior to placing the carcasses in a cold truck (typically with temperatures <6°C), the head, feet and red pluck are inspected by a health official (van Schalkwyk & Hoffman, 2010). After the truck has been filled, the truck is sealed and the carcasses are transported to the commercial abattoir/ deboning plant for further processing. Van Shalkwyk & Hoffman, (2010) give an in-depth description of the whole harvesting procedures.

In a commercial abattoir where live animals such as farmed deer are slaughtered, there is separation between the dirty working areas and the clean working areas (Hoffman & Wiklund, 2006; van Schalkwyk & Hoffman, 2010). This is mostly for hygiene purposes and to minimise the risk of cross contamination. Those working in the 'dirty' areas deal with removal of the hides and head and any other procedures which may lead to contamination of the carcasses. In the 'clean' areas the internal organs are removed, the carcasses are cleaned and placed in the coolers (Hoffman & Wiklund, 2006). Tags are placed on the carcass at the point of harvest, with information about the carcass to improve

traceability (Hoffman & Wiklund, 2006; van Schalkwyk & Hoffman, 2010). At this stage, the muscle still needs to undergo a series of biochemical reactions which transform muscle into meat.

2.3 Conversion of muscle into meat

Meat can be loosely defined as the musculature of animals which is edible. Firstly when an animal is slaughtered, blood circulation is stopped which consequently stops the circulation of oxygen and nutrients (Lawrie, 1998). To improve meat quality, it is necessary to completely bleed the animal because if bleeding is insufficient, blood provides a source of nutrients and an environment which encourages the growth of microorganisms (Lawrie, 1998). Even though the muscles do not actively contract after death, the tissues continue with metabolism and energy is utilised to maintain the relaxed state of muscles (Lawrie, 1998). The stoppage in blood-borne oxygen circulation leads to a fall in redox potential (Warriss, 2000). The cytochrome enzyme system ceases working and ATP regeneration also stops (Lawrie, 1998). Non-contractile ATPase of myosin depletes the ATP, causing an increase in the production of inorganic phosphate which results in the breakdown of glycogen anaerobically to lactic acid. As a result of lactic acid and hydrogen ion formation, the pH drops gradually (Lawrie, 1998). Also, these products remain in the muscles because of the stoppage in circulation. In live muscle, the role of ATP is to prevent the association of actin with myosin (Warriss, 2000). When the glycogen concentration falls, ATP production ceases (Lawrie, 1998). When ATP levels fall to approximately 5 $mmol \cdot kg^{-1}$, irreversible actomyosin is formed and this marks the beginning of rigor mortis (Lawrie, 1998). Greaser (2001) reported that post-mortem glycolysis stops before glycogen stores are depleted. This may be due to enzyme inactivation at low pH. Low levels of ATP, coupled with the accumulation of lactic acid make proteins more liable to disintegration (Greaser, 1986). Furthermore, the gradual fall in pH causes the release and activation of cathepsins (Warriss, 2000). Disintegration of proteins in the sarcoplasm increases their chance of being attacked by activated cathepsins (Warriss, 2000). Continued glycolysis results in a further fall in pH and also results in the accumulation of metabolites and flavour compounds.

Under normal circumstances, pH falls from 7 to between 5.4-5.6 within 24 hours post-mortem in red muscles (Lawrie, 1998; Warriss, 2000; Adzitey & Nurul, 2011). In the event that there is insufficient glycogen available for anaerobic breakdown (as experienced with wild animals when harvested under stressful conditions), the ultimate pH ends in the range of above 6, where the meat is then classified as DFD (Adzitey & Nurul, 2011). Some muscles are however more prone to DFD than others. For example, red, oxidative muscle fibres naturally have lower glycogen levels which can easily be

depleted post-mortem (Adzitey & Nurul, 2011). In such muscles, Warriss, (2000) suggested that pH values of 6.3 and below can be considered as normal.

Even though the process of slaughter has been optimized to produce high quality meat, some intrinsic factors, such as those responsible for DFD may result in defective meat. As mentioned before, DFD meat has a pH which is higher than 6. The impact of this phenomenon on microbial growth will be discussed further.

2.3.1 Role of pH in microbial growth

An internal factor which can influence microbial growth is muscle pH. Muscle pH also affects other quality parameters such as colour and drip loss (Lawrie, 1998). In the living animal, pH is maintained at neutral levels (Lawrie, 1998). After death, there is a shift from neutral to acidic. As explained earlier, some muscles fail to achieve acidic pH and tend to remain within the neutral range. Although most microorganisms can grow within pH ranges of 5.4-7.0, they prefer more neutral pH (Doulgeraki *et al*., 2012). In normal pH meat, some bacteria are inhibited and the growth of lactic acid bacteria is promoted (Borch *et al*., 1996). These have the effect of further lowering the pH and consequently suppressing growth of other microorganisms (Doulgeraki *et al*., 2012). In meat of higher pH (>6), growth of some bacteria including *Aeromonas* and *Enterobacteriaceae*, produce hydrogen sulphide which reacts with myoblobin, resulting in a green colour (Kamenik, 2012). In this scenario, the meat pH alters the metabolism of the microorganisms mentioned thereby indirectly affecting colour. Also, because DFD meat is deficient in glucose, this promotes amino acid breakdown for energy by the bacteria earlier than normal, thus resulting in quicker spoilage (Newton & Gill, 1981). Various strategies have been employed to improve the keeping quality of meat. The concept of meat packaging and how it is used to manipulate microbial growth will be discussed in a later section.

2.4 Quality characteristics of meat

Consumer awareness about environmental issues, health and diet has placed pressure on producers to supply products which apart from being natural, safe and healthy; have cost-effective production systems. While all these factors need to be adhered to, product quality should not be compromised at any point. Producers attempt to stay up to date with the consumer demands and it is imperative to know what the important meat quality attributes are in the consumer's opinion. Meat quality is usually defined in terms of visual appearance, texture and sensory characteristics (Lawrie, 1998). These include colour, drip loss, water-holding capacity, tenderness, flavour, odour and juiciness. Although quality is expressed in these individual factors, ultimate quality is dependent on complex interactions between the different quality factors (Muchenje *et al*., 2009). Knowledge of relationships which exist

between the quality factors can lead to predictions of the overall meat quality. For example, pH has an effect on tenderness and water-holding capacity, so based on the knowledge of ultimate pH, the other factors can be predicted (Muchenje *et al*., 2009). In addition to growth conditions of the animal, quality is also affected by handling prior to and post slaughter. In this review, focus will be placed on colour in general with an emphasis on the colour of game meat. Furthermore, the relationship between colour and meat safety will also be discussed.

2.4.1 Role of ultimate pH on meat colour

One of the most important factors which affect a consumer's purchasing decision is meat colour (Carpenter *et al*., 2001). The characteristic cherry red colour of beef is what makes it attractive to most consumers. When light hits the surface of meat, it can be reflected, absorbed or scattered (Hughes *et al*., 2014). Colour is a function of these three occurrences, but the more important one is reflected light as it determines perception and ultimately acceptance (Hughes *et al*., 2014). Colour can be measured instrumentally or through analysis by a trained panel. Both methods can be used in conjunction in instances where using only one method is not sufficient. Instrumental colour can be measured by a range of colorimeters and spectrophotometers (Mancini & Hunt, 2005). These usually use the Hunter, CIE or tristimulus methods, depending on the requirements of the project. The CIELab system is most commonly used where the colour is measured as L* (lightness, 0:black, 100:white), a*(red-green, positive values: red, negative values: green) and b*(blue-yellow, positive values: yellow, negative values: blue) (Commission International de L'Eclairage, 1976). Chroma and hue angle are calculated from the a* and b* values as follows: Hue angle = $\tan^{-1}$ (b*/a*) and C*= (a^{*2}+b^{*2}) (AMSA, 2012). Higher a* and b* values result in higher chroma or colour saturation, which is more desirable in meat (Onyango *et al*., 1998).

As mentioned, meat colour is one of the factors which influence consumer purchasing decision. One obvious indication of spoilage, besides odour is colour. Ultimate pH influences colour of meat, as well as protein structure and functionality (Swatland, 1995). In addition, high ultimate pH can have an effect on the colour stability of fresh meat because it affects enzyme activity and the rate of oxygenation (Swatland, 1995). Functionality of the enzymes responsible for converting metmyoglobin to oxymyoglobin is diminished by high pH, thereby slowing down the process (Barbut *et al*., 2008). This can partially explain the colour defect in meat with higher pH. Furthermore, meat with high ultimate pH binds moisture strongly, resulting in a dry surface which slows down oxygen penetration into the meat (Barbut *et al*., 2008). In addition to binding moisture, the muscle fibres associate more with each other, being closely packed and thereby reducing the reflectance of light (Huff-Lonergan & Lonergan, 2005; Hughes, 2014). This causes DFD meat to appear darker. With pale, soft, exudative (PSE) meat on the other hand, the meat appears lighter as a result of increased reflectance of light as

a result of sarcoplasmic protein denaturation as well as a weaker binding of water allowing water to flow to the cuts surface of the meat (Swatland, 1995).

Meat colour can be influenced by a number of factors including but not limited to diet, age, genetics and pre slaughter handling (Muchenje *et al.,* 2009). For example, in animals subjected to stress prior to slaughter, high ultimate pH and lower L* values were seen (Zhang *et al*., 2005). The high ultimate pH encourages the proliferation of microorganisms and consequently shortens the shelf-life (Zhang et al., 2005). High pH meat has L*, a* and b* values which are lower than normal, and they also vary between different muscles (Zhang *et al*., 2005; Adzitey & Nurul, 2011). The pigments mainly responsible for colour are myoglobin and haemoglobin and cytochrome C (Lawrie, 1998). Myoglobin has a structure similar to haemoglobin and is found predominantly in the muscle whereas haemoglobin is mainly found in the blood (Mancini & Hunt, 2005). The condition of myoglobin, which varies depending on oxygen saturation influences meat colour to a greater extent; when subjected to high concentrations of oxygen, myoglobin is oxidised to oxymyoglobin which is red in colour (Lawrie, 1998). In the absence of oxygen, myoglobin is reduced to deoxymyoglobin, which is purple in colour.

Prolonged exposure to low oxygen concentrations leads to the formation of brown metmyoglobin; which is associated with low quality or 'stale' meat. Metmyoglobin forms on the surface of meat when the iron group is oxidised to the ferric state (Fe^{3+}) and the layer can become thicker in the absence of oxygen (Mancini & Hunt, 2005). Lawrie (1998) found that when approximately 60% of the myoglobin is present as metmyoglobin, the brown colour becomes evident. When the levels of oxymyoglobin are about 30-40%, consumers will refrain from purchasing the meat (Carpenter *et al*., 2001). Metmyoglobin formation is influenced by various factors such as microbial load, low pH, heat, and salts. Mancini & Hunt (2005) found that even though metmyoglobin can form beneath the surface, it can affect meat quality as the layer thickens and migrates to the surface. In the presence of carbon monoxide as seen in modified atmosphere packaging, carboxymyoglobin is present, which is bright pink in colour (Wilkinson *et al*., 2006). Consumers usually prefer red and pink meat, which they associate with quality and freshness (Carpenter *et al.,* 2001).

Game meat has a colour that is darker than other red meat (Hoffman, 2005). This is viewed as undesirable by consumers and can result in game meat being less accepted when compared to other red meats. Wild game animals are exposed to predators in the veld and they are used to taking flight. Because of this increased activity, their muscles accumulate more myoglobin than normal, resulting in darker meat (Lawrie, 1998; Hoffman, 2001). While this is just biochemical specialisation, consumers can associate dark meat with spoilage or as meat of a lower quality (Hoffman, 2005; Bekker *et al*., 2011). To reiterate the importance of colour, in the United States, up to 15% of fresh meat discolours

and is sold at discounted prices. This results in losses of up to 1 billion dollars revenue (Mancini & Hunt, 2005).

Closely related to colour is the microbial condition of meat. This will be explored further in a section on meat packaging. Sanitation and hygiene used during slaughter and processing thereafter have a direct impact on the microbial quality of meat. The general microbial quality of meat will be discussed and then some scenarios involving the microbial quality of game meat will also be mentioned.

2.5 Microbial quality of meat

While it is rather complicated to completely eradicate food borne illnesses, a number of measures have been put in place by manufacturers to reduce their occurrence. One such measure is the development of HACCP (Hazard Analysis and Critical Control Point) and GMP (Good Manufacturing Practices). HACCP is a preventative measure in production systems and aims at stopping incidents before they happen. It focuses on biological, chemical and physical hazards (USDA, 1997). Examples for these hazards are presented in Table 2.2.

Table 2.2: Examples of hazards for a HACCP plan, categorised according to chemical, physical and biological hazards (USDA, 1997).

Chemical Hazards	**Biological Hazards**	**Physical Hazards**
Pesticides	Bacteria	Foreign objects (glass, sticks, stones)
Fertilizers	Viruses	
Sanitizers	Nematodes	
Environmental contaminants (lead, arsenic, mercury)	Yeasts and moulds	
Aflatoxins	Protozoa	
Food additives (preservatives, colourants)	Algae	

Of interest in this review are the biological hazards, which lead to intoxication or infection. Bacterial infection is as a result of consuming a high enough number of bacteria to cause illness. Intoxication on the other hand is the ingestion of bacteria which produce toxins in the gut or consuming the toxins themselves. The resulting symptoms are different for each microorganism and cannot be generalized. The major pathogenic bacteria involved in contamination of meat include *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli* O157:H7,

Listeria monocytogenes, *Salmonella* spp., *Staphylococcus aureus* and *Yersinia enterocolitica* (USDA, 1997). Microbial loads of meat or any other food can be reduced by employing GMP.

Of importance in GMP is the maintenance of sanitized environments. The main objectives of maintaining meat hygiene are to minimise contamination, maintain wholesomeness and inhibit the growth of the contaminating microorganisms (Bell, 1996; Barros *et al*., 2007). The practice of rapid chilling of carcasses is encouraged because it has been shown to reduce the growth and multiplication of most pathogens and spoilage microorganisms on the carcass surface (Nychas & Drosinos, 2014). Sanitation may be either removing visible, physical dirt or aimed at unseen microorganisms. Levels of organisms are not meant to be more than a stipulated number because beyond that level, they start producing by-products which are offensive to the consumers and cause deterioration in quality.

When considering microbial quality of meat, it is imperative to consider the identity of the microorganisms present and how to enumerate them, the part of the carcass to be examined, how to sample and when to sample. The most accurate way of enumerating and identifying microorganisms would be to isolate all the organisms on the samples involved. However, due to the impracticality of this task, indicator microorganisms are usually used as an estimate (Barros *et al*., 2007). It is common practice to enumerate indicators of faecal pollution, which are the enterobacteria such as *E. coli* and *Salmonella* (Ingram & Roberts, 1976). Indicator organisms give valuable information on the sanitation during handling and processing, ensuring maintenance of the keeping quality of the food and microbiological safety (Mead, 2007). Testing for indicator organisms can be done at any point in the production process or initially on the raw materials. In addition, they are now being used in shelf-life assessment because of the economic implication of products with longer shelf-life in industry (Mead, 2007).

Selection of indicator organisms to test for depends on the type of product and storage conditions. For instance, in fresh meats, spoilage will mainly be as a result of attack by *Pseudomonads* and therefore a total count can be done at 25°C for 72hrs (Mayr *et al*., 2003). This will allow for growth of both mesophilic and psychrotrophic microorganisms. Mayr *et al*. (2003) found that in meat stored aerobically for 11 days, 83 to 100% of *Pseudomonad* spp. could be isolated. In instances where there is a lot of handling, it would be advisable to also test for *Staphylococcus* spp. which are present on the skin of all humans and can be passed on to the product through unhygienic practices (Postgate, 2000). In meats which are vacuum packaged where there are trace amounts of oxygen, it would be wise to test for anaerobic organisms such as *Clostridium* spp. which would thrive in such environments.

When an animal is slaughtered, the muscles maintain their sterility and will only become contaminated from external sources (Hernandez-Macedo *et al*., 2011). Gill *et al*. (1976) found that

delayed evisceration did not increase microbial contamination in meat. Contrary to popular belief, the intestine walls in this study were found to be impermeable to bacterial spores. However, from a microorganism's point of view, meat is a perfect environment for growth as it is nutrient dense.

Microbiological quality of meat is important in terms of shelf-life of meat, meat safety and public health concerns. It is the responsibility of producers to provide meat which is wholesome and safe. Concerns over meat safety have increased because of a number of food-borne disease outbreaks related to meat. For instance, in 1996, the incidence of BSE (Bovine Spongiform Encephalopathy) led to the ban in all beef imports from the United Kingdom to South Africa (FAO, 2002). Another common pathogen is *Escherichia coli* O157:H7, which has been responsible for a number of meat recalls and human cases of infection; and even death over the years. In Africa, a number of outbreaks have been recorded including South Africa (Browning *et al*., 1990), Nigeria (Olorunshola, 2000), Gabon (Presterl *et al*., 2003) and Ivory Coast (Dadie *et al*., 2000). The largest outbreak in Africa was recorded in Swaziland in 1992 (Effler *et al*., 2001). Although cases of *E. coli* outbreaks are widespread, a large number of laboratories in Africa still do not have means of isolating and identifying the strain, which leads to many infections going unnoticed (Raji *et al*., 2006). To assist with identification and prevention of food borne illnesses as a result of *E. coli* O157:H7 in Africa, Raji *et al*. (2006) suggested that the public be educated on the dangers of consuming meat that is not properly cooked. Furthermore, awareness should be increased in medical practitioners so that they report and document any cases of such illnesses.

2.5.1 Different storage conditions and typical microorganisms

Microbes require different environments for optimal growth. This is one of the factors that has been used in the development of packaging for food, including meat and meat products. The differences between microorganisms in relation to temperature and air requirements are shown in Table 2.3.

The nutritional factors which make meat attractive as a nutrient source for humans also render it attractive for microorganisms. In fresh, unprocessed products, microorganisms are capable of rapid multiplication, especially at ambient temperatures resulting in spoilage (Sofos, 1994). Packaging serves as a barrier to microorganisms and retards the biochemical processes occurring in food. It is therefore noteworthy to highlight the external factors which affect growth of microorganisms, which are targeted in most packaging systems.

Table 2.3: Typical microorganisms found in meat (adapted from Pommerville, 2009)

Group	Aerobes	Facultative anaerobe	Anaerobes
Mesophiles (30-45°C)	*Bacillus* spp.	*Salmonella* spp.	*Cl. sporogens*
		Staphylococcus spp.	*Cl. perfringens*
		Enterobacteriaceae	*Cl. botulinum*
Psychrotrophs (15-30°C)	*Micrococcus* spp.	*Lactobacillus* spp.	
		Streptococcus spp.	
Psychrophiles (5-15°C)	*Pseudomonas* spp.	*B. thermosphactum*	*Cl. putrefacians*
	Acinetobacter spp.		
	Moraxella spp.		

Temperature is one of the most important factors affecting microbial growth. Hamad, (2012) reported that growth rates at 0-1°C are half of those at 5°C and continue to fall as temperature decreases. Most microorganisms have optimal growth temperatures of between 30 to 45°C and this is used as one of the major hurdles in processing technology. Microorganisms require water to grow and reducing water content consequently lowers the growth rate. Meat has a water activity of approximately 0.99 which makes it liable to attack from microorganisms (Lawrie, 1998). This can be manipulated by either drying or increasing salt concentrations in the product, which is the case for cured products. Apart from temperature and water activity, another important factor which affects microbial growth is the gas atmosphere. Most organisms prefer environments rich in oxygen and are inhibited by carbon dioxide. Different ratios of gases are utilised in modified atmosphere packaging, to inhibit microbial growth and simultaneously maintain other quality characteristics such as colour (Wilkinson *et al*., 2006).

2.5.2 Types of meat packaging

Under aerobic packaging, meat is placed in Styrofoam trays, covered by plastic films which are permeable to oxygen and water vapour. Normal air composition consists of 78% nitrogen, 21% oxygen, 0.01% carbon dioxide and trace amounts of water vapour and inert gases. Although this is the easiest way of packaging meat, it does not result in improved shelf-life. This is because the overwrap package does not serve as an effective barrier for moisture, oxygen or light. Apart from increased microbial growth, shelf-life is not maintained because of lipid oxidation (Sivertsvik *et al*., 2002). In meat exposed to air during chilling, Ercolini *et al*., (2006) found that the growth of *Pseudomonas* spp. was dominant. When meat was subjected to aerobic packaging, the shelf-life did not exceed 7 days at 4°C (Ercolini *et al*., 2006).

Vacuum packaging has been used successfully to lengthen the shelf-life of meat and meat products. In foods packaged in this way, air is first expelled from the container prior to sealing. The type of packaging film is impermeable to gases and water vapour. Kiermeir *et al*., (2013) found that lamb stored under vacuum storage was still acceptable after 12 weeks although the microbial numbers exceeded 10^8 $cfu \cdot g^{-1}$. Furthermore, the microbial species dominant in this study were *Carnobacteria* spp.

Modified atmosphere packaging (MAP) involves the use of different gas ratios to prolong shelf-life. Type of gas combination used depends on the product, temperature of storage and the type of film used. Carbon dioxide is mostly used in high concentrations for MAP of meat due to its ability to increase shelf-life (Viana *et al*., 2005; Ozturk *et al*., 2009). Carbon dioxide is colourless and is soluble in water, producing a slightly acidic solution (Sandhya, 2010). The extent to which carbon dioxide will be effective against microorganisms is affected by the presence of other gases in the mixture as well as the growth phase of the microorganisms (Viana *et al*., 2005). A disadvantage of carbon dioxide in MAP however is the promotion of metmyoglobin formation especially in low oxygen concentrations (Ozturk *et al*., 2009). This is usually countered by the use of trace amounts of carbon monoxide in MAP. MAP which uses these two gases in combination has been shown to improve the colour of beef and pork. The main role of carbon monoxide in MAP is for the maintenance of colour. Myoglobin has a greater affinity for carbon monoxide than for oxygen and results in a more stable pink colour called carboxymyoglobin (Wilkinson *et al*., 2006). Carbon monoxide can however give a false impression of the microbial quality/safety of meat as the colour can remain stable even after the meat is microbiologically not suitable for consumption (Venturini *et al*., 2010). As a result, the use of carbon monoxide for colour enhancement in meat has been banned in the European Union (Venturini *et al*., 2010).

High oxygen environments are recommended for fresh meat for the maintenance of colour (Ozturk *et al.*, 2009). An obvious drawback of using MAP systems high in oxygen is the oxidation of fats and promotion of microbial growth. Therefore, oxygen can be used in combination with carbon dioxide so that both colour and microbial quality can be maintained. Presence of oxygen has also been shown to inhibit the growth of strict anaerobes which thrive in environments which are devoid of oxygen (Cutter, 2002). Nitrogen is an inert gas that is mostly used as a filler gas to prevent collapse of the package (Venturini *et al*., 2010).

2.5.2.1 Spoilage under vacuum storage

Under oxygen impermeable vacuum storage, the growth of lactic acid bacteria (LAB) will be favoured due to the increased carbon dioxide and low redox potential (Borch *et al*., 1996). Vacuum packaging

has been employed as a meat preservation technique due to the shift in microbial populations it creates. The growth of Pseudomonads, which have high spoilage potential, will be replaced by lactic acid bacteria (LAB), which have low spoilage potential (Borch *et al*., 1996). LAB levels can reach 10^8 cfu·g^{-1} without causing spoilage; Bell, (1996) showed that LAB levels can be high for several weeks without any visible signs of spoilage. Spoilage as a result of LAB is characterised by sour flavours, carbon dioxide formation, slime formation and colour changes in the meat (Schillinger & Holzapfel, 2006).

Although vacuum storage is an effective way of preserving meat, the conditions can possibly encourage the growth of facultative anaerobes and anaerobes. Anaerobic bacteria such as *Clostridium* spp*.* are known for producing gas and could result in early spoilage if they are present in the package (Moschonas & Bolton, 2012). In the case where there is residual oxygen in the vacuum pack; there is an increased growth of microorganisms such as *Broncothrix thermosphacta* and *Shewanella putrefaciens* (Adzitey & Nurul, 2011). In addition, if the pH is >6, as is observed for DFD meat, off odours and flavours will be observed at counts of approximately 1 million/gram (Food Science Australia, 2003). Spoilage by *Shewanella putrefaciens* is characterised by a green colour on the meat surface and a distinctive hydrogen sulphide odour (Adzitey & Nurul, 2011).

2.5.2.2 Spoilage under MAP storage

Ercolini *et al*. (2006) used different gaseous compositions to investigate microbial growth over a period of 14 days. They found that in meat exposed to air during chilling, the growth of Pseudomonads was dominant. Furthermore, when meat was subjected to 20% oxygen and 40% carbon dioxide, Pseudomonads and *Lactobacillus sakei* dominated the population. Pseudomonads are unaffected by the normal pH in meat and will be dominant at chill temperatures. High levels of carbon dioxide are used in modified atmosphere packaging due to its effect of retarding microbial growth (Ercolini *et al*., 2006). Using concentrations of up to 100% CO_2 can double shelf-life of meat in chilled storage (Narendran, 2003). The combination of chill temperatures and carbon dioxide has an effect of lengthening the lag phase thereby slowing down the growth of psychotropic organisms (Narendran, 2003).

In a study where MAP (80% O_2 and 20% CO_2), vacuum packaging and overwrap packaging were compared, MAP was found to be the most effective inhibitor of most microorganisms (Lorenzo & Gomez, 2012). Venturini *et al*. (2010) found that colour and odour of beef was best maintained through the use of anoxic environments.

2.5.2.3 Spoilage under aerobic storage

As expected, spoilage under aerobic storage is dominated by aerobic microorganisms. Although aerobic storage is preferred for the maintenance of colour at the point of sale, the meat has a significantly lower shelf-life than meat packed in any other way. Aerobic microbial spoilage leads to a more rapid formation of offensive by-products when compared to anaerobic spoilage. Apart from microbial spoilage, meat packaged this way has a shorter shelf-life due to an increase in oxidation. Myoglobin oxidation to metmyoglobin is accelerated at high oxygen concentrations and this leads to a permanent loss in colour.

2.5.2.4 Spoilage at freezing temperatures

Referring to Table 2.3, it can be seen that most of the microorganisms found in meat grow at a temperature range of 5 to 45°C. Fresh meat can either be stored at chill temperatures (-1.5 to 7°C) or frozen (Hernandez-Macedo *et al*., 2011). When stored at chill temperatures, maintenance of the temperature must be enforced to ensure minimal microbial growth. At freezing temperatures, which are typically lower than -15°C, growth of microorganisms is almost non-existent. In that temperature range however, the growth of some fungi has been reported (Narendran, 2003).

2.5.3 Meat spoilage mechanisms

Meat is composed of mostly water (about 75%) and various metabolites such as amino acids, peptides, sugars and nucleotides (Labadie, 1996). Although it is a poor source of sugars, the proteins abundant in meat can be utilised by microorganisms. Apart from the nutrition that meat can provide to microorganisms, other factors play a role in whether the microbes will be able to flourish or not. These include pH, water activity and storage conditions (Labadie, 1996).

The complexity of meat as an ecosystem renders it suitable for the growth of a number of microorganisms (Holzapfel, 1998). However, before microorganisms can grow they have to be able to attach to a particular surface. It is generally accepted that bacterial attachment is a two stage process, namely reversible and irreversible attachment (Holzapfel, 1998; Narendran, 2003). Reversible attachment is facilitated by physical forces whereas irreversible attachment is cemented by extracellular polysaccharides (Davies & Geesey, 1995). Following attachment to the surface, penetration into the muscle is necessary to facilitate growth. As rigor progresses, the muscle fibres pull away from the endomysia, producing crevices through which bacteria can penetrate (Gill *et al*., 1984). Proteolysis also increases the ease of bacterial penetration (Gill *et al*., 1984). The major nutrients found in meat (protein and fat) are mostly insoluble and not readily attacked by microorganisms. During rigor and post-rigor, there is formation of low molecular weight compounds, including creatine and some nucleotides which can be used immediately by microorganisms to

support their metabolism (Gill, 1983). As time progresses, proteins are broken down and yield amino acids and peptides which facilitate microbial growth.

Pseudomonads are ubiquitous in nature, where they are mainly involved in organic matter mineralisation (Hernandez-Macedo *et al*., 2011). Due to the nature of an animal's surroundings, it is easy to see that these microorganisms could possibly contaminate meat. In their metabolism, glucose is preferred and utilised first and amino acid breakdown is suppressed until all the glucose is exhausted (Hernandez-Macedo *et al*., 2011). During this initial stage where Pseudomonads utilise glucose, they also have an effect of subduing growth of other microorganisms and at this point they are dominant (Food Science Australia, 2003). However as time progresses and muscle glucose becomes limited, Pseudomonads' metabolism changes and they break down amino acids (Hernandez-Macedo *et al*., 2011). Usually at this stage, cell density increases and can be in excess of 10^8 cfu·ml^{-1} (Food Science Australia, 2003). The high microbial load renders the meat incapable of supporting all the microorganisms and competition for amino acids occurs. Microbial breakdown of amino acids results in a rise in pH because of ammonia production (Borch *et al*., 1996). This is accompanied by off flavours and taste. Environmental factors such as temperature, pH, and gaseous composition play a major role in determining which organisms will flourish (Doulgeraki *et al*., 2012). Such examples have been discussed in the preceding section.

In the case of DFD meat, Pseudomonads are the key spoilage microorganisms; as DFD meat has very low glucose levels initially, it is exhausted in a short period of time and microbial attack on amino acids then occurs and results in a further rise in pH. Spoilage becomes obvious when the bacterial numbers are high enough to produce by-products which can be perceived. This is characterised by slime production, colour changes on the meat surface, gas production and off odours (Borch *et al*., 1996). It is however noteworthy that in some cases, bacterial levels may be as high as 10^7 without any visible spoilage sign which is usually the case when bacterial populations are dominated by bacteria such as lactic acid bacteria.

2.6 Meat preservation techniques

Meat is naturally perishable due to its biological composition (Zhou *et al*., 2010). Packaging techniques have been developed to maintain the wholesomeness of meat. Other methods of preservation include hydrostatic pressure, super-chilling, freezing and chemical preservatives (Zhou *et al*., 2010). Apart from eliminating even the most resistant microorganisms, recent technologies focus on maintaining food in its natural state, while being mild, environmentally friendly and energy conserving (Zhou *et al*., 2010). Although most preservation methods can be used individually, more effectivity can be achieved when used in combination. Liao (2006) reported that when high CO_2 concentrations are used

in MAP, maximum inhibition of microorganisms was achieved when the meat was stored at temperatures below 10°C. Hurdle technology has been applied to food to reduce spoilage and pathogenic bacteria and ultimately lengthen shelf-life. Huffman (2002) reported on the use of hurdle technology both pre- and post-slaughter. Under pre-slaughter conditions, feeds with lower counts of *E. coli* were used. In addition drinking water was treated and feeds were also fortified with probiotics and natural antimicrobials. Post-harvest elimination of microorganisms was achieved by rinsing carcasses in hot water or chemicals such as organic acids, or by steam sterilization (Huffman, 2002).

In meat products meant for marketing, chemical preservatives have been used successfully. However, excessive use of chemical preservatives such as butylated hydroxianisole, butylated hydroxytoulene and sulphur dioxide is now being regulated due to possible carcinogenic and chronic toxicity (Baratta *et al*., 1998; Aymerich *et al*., 2008). Furthermore, the modern consumer demands foods that are low in salt, low in chemicals and less acidified (Dadalioglu & Evrendilek, 2004; Aymerich *et al*., 2008). This has increased pressure on food manufacturers to limit or eliminate the use of chemical preservatives. Therefore, there has been an increased interest in natural preservation techniques, which include medicinal plants (essential oils, herbs and spices) and bio-preservatives such as LAB and their metabolites (Mauriello *et al*., 2004; Nes & Johnsborg, 2004).

2.6.1 Essential oils in the preservation of meat

In spite of being generally regarded as safe (GRAS) and being used successfully for their antibacterial, antifungal and insecticidal properties, essential oils have only been used in food as flavour enhancers (Dadalioglu & Evrendilek, 2004). Recent trends in consumer behaviour have led to more research being focused on the use of essential oils as natural alternatives to conventional preservatives. However, essential oils have been shown to exhibit more bactericidal properties *in vitro* rather than *in vivo* and this has limited their use (Nychas & Tassou, 2000). Furthermore, bactericidal effects are achieved at high doses and their use is limited due to strong flavour at high doses (Nychas *et al*., 2003). Plant essential oils (sage, rosemary, oreganum, coriander, laurel) have been found to be active against pathogens such *as Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and *E. coli* (Skandamis & Nychas, 2000; Tsigarida *et al*., 2000; Dadalioglu & Evrendilek, 2004). Bactericidal activity for most essential oils has been shown more against gram positive bacteria than gram negative bacteria (Mangena & Muyima, 1999). This is supposedly due to the more resistant bacterial cell wall for gram negative bacteria (Burt, 2004). Baratta *et al*. (1998) and Burt (2004) however reported that the gram reaction seemed to have no influence on microorganisms' sensitivity to oregano essential oil. Also, *in vitro*, oregano essential oil had the highest and broadest activity against the microorganisms tested (Baratta *et al*., 1998).

Lamiaceae species' essential oils (Oreganum and Thyme) have been shown to possess strong antimicrobial properties (Aligiannis *et al*., 2001). Burt (2004) suggested that oregano essential oil's active components increase cell membrane permeability, thereby leaking cell contents into the environment and disrupting ionic balance of the bacteria. Sivropoulou *et al*., (1996) showed that oregano essential oils were effective against a selection of gram positive and gram negative microorganisms *in vitro*. In meat, numerous studies found that oregano essential oil was one of the most potent essential oils (Skandamis *et al*., 2002; Chouliara *et al*., 2007; Karabagias *et al*., 2011; Hulankova *et al*., 2013). However, although higher concentrations of the essential oil resulted in bactericidal effects, it was not acceptable during sensory analysis (Chouliara *et al*., 2007). Therefore, a compromise should be reached between antimicrobial action and sensory acceptability when using essential oils as preservatives.

2.7 Conclusions and objectives

Game animals, due to their wild nature are more likely to be stressed during the harvesting process. As such, this leads to quality defects in the meat produced. These range from physical quality to sensory quality and ultimately affect other quality characteristics. The role of this study is to investigate how ultimate muscle pH, which is directly related to ante-mortem stress influences microbial growth in black wildebeest meat; thereby broadening the knowledge pool. Also, there is limited knowledge on the microbial quality of local game meat. Furthermore, because game meat is viewed as being organic, a second aim was to evaluate how applying a natural preservative would affect microbial growth in the meat studied.

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Chapter 3: The influence of muscle ultimate pH on the microbial growth and colour stability of previously frozen black wildebeest (*Connochaetes gnou*) meat.

Abstract

Although harvesting procedures have been developed for wild meat animals, the stress experienced as a result thereof can be unpredictable, leading to some quality differences in the meat. When exposed to stressful conditions during slaughter, wild animals become prone to producing meat with high ultimate pH. The aim of this study was to determine how high ultimate muscle pH (pH >6) affected the microbial quality of previously frozen black wildebeest meat stored under anaerobic refrigerated (4.2±0.8°C) conditions. Under the aforementioned conditions, it was found that the growth curves for total viable counts (TVC) and lactic acid bacteria (LAB) were similar; LAB was most likely the major microorganism present. Total coliforms had slower growth and this was attributed to vacuum storage and refrigeration temperature. Meat with normal pH values (pH <6) had slower growth rates of all microorganisms tested compared to high pH (pH >6) meat. *Salmonella* spp. was not detected in any of the samples tested. Normal pH meat was also found to be lighter than DFD meat and more prone to browning under anaerobic storage conditions. The conclusion reached was that DFD meat had higher growth rates for the microorganisms tested and ultimately had a shorter shelf-life than normal pH meat.

3.1 Introduction

Meat has been consumed for a long time, with archaeological studies showing that meat consumption started approximately 2.6 million years ago (Cawthorn & Hoffman, 2014). Meat popularity continues to increase due to its nutritional profile. Apart from providing essential amino acids meat is also a good source of fatty acids, vitamins and minerals (Smolin & Grosvenor, 2003). According to the United Nations (2012) forecast, the world population will continue to increase also causing an increase in urbanisation. Consequently, population increase will cause an increased demand for food in general and meat is not an exception. FAO (2011) reported that 925 million people (greater than 10% of the world population) are undernourished, with the majority being in developing countries. As a result of food insecurity, humans continue to consider a variety of alternative protein sources, such as those derived from wild animals.

Game meat has been defined as the meat originating from any terrestrial wild animal, whether used for commercial or subsistence purposes (Hoffman & Cawthorn, 2012). This ranges from large to small animals, for example eland down to rodents (Hoffman & Cawthorn, 2012). One of the most important

factors influencing purchasing decision of the modern consumer is the impact of the product on their health (Mathijs, 2015). Based on this viewpoint, game meat appeals to the modern consumer for a number of reasons. Firstly, game meat is naturally low in fat; it seldom exceeds 3% and it can be classified as lean meat (Jansen van Rensburg, 2001; Hoffman, 2007). In addition, game meat is naturally low in energy (Jansen van Rensburg, 2001). Hoffman, (2007) acknowledges that the production potential of game meat/venison has been recognised for a long time. Although the game meat industry has the potential to provide a partial solution to the problem of protein malnutrition, research still needs to be done globally on the suitability, especially quality and safety of game meat. Hoffman *et al*., (2004) found that the quality of game meat sold in butcheries varied and therefore there is need for the development of quality grading systems tailor-made for game meat. Physical quality is only one aspect of game meat and it cannot be considered in isolation as there are other factors such as microbial quality and safety, which are equally important.

Currently, there is limited literature on the microbiological quality of South African game meat. Furthermore, the standards used for microbiological quality control in this regard are not specific to game meat but have been adapted from those applicable to other traditionally farmed species. Increased use of game meat as an alternative protein source necessitates research to ensure that the meat is safe for consumption and is also microbiologically stable during storage. Due to the wild nature of game animals, they are frequently stressed during the harvesting process (Kritzinger *et al*., 2003; Hoffman & Wiklund, 2006); a situation that frequently results in the muscles using up all their glycogen reserves and as a result having high ultimate muscle pH values. Different game species differ in their susceptibility to ante-mortem stress, with the black wildebeest being an animal that stresses readily during the harvesting procedures. In instances where the ultimate muscle pH exceeds 6, the meat can be referred to as dark, firm and dry (DFD). Beef and pork studies have shown that ultimate muscle pH higher than 6 shortens the shelf-life of meat by encouraging microbial growth (van der Wal *et al*., 1988; Adzitey & Nurul, 2011). Meat with ultimate pH higher than 6 has also been shown to be darker and firmer than meat with normal pH (Lawrie, 1998). Although classification of DFD has been set at pH of 6 and above, some meat with intermediate pH can possibly spoil quicker than normal pH meat. The determination of spoilage for meat at intermediate pH has not been explored in literature. In this chapter, the effect of different ultimate pH ranges on naturally occurring microbial growth was investigated on black wildebeest meat under vacuum storage. The effect of ultimate muscle pH on the colour of black wildebeest meat was also investigated.

3.2 Materials and methods

The 12 animals used in this study were hunted as part of a larger study on the effect of season on the meat quality of game species. The trial was approved by the Animal ethics committee at Stellenbosch University (Ethical clearance number: SU-ACUM14-001SOP). Processing of the carcasses was performed at an experimental facility in Kimberley, South Africa. After slaughter, the carcasses were eviscerated and cleaned and rapidly chilled. After the development of rigor mortis (24 h post-mortem), the carcasses were deboned. The *Biceps femoris* (BF) muscle was removed and ultimate pH was measured in this muscle using a Crison 25 pH meter (Crison instruments S.A, Barcelona, Spain, purchased from Lasec, South Africa). The pH meter was calibrated before each reading using standard buffers of pH 7 and 4. Thereafter, the meat was transported to the Meat Science Laboratory at SU where it was kept frozen at -20°C for 12 months, prior to subsequent analysis.

The BF muscles (right side) were defrosted at 4.2±0.8^{o}C for 48 h prior to analysis. Following defrosting, the meat was cut into steaks of 1.5 cm thickness and weighing approximately 200 g. The meat was packed in high-barrier (moisture vapour transfer rate of 2.2 $g \cdot m^{-2} \cdot 24\ h^{-1} \cdot 1\ atm^{-1}$, oxygen permeability of 30 $cm^{-3} \cdot m^{-2} \cdot 24\ h^{-1} \cdot 1\ atm^{-1}$ and carbon dioxide permeability of 105 $cm^{-3} \cdot m^{-2} \cdot 24\ h^{-1} \cdot 1\ atm^{-1}$), polyethylene and nylon film vacuum bags (70µm thickness) (Multivac packaging machine, USA). The steaks were randomly assigned to a sampling day. The meat was stored at refrigeration temperatures (4.2±0.8^{o}C) for the duration of the shelf-life study.

3.2.1 pH measurements

The pH was measured after each storage time using the iodoacetate method (Warriss, 2010; Zhu *et al.,* 2011). The meat sample was cut off from the core of the main piece and weighed. The meat sample (0.5 g) was homogenised in previously prepared iodoacetate/KCl reagent in a ratio of 1:10. The pH was then measured by inserting the probe (Jenway 3510 pH meter, Lasec, South Africa) into the homogenate and waiting for the reading to stabilise. The pH meter was calibrated using standard buffers of pH 7 and 4. The homogenised samples were placed on ice to reduce temperature fluctuations as these affect pH readings.

3.2.2 Microbiological analysis

Microbial analysis was done on day 0, 3, 6, 9 and 12 of vacuum storage. Twenty-five grams of meat samples were weighed aseptically. Thereafter, the meat was mixed with 225 mL buffered peptone water (Merck, South Africa) and digested in a stomacher for 60 seconds (BagMixer 400CC, Interscience). Dilution series was then made for the meat in 0.85% $v \cdot v^{-1}$ physiological salt solution, and 1 mL of the corresponding dilution was transferred aseptically to labelled petri dishes. For each dilution, all the samples were plated out in duplicate.

Total aerobic microorganisms (TVC) were identified through the use of plate count agar (PCA) (Merck, 2007). The plates were incubated at 37°C for 48 hours. After incubation, colonies were counted manually. Lactic acid bacteria (LAB) were selected for using De Man, Rogosa, Sharpe agar (MRS) according to the method outlined by Merck, (2007). Incubation was at 37°C for 48 hours. Counts were done thereafter and the results expressed as colony forming unit (cfu) per gram of meat. Total coliforms were enumerated through the use of violet red bile agar (VRBA) (Merck, 2007). The plates were incubated at 37°C for 24 hours and thereafter the visible colonies were counted (Da Silva *et al*., 2012). *Salmonella* was tested for using the enrichment method outlined by SANS, (2003) and the result only indicated presence or absence of *Salmonella*.

3.2.3 Colour analysis

At each storage period, after samples had been taken for microbial analysis, the meat was left to bloom for 30 min. Instrumental colour was then taken using a Colour guide 45°/0° Colorimeter on the steak surface (Catalogue no: 6805; BYK-Gardner, USA). The measurements were taken using an illuminant/observer of D65/10° (Neethling *et al*., 2014). The colour guide was calibrated using the standards provided (BYK-Gardner). The L*, a* and b* values were taken on five different points on the meat surface after blooming for 30 minutes. At each sampling period (days 0, 3, 6, 9, 12), notes were taken based on visual inspection of the meat.

3.2.4 Statistical analysis

Firstly, the growth for total viable counts, lactic acid bacteria and total coliforms were analysed at every time period (days 0, 3, 6, 9, 12) and for every muscle pH reading. Linear regressions were calculated, for the growth of the different microorganisms tested (SAS Inc, USA). Thereafter the meat was grouped based on ultimate pH and pH at day 0 of analysis. Grouping was then done according to the criteria that high pH meat (DFD) had values higher than 6 whereas low pH meat (Normal) had pH values below 6. Differences among the groups (DFD or Normal) were statistically analysed using the analysis of variance (ANOVA), general linear procedure (Proc GLM) of SAS version 8.2 (SAS Inc, USA). Thereafter, differences between the means were separated using t-tests for least significance differences. For all analysis, the confidence interval was 95%.

3.3 Results

3.3.1 Initial pH range in the samples

The pH of the meat ranged from 5.54 to 6.49 at day 0 as shown in Figure 3.1. Seven animals were classified as DFD (pH >6) and the rest were classified as Normal (pH <6) based on these pH values.

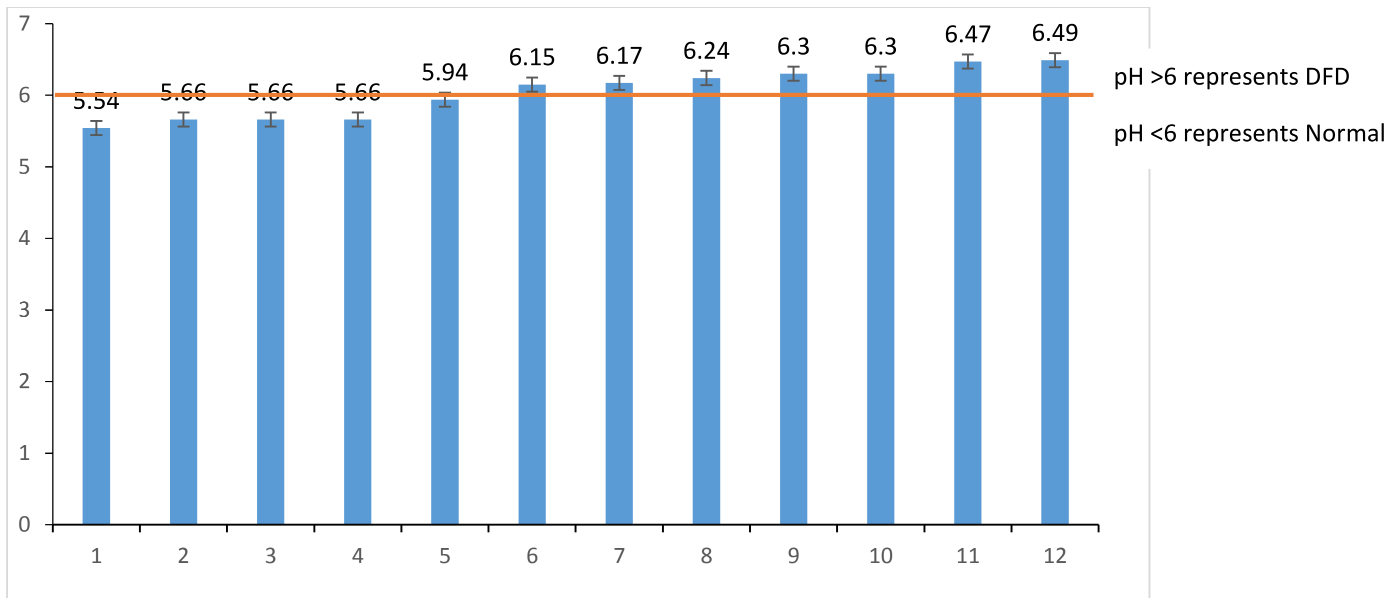


Figure 3.1: The range of pH in black wildebeest meat at day 0 arranged from lowest to highest (Line indicates pH categorising samples into Normal or DFD).

3.3.2 pH changes during storage

The trends observed for pH during the 12 day vacuum-storage period were different for Normal and DFD meat (Figure 3.2). In DFD meat, the pH remained fairly constant over the 12 d storage period; there were no noticeable differences between pH at day 0 and pH at day 12 (p>0.05). However, the pH dropped at day 6, although the drop was minor. For Normal meat however, the pH was constant from day 0 to day 6 and thereafter the pH dropped from approximately 5.78 to 5.48 during the storage period (Figure 3.2).

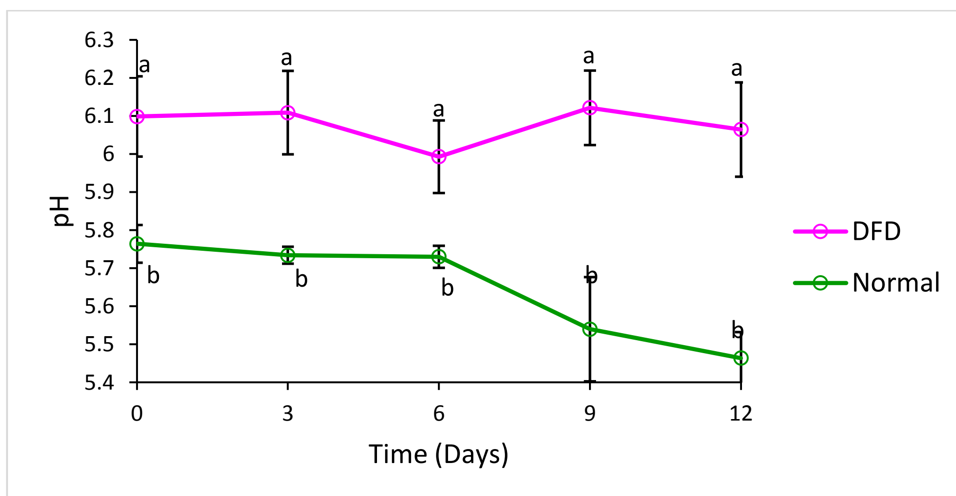


Figure 3.2: pH changes observed during 12 days of vacuum storage for black wildebeest meat, classified according to pH level.

[a]/[b] Means with different letters show significant differences between DFD and Normal groups

3.3.3 Microbiological results

The regression equations fitted for each individual animal/actual muscle pH showed that the growth in bacteria over the 12 day shelf-life period for DFD meat was significantly faster than that of Normal meat for TVC, LAB and total coliforms (Table 3.1).

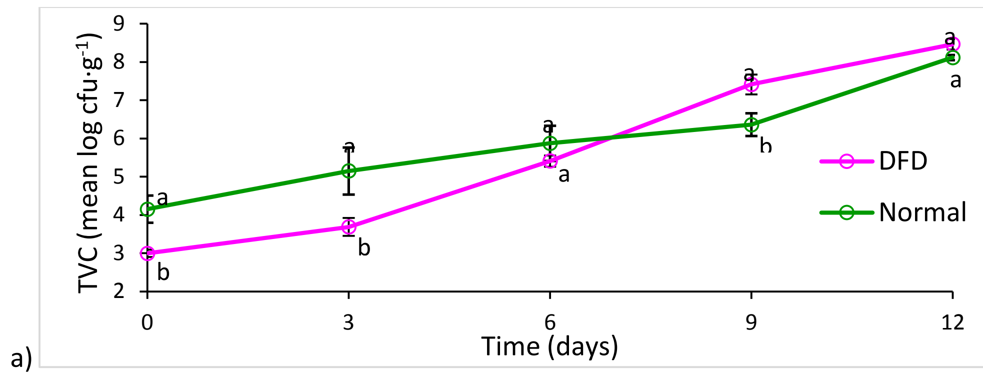
Table 3.1 Linear regression equations for growth of TVC, LAB AND coliforms in black wildebeest meat over 12 days vacuum storage (4.2±0.8°C).

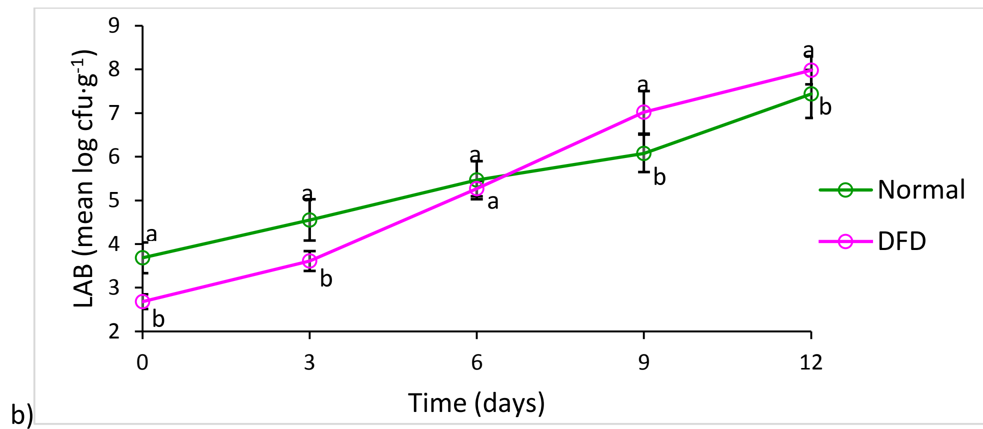
	TVC	Lactic acid bacteria	Coliforms
DFD	Y=0.49x+2.63	Y=0.47x+2.49	Y=0.26x+1.69
Normal	Y=0.31x+3.99	Y=0.30x+3.56	Y=0.18x+3.59
p-value	0.0001	0.0006	0.04

Y=TVC or Lactic acid bacteria or Coliforms; X=Time in days

When the meat was split into two groups based on pH level, TVC increased steadily in both DFD and Normal groups over the 12 day storage period (Figure 3.3). At the beginning of storage (day 0), Normal meat had significantly higher counts than DFD pH meat ($p<0.05$). At approximately day 7, TVC were the same for both DFD and Normal meat (Figure 3.3a). At the end of the 12 day storage period, both groups had exceeded 8 log cfu/g for TVC. At this stage, there was no significant difference between total viable counts for both DFD and Normal meat.

Lactic acid bacteria exhibited a similar growth pattern to TVC. Initially, Normal meat had significantly higher counts (1 log cfu·g^{-1} difference; $p<0.05$) than DFD meat. At day 7 of vacuum storage, both groups had similar counts. Thereafter, the counts for DFD meat were higher than Normal meat and this trend was observed till the end of the 12 day storage period ($p<0.05$). On day 12, DFD meat had 0.5 log cfu·g^{-1} more than Normal meat. Also, the similar trend observed for lactic acid bacteria and total viable counts suggested that lactic acid bacteria dominated the flora in the vacuum package. Therefore, although the meat reached levels of 6 log cfu·g^{-1} within 6 days of storage, there was no visible spoilage at the end of storage. At day 12, most samples gave a slightly acidic odour upon opening the package.





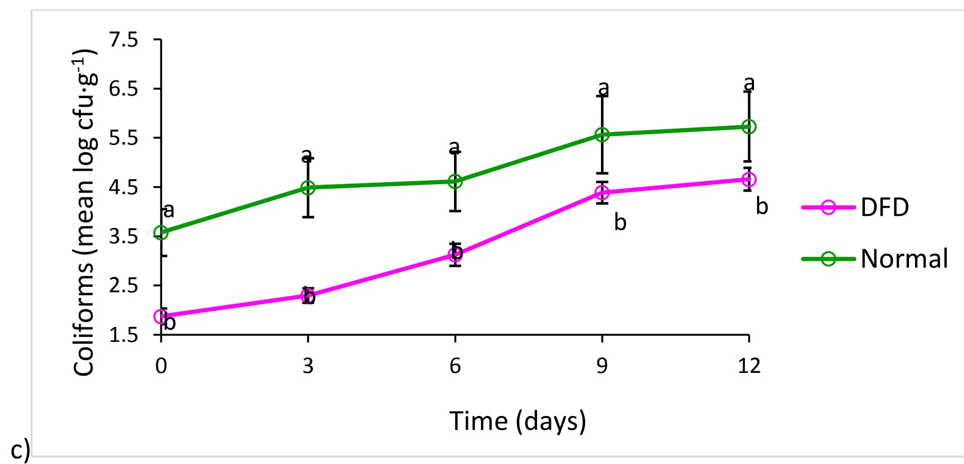


Figure 3.3: Total viable count (log cfu·g^{-1}) (a), lactic acid bacteria (b) and total coliforms (c) growth for Normal and DFD black wildebeest (BF) meat over 12 d of vacuum storage at 4.2±0.8°C.

[a]/[b] Means with different letters show differences between the DFD and Normal groups

Although total coliforms also experienced a general growth trend, the growth rate was slower under vacuum conditions and at 4.2±0.8°C, than that of total viable counts and lactic acid bacteria (Table 3.1). Similar to lactic acid bacteria and total viable counts, initial coliform counts of the DFD and Normal groups differed ($p<0.05$); there was a difference of approximately 2 log cfu·g^{-1} between the lower DFD and higher Normal groups at the beginning of storage. At the end of the 12 d storage period, there was a 1 log cfu·g^{-1} difference between the DFD and Normal meat ($p<0.05$). DFD meat experienced an increase of approximately 3 log cfu·g^{-1}, while Normal meat experienced an average growth increase of 2 log cfu·g^{-1} throughout the storage period.

3.3.4 Effect of vacuum storage on colour parameters

The meat in this study maintained a dark colour throughout the storage period. Once the meat was taken out of the vacuum package and blooming was allowed, visual examination showed that there were differences in colour between the samples. The DFD meat samples tended to maintain a dark red colour whereas the Normal meat appeared browner. At day 0 and 3, the meat from all the samples was still dark red. However, at day 6 there was a noticeable browning in the Normal meat samples. At the end of storage, although there was a noticeable difference in colour between all the meat samples, the colour was still attractive after the 30 mins blooming period.

Table 3.2: Mean (±SE[1]) of colour ordinates (L*, a*, b*) over 12 days of storage for the bloomed black wildebeest meat samples.

Colour ordinates	**pH level**	**Time (days)**				
		0	3	6	9	12
L*	DFD	28.6^{bw}±0.63	28.2^{bw}±0.63	29.1^{bw}±0.97	28.1^{bw}±1.41	29.4^{bw}±1.02
	Normal	34.3^{ax}±1.46	32.0^{ax}±1.03	32.8^{ax}±1.41	33.3^{ax}±1.40	33.1^{ax}±1.34
a*	DFD	11.1^{ay}±0.76	12.2^{ay}±0.57	11.6^{ay}±0.51	11.1^{ay}±0.56	12.0^{ay}±0.68
	Normal	12.5^{ay}±0.87	12.6^{ay}±0.33	12.1^{ay}±0.64	11.9^{ay}±0.64	12.5^{ay}±0.66
b*	DFD	7.8^{bz}±0.79	8.4^{by}±0.92	8.9^{by}±0.89	8.7^{bw}±0.80	9.4^{bw}±0.83
	Normal	9.9^{az}±1.20	11.0^{ay}±0.81	11.4^{aw}±0.95	11.3^{aw}±0.63	11.4^{aw}±1.28

[a/b] Means with different letters show differences ($p<0.05$) between pH groups within day.

[w-z] Means with different letters show differences ($p<0.05$) between time/day intervals.

[1]SE (standard error of the mean).

At the beginning of storage, L* for the Normal group was higher than that of the DFD group, which confirms the visual observation (lighter meat in the Normal group after blooming). There were no differences in a* at day 0 whereas b* was higher in Normal meat than in the DFD meat. During the storage period, there was no significant change in L* or a* values over time for both pH groups ($p>0.05$). There was however a significant increase in b* values noted for both pH groups (Table 3.2).

3.4 Discussion

In DFD meat, the pH remained unchanged throughout the storage period (Figure 3.2). In the Normal meat however, there was a decline in pH observed after 6 days of vacuum storage. Whyte & Fanning (2011) mentioned that for high protein foods, the buffering capacity is high. In meat, buffering systems such as phosphate compounds, histidylimidazole residues of myofibrillar proteins and the dipeptides carnosine and anserine (Puolanne & Kivikari, 2000) which regulate pH in living muscle would also be expected to be active in post-rigor meat. Vacuum packaged meats are dominated by lactic acid bacteria, which produce lactic acid in their metabolism; the meat in the Normal pH category had a high initial LAB load and this could be one cause for the pH fall observed in the black wildebeest meat (Figure 3.2). In addition, glucose available in meat can be broken down further during anaerobic glycolysis forming lactic acid, which further causes a fall in pH. In DFD meat, the levels of glycogen in the living animal's muscles are typically low prior to death of the animal (Adzitey & Nurul, 2011). Consequently, during storage there will be virtually no glycogen to be broken down into glucose and ultimately lactic acid (Lawrie, 1998). Therefore, the only changes in pH in DFD meat in this case would possibly be from lactic acid bacteria metabolism. This change in pH would most likely be resisted by the buffering systems mentioned in the meat. With Normal meat on the other hand, residual glycogen can be broken down further into glucose and lactic acid both during anaerobic glycolysis and by microorganisms. Although buffering could have occurred in both instances, the cumulative effect of lactic acid metabolism and glycogen breakdown possibly resulted in a significant dropping of the pH in low pH (Normal) meat as compared to high pH (DFD) meat.

However, results on the effect of storage time on the pH of meat is not conclusive; Jouki & Khazaei (2011) found no significant pH changes in camel meat stored under vacuum storage for 18 days. Sekar *et al*. (2006) on the other hand found a decline in pH over 3 days of vacuum storage for buffalo meat; thereafter the pH began to increase gradually. Different to the aforementioned studies, Fernandez-Lopez *et al*. (2008) reported a decline in pH of ostrich meat stored under vacuum storage for 18 days. Considering the differences in pH trends observed for vacuum storage meat for different studies, it could be proposed that differences exist due to the biochemical condition of the meat during storage; for example, meat with a higher concentration of residual glycogen would be expected to experience a greater fall in pH than meat with lower glycogen concentrations. Therefore, in such studies it is valuable to also include measurements of such compounds (glucose and lactate) during storage.

Vacuum packaging has been used to extend meat shelf-life successfully (John *et al*., 2005). Vacuum storage eliminates oxygen and when coupled with low storage temperatures, promotes the growth of facultative anaerobes and psychrophilic and psychrotrophic microorganisms such as lactic acid bacteria (Doulgeraki, 2010; Doulgeraki *et al*., 2012). According to Food Science Australia (2003),

spoilage in meat becomes evident when the total viable counts exceed 6 log cfu/g. Under vacuum storage however, total viable count is not a reliable indicator of microbial quality as the populations are dominated by lactic acid bacteria. In such instances, spoilage only occurs when TVC levels exceed 8 log cfu·g^{-1} (Food Science Australia, 2003). Kiermeier *et al*. (2013) found that lamb was still acceptable after 12 weeks of storage, despite reaching TVC levels of 8 log cfu·g^{-1} after 8 weeks of storage. When beef is stored under vacuum storage in combination with chill temperatures, the shelf-life is approximately 10 to 12 weeks (Food Science Australia, 2003).

In the current study, although composition of TVC was not measured, it was probably composed of LAB; due to the similar growth curves observed (Figure 3.3a and 3.3b). For both DFD and Normal meat, levels of TVC and LAB exceeded 8 log cfu·g^{-1} within 12 days, compared to the 8 weeks reported by Kiermeir *et al*. (2013). Daszkiewicz *et al*. (2011) reported a 1 log cfu·g^{-1} increase in TVC over 7 days of vacuum storage of roe deer and a 2.55 log cfu·g^{-1} increase over 21 days of vacuum storage. In comparison, the current study had an increase of 2 log cfu·g^{-1} over 7 days of storage and an increase of 4 log cfu·g^{-1} over 12 days of storage (Figure 3.3a; Normal meat). This shows that even in the Normal meat, the growth rate exhibited for TVC was higher than what would otherwise be expected. Wiklund (2011) found that when vacuum packed reindeer meat was stored at chill temperatures, the quality at 2 and 3 weeks of storage went from borderline to poor (>6 log cfu·g^{-1}). Lorenzo & Gomez (2012) reported an increase of 2 log cfu·g^{-1} for TVC, over 14 days of vacuum packed, high pH ostrich meat. This differed from the increase observed in the current study of 5 log cfu·g^{-1} over 12 days for the DFD meat. Allonso-Calleja *et al*. (2004) on the other hand experienced an increase of approximately 3 log cfu·g^{-1} over 7 days for high pH ostrich meat; these results are almost similar to the current study.

Knox *et al.* (2008) reported an average of 5 log cfu·g^{-1} increase in lactic acid bacteria over a period of 34 days for vacuum packed pork stored at 4°C. This is lower than the increase of approximately 6 log cfu·g^{-1} observed in the current study. Furthermore, Knox *et al*. (2008) reported that the spoilage flora was not dominated by lactic acid bacteria but by aerobic plate counts, psychrotrophic plate counts and Enterobacteria. In contrast, Alonso-Calleja (2004) found that the LAB levels reached 7 log cfu·g^{-1} within 7 days of packaging and also dominated the spoilage microflora in the package. When the meat packages in this investigation were opened on day 12, there was a slight acidic smell observed for most of the meat samples. Similarly Alonso-Calleja *et al*. (2004) reported an acidic odour upon opening vacuum packed meat and attributed this to lactic acid formed by LAB.

In the current study, the growth of total coliforms was slower than that of both LAB and TVC. Also, in Normal meat, only a 2 log increase was observed for total coliforms (Figure 3.3c). The slower growth rate is mostly attributed to the growth requirements for total coliforms. In environments of limited

oxygen, the growth of coliforms is limited (Sun & Holley, 2012). Furthermore, the chill temperatures (4.2±0.8°C) used in the study further inhibited the growth of coliforms. Assuming that the microflora was dominated by LAB, this could have also led to an inhibition in the growth of total coliforms.

Spoilage of meat occurs when glucose in the muscle is depleted (Gill & Newton, 1979; Newton & Gill, 1980). In normal pH meat, spoilage has been shown to be delayed until glucose depletion. As such, shelf-life extension has been achieved by adding glucose to meat (Gill & Newton, 1979). Glucose depletion leads to the onset of microbial attack on amino acids. DFD meat is deficient in glucose therefore amino acid attack occurs earlier than it would for normal pH meat (Food Science Australia, 2003). Also, this explains possibly why spoilage in DFD meat has been shown to occur at fewer cell numbers (Gill & Newton, 1979). Apart from glucose, lactate concentration also affects microbial growth. Knox *et al.,* (2008) observed no change in glucose concentration over 34 days of vacuum storage. However, there was a significant difference between the pH groups at the end of storage; the lowest pH group had the highest amount of residual glucose and lactate.

Salmonella tests were negative for all the samples throughout the storage period. Sagoo *et al*. (2007) did not detect *Salmonella* in any of the turkey samples they tested. El-Ghareeb *et al*., (2009) and Wiklund, (2011) also did not detect *Salmonella* in hunted game birds and reindeer stored under vacuum storage and chill temperatures. Obeng (2013) however reported having isolated *Salmonella* spp. in meat sold at a Ghanaian market and the cause of this detection was assumed to be poor hygiene practices.

What makes comparisons between different studies difficult is the fact that numerous factors could influence the microbial growth; initial microbial load and storage temperature being two of the more important factors. Although the increase in TVC differs across different studies, the trend observed is that DFD meat experiences higher growth rates than normal pH meat. However, meat used in the current study (both DFD and Normal) had higher growth rates for total viable counts when compared to meat from other studies – results were mostly linked to the storage temperature as well as initial microbial contamination/load.

Although it can be deduced that pH had an effect of promoting microbial growth in DFD meat, both DFD and Normal meat seemed to experience faster growth rates when compared to other meat. A possible explanation for this occurrence is the frozen storage of meat. Although at temperatures of -20°C, any form of microbial growth is stopped, this may cause damage to the meat structure resulting in easier proliferation of microorganisms after thawing. During freezing, ice crystals form within the muscle structure and the size thereof is determined by the rate of freezing (Leygonie *et al*., 2012;

Aidani *et al*., 2014). Rapid freezing has been shown to have less impact on quality parameters of meat, as there is less damage to the muscle fibres and myofibrils (Aidani *et al*., 2014).

Leygonie *et al*. (2012) found that neither freezing nor thawing decreased the number of viable microbes present in meat. Although the microorganisms are dormant at freezing temperatures, they recover during thawing (Londahl & Nilaaon, 1993). The drip which is released during thawing is rich in nutrients which promote microbial growth. However, thawing is not uniform and some portions of the meat, particularly close to the surface may experience more microbial growth when compared to meat at the core (Leygonie *et al*., 2012). Increase in temperature during thawing is also not uniform and results in the surface defrosting at a faster rate than the core of the meat. As time progresses, the microorganisms may also move to the core of the meat, with more ease as the physically inhibiting muscle structure will have been damaged by ice crystals (Leygonie *et al*., 2012). There the microorganisms also encounter proteins, minerals and vitamins which would have been released from the meat structure due to the freeze-thaw process (Leygonie *et al*., 2012). However, Cox *et al*. (1998) found that chicken carcasses which were frozen for two months and thawed had similar shelf-life when compared to unfrozen meat. Vieira *et al*. (2009) also found that beef frozen for three months, following an ageing period of 3 and 10 days, did not spoil due to microbial growth. However, an increase in psychrotrophic bacteria was observed during the three month frozen storage period. These were then probably favoured above the other bacteria by thawing at 4.2±0.8°C for 48 hours. This could explain the increased growth in lactic acid bacteria but not total coliforms noted in this investigation. Most studies however focus on the quality effects of freezing and thawing and not necessarily on the effect thereof on microbiological growth.

Although there are limited studies on the colour of black wildebeest meat, Hoffman *et al*. (2009) reported similar initial L*, a* and b* values for this species as those reported in this study. There were some differences observed between the two pH categories for L* and b* values. Normal meat consistently had higher L* and b* values than DFD meat (Table 3.2). Game meat is considered to be darker than other types of red meat due to an increased concentration of myoglobin in the muscle (Lawrie, 1998; Hoffman, 2005). On average, the L* values were lower than what would otherwise be expected for red meat which confirms that black wildebeest meat is darker than other red meat. Muchenje *et al*., (2009) reported L* values ranging from 37-40.4 for DFD meat whereas Owens *et al*., (2000) reported L* values of 47.31-48.99 for normal meat. Seydim *et al*. (2006) mentioned that at least a 5 point difference is required in L* values to show differences between samples; the differences found in this study between DFD and Normal meat exceeded this requirement (Table 3.2). Warriss (2000) attributed the colour observed in DFD meat to insufficient protein denaturation. At pH close to neutral, the protein structure remains intact and limited denaturation occurs. Therefore, the protein

structure has a high affinity for intracellular water which in turn reflects less light. More light absorption makes DFD meat to be perceived as darker than normal meat (Warriss, 2000). Zhang *et al*., (2005) reported that DFD or high pH meat had lower L*, a*and b* values than normal pH meat; this is in agreement with the results obtained in this study for L* values.

Under normal vacuum storage, there is less than 1% residual oxygen in the package (Mancini & Hunt, 2005) which is consumed by microorganisms or biochemical reactions in the meat. In this study, the percentage of residual oxygen in the vacuum package was not measured. However, the anoxic environment causes vacuum packaged fresh meat to have a dark, purplish red colour because the reducing enzymes have converted the meat pigment to deoxymyoglobin (AMSA, 2012). Changes in the myoglobin condition are mostly responsible for colour changes in meat during storage. When subjected to high concentrations of oxygen, myoglobin is oxidised to oxymyoglobin which is red in colour. In the absence of oxygen, myoglobin is reduced to deoxymyoglobin, which is purple in colour. Prolonged exposure to low oxygen concentrations leads to the formation of brown metmyoglobin; which is associated with low quality or 'stale' fresh meat. Metmyoglobin forms on the surface of meat when the iron group is oxidised to the ferric state (Fe^{3+}) and the layer can become thicker in the absence of oxygen (Mancini & Hunt, 2005). Adzitey & Nurul, (2011) reported that in high pH (DFD) meats, oxygen penetration is reduced due to the intact protein structure. Therefore, any oxygen which manages to penetrate the muscle is removed by the high cytochrome activity which is encouraged by the high pH. This results in a thin surface layer of bright red oxygenated myoglobin, allowing visibility of the purple colour of the underlying reduced myoglobin.

In this case, due to the packaging effect, there were no changes (after blooming) in lightness and redness observed over time. Daszkiewicz *et al*., (2011) however reported a significant increase in L* values for roe deer meat stored under vacuum storage for up to 21 days. Other authors also reported an increase in L* values during vacuum storage ranging from 12 to 18 days (Kusmider *et al*., 2002; Fernandez-Lopez *et al*., 2008). In the above-mentioned studies, it was not clear whether the meat was bloomed prior to taking colour measurements.

Similar to this study, Daszkiewicz *et al*. (2011) also reported a significant increase in b* values over the storage period. Also, although there were no noticeable changes in a* ordinates (Table 3.2), there was a slight decrease noted. An increase in b* over the storage period is correlated to an increase in metmyoglobin formation in the muscle tissue (Neethling *et al*., 2014); a significant increase was observed for the b* ordinates over time (Table 3.2). Storage time affects colour stability; the co-factors and reducing enzymes which are necessary for the conversion of metmyoglobin back to myoglobin are depleted as storage time increases (Boles & Pegg). Also, freezing the meat might have led to

microstructure damage, such that the ability of metmyoglobin to revert back to myoglobin would be reduced (Boles & Pegg). Fernandez-Lopez *et al*., (2008) reported a decrease in a* values over 18 d of vacuum storage whereas Seydim *et al*., (2006) did not observe changes in a*. The comparison between studies is difficult for colour as the meat storage and handling differ.

According to Daszkiewicz *et al*. (2011), the amount of metmyoglobin formed is also related to tissue pH. High pH (DFD) meat consistently had lower b* values than Normal meat throughout the storage period. At lower pH, myoglobin becomes more prone to oxidation and metmyoglobin formation increases. Thus higher levels of metmyoglobin would be expected to be formed in Normal meat as compared to DFD meat. Zhang *et al*. (2005) also reported that DFD meat had less browning than Normal meat over time.

Some studies reported that high pH meat stored under vacuum storage was more prone to greening as a result of microbial production of hydrogen sulphide (Adzitey & Nurul, 2011). Hernandez-Macedo *et al*. (2011) reported that the growth of *Alteromonas putrefaciens, Aeromonas* spp. and *Enterobacteriaceae* was responsible for greening in vacuum packed meats. The breakdown of the amino acid cysteine forms hydrogen sulphide which reacts with myoglobin to form sulphomyoglobin (Broda *et al*., 2002). Consequently, meat with high concentrations of myoglobin would experience more intense greening. Furthermore, for high pH meat, the attack on amino acids occurs prematurely, due to the depletion of glucose (Hernandez-Macedo *et al*., 2011). The conclusion reached was that vacuum storage was not suitable for DFD meat due to premature greening. In this study however, the meat did not turn green during the 12 day storage period; although this green phenomenon has been noticed in random vacuum packed muscle samples from this species that was also classified as DFD (data not shown). This could be as a result of the initial populations of microorganisms present and is an aspect that warrants further research.

3.5 Conclusions

In this study, the pH changes, microbial growth and colour stability were investigated for vacuum packaged black wildebeest meat under chilled storage. The investigation centred on the role of ultimate pH on the shelf-life of the meat. DFD (pH >6.0) meat exhibited higher growth rates for lactic acid bacteria and total viable counts when compared to Normal (pH <6.0) meat. This confirms the hypothesis that DFD meat spoils more rapidly than Normal meat. However, the initial microbial load of the meat varied which could be as a result of inconsistency during field dressing of the carcasses. The process needs to be regulated to ensure that the meat produced is of standard quality. Vacuum packaging could potentially be used in the distribution and storage of DFD meat as it prolongs the shelf-life.

Further research could focus on a larger sample size, consisting of meat with a continuum of pH; ranging from normal to intermediate, then to high ultimate pH whilst ensuring that the meat all had a similar initial microbial contamination and species structure. The range at which significant differences in microbial growth and ultimately spoilage occur can be determined in this way. Also, the concentrations of biochemical constituents of the meat could be determined, to find out at which levels of glucose spoilage begins to occur.

3.6 References

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Chapter 4: Preservation of previously frozen black wildebeest meat (*Connochaetes gnou*) using oregano (*Oreganum vulgare*) essential oil.

Abstract

This study investigated the effects of oregano essential oil (1% $v{\cdot}v^{-1}$) on the microbial shelf-life, lipid oxidation and colour stability in black wildebeest meat under refrigerated aerobic storage. The essential oil lowered initial counts for all microorganisms tested. Furthermore, meat treated with the essential oil had slower growth rates of lactic acid bacteria and total viable counts. The microbial shelf-life was determined to be 6 days for the untreated meat and 9 days for the treated meat; translating to a shelf-life extension of 3 days by the oil. Lipid oxidation was lower in the treated meat, throughout the storage period. However, lipid oxidation in both the treated and untreated meat was high initially; this was attributed to previously freezing the meat. Therefore, no deduction was made on this aspect of shelf-life. Similarly, colour for both treatment groups was relatively unstable; at the beginning of storage the metmyoglobin percentage was higher than the level at which visible browning occurs. Therefore, no deductions of shelf-life were made based on the colour attributes. Overall, adding oregano essential oil significantly lowered microbial growth, lipid oxidation and reduced metmyoglobin percentage. A recommendation is however being made for further studies on fresh meat, to effectively state the efficacy of the oil in elongating shelf-life.

4.1 Introduction

The modern consumer has become increasingly aware of their health and the external environment (Hoffman & Wiklund, 2006; Mathijs, 2015). This has been a response to the increase in lifestyle diseases and also environmental issues such as climate change and global warming. Consequently, the demand for organic or responsibly sourced products has increased (Dransfield, 2003; Verbeke *et al*., 2010). Game meat is produced with minimal agricultural input and can be classified as being organic or natural (Hoffman, 2007). With the increase in popularity of organic products comes the need for different formats of marketing.

Consumers are also increasingly concerned with their safety and therefore product safety is one of the minimum requirements for the success of a food product. In meat products, preservatives such as nitrites, butylated hydroxytoulene, acetic acid, SO_2 and butylated hydroxyanisole have been used successfully for shelf-life extension (Baratta *et al*., 1998). Although most of these preservatives have been successful in improving safety, concerns are being raised on the safety of the preservatives themselves. Mepham (2011), reported that a number of additives used in meat have been linked to

cancer in severe cases, and allergic reactions. The reality is that although these preservatives may be implicated in some diseases, their role in preservation has been successful. There is therefore need for research on compounds which are both safe for consumption while effectively inhibiting microbial growth and quality deterioration. Burt (2004) mentioned that there continues to be an increase in the demand for natural additives.

Considering that game meat is organic, it would be beneficial to use natural additives in its preservation. Some research has been done on the potential of natural preservatives and the essential oils of some plants have been found to have strong antimicrobial, antifungal and antioxidant properties (Burt, 2004; Seow *et al*., 2014). Essential oils are complex mixtures of volatile compounds derived from plants (Sanchez-Ortega *et al*., 2014). Oils which are mainly made up of thymol, carvacrol and eugenol have been shown to have most inhibition on microorganisms (Sanchez-Ortega *et al*., 2014). *Oregano vulgare* essential oil has been shown to contain thymol and carvacrol and is one of the most potent essential oils (Sivropoulou *et al*., 1996; Baratta *et al*., 1998). *In vitro* and *in vivo* studies have shown that oregano essential oil is active against a broad spectrum of microorganisms in meat (Hammer *et al*., 1999; Chouliara *et al*., 2007; Schirmer & Langsrud, 2010). However, Schirmer & Langsrud (2010) also mentioned that although an oil may be effective in a particular food system, the same cannot be implied in a different food system. For instance, if an oil is successful in inhibiting *Salmonella* in pork, the same might not apply to *Salmonella* in chicken, due to the intrinsic differences in the meat.

Therefore, the aim of this study was to determine the preservative effects of oregano essential oil in freeze-thawed, refrigerated black wildebeest meat. This was done by testing the effect of the oil on the natural flora of black wildebeest (*Connochaetes gnou*) meat. The effect of the oil on lipid oxidation, pH and colour was also investigated.

4.2 Materials and methods

The 11 animals used in this study were hunted as part of a larger study on the effect of season on the meat quality of game species. The trial was approved by the Animal ethics committee at Stellenbosch University (Ethical clearance number: SU-ACUM14-001SOP). Processing of the carcasses was performed at an experimental facility in Kimberley, South Africa. After slaughter, the carcasses were eviscerated and cleaned and rapidly chilled. After the development of rigor mortis (24 hours post mortem), the carcasses were deboned. The *Biceps femoris* (BF) muscle was removed and ultimate pH was measured in this muscle using a Crison 25 pH meter (Crison instruments S.A, Barcelona, Spain, purchased from Lasec, South Africa). The pH meter was calibrated before each reading using standard

buffers of pH 7 and 4. Thereafter, the meat was transported to Stellenbosch University's meat science laboratory where it was kept frozen at -20°C for 12 months, prior to subsequent analysis.

For the trial, 11 animals were used; the BF muscles (left side) were defrosted at 2.6±0.6^{o}C for 48 hours prior to analysis. Following defrosting, the meat was cut into steaks of 1.5 cm thickness and weighing approximately 150 grams. Oregano essential oil (Clive Tuebes, South Africa) was diluted to a concentration of 1% $v.v^{-1}$ in sterilised spring water. Half of the steaks were dipped into the essential oil solution for 60 s and the rest were left untreated. Thereafter the meat was packed in polystyrene trays, overwrapped with low density polyethylene wrap (moisture vapour transfer rate of 585 $g{\cdot}m^{-2}{\cdot}24\ h^{-1}{\cdot}1\ atm^{-1}$, oxygen permeability of 25 000 $cm^{-3}{\cdot}m^{-2}{\cdot}24\ h^{-1}{\cdot}1\ atm^{-1}$ and carbon dioxide permeability of 180 000 $cm^{-3}{\cdot}m^{-2}{\cdot}24\ h^{-1}{\cdot}1\ atm^{-1}$). The meat was stored at refrigeration temperatures (2.6±0.6^{o}C) for the duration of the shelf-life study.

For microbial analysis, sampling was done at days 1, 3, 5, 7 and 9 to allow for the microorganisms to grow. On the microbial sampling days, surface colour and pH of the samples was also taken. However, for lipid oxidation, due to the unstable nature of the measured compounds, sub-samples were collected on days 0, 1, 2, 3, 4, 5, 7 and 9 of storage. The steaks where lipid oxidation sub-samples were taken were packaged separately to allow for sampling without contaminating the meat. Immediately after sampling, the lipid oxidation and pH samples were frozen in liquid nitrogen and frozen at -80°C until further analysis

4.2.1 Oregano essential oil analysis

The essential oil was kept refrigerated and in an opaque glass jar for the duration of the experiment. To determine the amounts of active compounds in the oil, GC-FID (Gerchman *et al*., 2011) was used (CAF Stellenbosch University). Standard solutions of the active compounds (carvacrol, thymol, p-cymene, gamma terpinene, alpha terpinene, beta caryophyllene, alpha humulene) were prepared and injected without extra treatment (Sigma Aldrich, South Africa). The method of external standard calibration as outlined by Ntsomboh-Ntsefong *et al*. (2014) was used. The standard curves for each standard solution were prepared using the standard solutions at known concentrations. The peaks in the chromatogram were plotted against the initial amounts injected. Separation of the active compounds was accomplished by gas chromatography (equipped with DB-5 capillary column of 50 m length and 0.25 mm i.d) (Chrompack 9001, Netherlands) fitted with a flame ionization detector, and an automatic liquid sampler (CP-9050, Chrompack). The injector was maintained at 250°C and the detector at 280°C. Nitrogen was used as a carrier gas at a constant flow rate (1 ml/min). The oil was then injected and separate compounds within were identified by their retention times and

chromatographic comparison with the standards. The percentage composition of the individual components was computed from the GC-FID peak areas without using correction factors.

4.2.2 pH measurements

The pH of meat was measured after each storage period (days 1, 3, 5, 7 and 9) using the iodoacetate method (Warriss, 2010; Zhu *et al.,* 2011). The meat sample was cut off from the core of the sub-sample and weighed; the sample (0.5 g) was then homogenised in previously prepared iodoacetate/KCl reagent in a ratio of 1:10. The pH was measured by inserting the probe (Jenway 3510 pH meter, Lasec, South Africa) into the homogenate and waiting for the reading to stabilise. The pH meter was calibrated using standard buffers of pH 7 and 4. The homogenised samples were placed on ice to reduce temperature fluctuations as these affect pH readings.

4.2.3 Lipid oxidation

The extent to which lipid oxidation occurred was measured through the 2-thiobarbituric acid extraction method (Lynch & Frei, 1993; Botsoglou *et al*., 1994). One gram of meat was weighed from the core of the steak sub-sample. The meat sample was placed in 10 ml of 0.15 M KCl with 0.1 mmol butylated hydroxytoulene (BHT). Thereafter, the meat was homogenised for 30 s (P-8; Kinematica, Littau, Switzerland). An aliquot (0.5 mL) of the homogenised meat was transferred to tubes containing 0.25 mL of NaOH (50 mmol) combined with 0.25 mL of 2-thiobarbituric acid and 0.25 mL trichloroacetic acid (TCA). The tubes were then incubated in a 100°C water-bath for an hour. Upon removal from the water-bath, the tubes were left to cool to room temperature. Thereafter 2 mL of n-butanol was added to the tube and vortexed and then centrifuged at 4°C, 4 000 rpm for 30 min (Allegra X22R; Beckman Coulter, Germany). The extract (0.2 mL) was then removed from the tube and transferred to microplate wells and the absorbance was measured at 532 nm (Cecil CE2012 2000Series, Lasec, South Africa). The standard curve was constructed using known concentrations of 1,1,3,3-tetramethoxypropane (TMP). The concentrations of TMP were varied by creating a dilution of TMP in distilled water. Thereafter 0.5 mL of TMP dilution was added to tubes containing 0.25 mL NaOH, 2-thiobarbituric acid and TCA. The standard curve tubes were also incubated, centrifuged and their absorbance measured in a similar way to the meat samples. The TBARS were then calculated using the standard curve and expressed as mg of malonaldehyde per kg of meat (mg MDA$\cdot$Kg^{-1}).

4.2.4 Microbiological analysis

Microbial analysis was done on day 1, 3, 5, 7 and 9 of aerobic storage. Twenty-five grams of meat samples were weighed aseptically. Thereafter, the meat was mixed with 225 mL buffered peptone water (Merck, South Africa) and digested in a stomacher for 60 seconds (BagMixer 400CC, Interscience). A dilution series was then prepared for the meat in physiological salt solution (0.85%

NaCl), and 1 mL of the corresponding dilution was transferred aseptically to labelled petri dishes. For each dilution, all the samples were plated out in triplicate.

For total viable counts (TVC) plate count agar (PCA) (Merck, 2007) were used. The plates were incubated at 37°C for 48 h. After incubation, colonies were counted manually. Lactic acid bacteria (LAB) were selected for using De Man, Rogosa, Sharpe agar (MRS) according to the method outlined by Merck, (2007). Incubation was at 37°C for 48 hours. Counts were performed thereafter and the results expressed as colony forming unit (cfu) per gram of meat. Total coliforms were enumerated using violet red bile agar (VRBA) (Merck, 2007). The plates were incubated at 37°C for 24 hours and thereafter the visible colonies were counted (Da Silva *et al*., 2012).

4.2.5 Colour analysis

At each storage period (days 1,3,5,7 and 9), after samples had been taken out for microbial analysis, instrumental colour was then taken using a Colour guide 45°/0° colorimeter on the steak surface (Catalogue no: 6805; BYK-Gardner, USA). The measurements were taken using an illuminant/observer of D65/10° (Neethling *et al*., 2014). The colour guide was calibrated using the standards provided (BYK-Gardner). L*, a* and b* values were taken on five different points on the meat surface. Hue angle and Chroma were then calculated from the samples according to the CIE Lab system. The following formulas were used: Hue angle = $\tan^{-1}(b^*/a^*)$ and $C^* = \sqrt{(a^{*2}+b^{*2})}$ (AMSA, 2012). The concentrations of metmyoglobin, deoxymyoglobin and oxymyoglobin at the meat surface were calculated from the spectral data as outlined in AMSA (2012). At each sampling period, notes were taken based on visual inspection of the meat.

4.2.6 Statistical analysis

The results for pH, lipid oxidation, microbial analysis and colour were analysed using the analysis of variance (ANOVA), general linear procedure of SAS (SAS Inc, USA). Thereafter the least significant differences for the means for the treatment and control were determined using t-tests. For all analysis, the confidence interval was 95%. Microbial analysis data was further analysed through linear regressions to compare the growth rates between the control and the treatment.

4.3 Results

4.3.1 Active compounds in Oregano essential oil

The major compound detected in oregano essential oil was carvacrol, followed by thymol (Table 4.1). The rest of the compounds were detected in trace amounts.

Table 4.1: Compounds detected in a food grade commercial *Origanum vulgare* essential oil.

Compound	Carvacrol	Thymol	p-cymene	Beta caryophyllene	Gamma-terpinene	Alpha humulene	Alpha-pinene
Concentration %	42.94	17.4	8.04	2.35	1.82	1.21	1.18

4.3.2 pH changes during aerobic storage

Changes in the pH of the meat throughout storage were monitored and the results are shown in Figure 4.1. The addition of the oregano essential oil caused a decrease in the meat's pH (Figure 4.1).

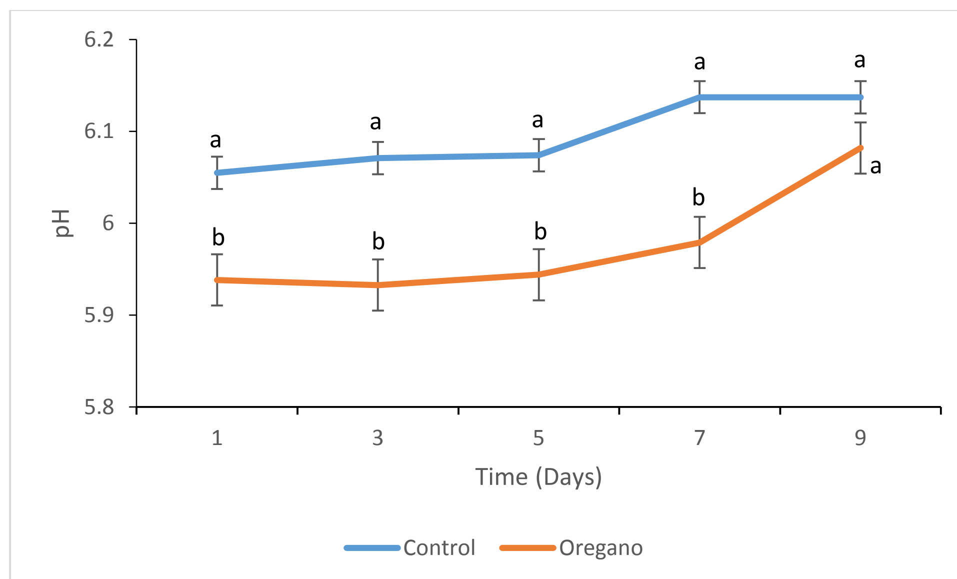

Figure 4.1: pH changes in refrigerated (2.6±0.6°C) black wildebeest meat treated with either 1% $v \cdot v^{-1}$ oregano essential oil or none (control) under aerobic storage

[a]/[b] Means with different letters represent significant differences ($p<0.05$) between oregano treatment and control groups

The pH for the control samples was significantly higher than that of treatment samples throughout the first 7 days of the storage period (Figure 4.1). The pH did not change noticeably between the two treatments for the first five days of storage (Figure 4.1). However, there was an increase in pH in the control samples between day 5 and day 7, followed by no increase between day 7 and day 9. For the treatment samples, there was also an increase in pH between day 5 and 7, albeit insignificant. Thereafter, there was a sharp increase in pH between day 7 and day 9.

4.3.3 Lipid oxidation

TBARS for the meat samples obtained throughout the storage period are presented in Figure 4.2.

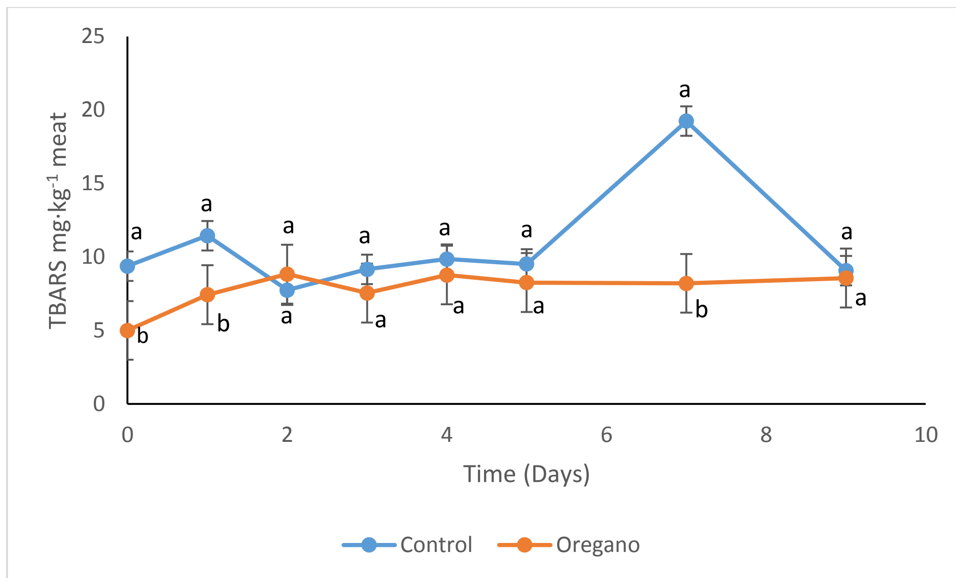

Figure 4.2: TBARS for refrigerated black wildebeest meat treated with 1%v/v oregano essential oil and the control under aerobic storage

[a]/[b] Means with different letters show significant differences (p<0.05) between the oregano treatment and control groups

At the beginning of storage, TBARS for the oregano treatment samples were lower (p<0.05) than that of the control samples (Figure 4.2). The trends observed for the treatment samples included a noteworthy increase between day 0 and day 2, followed by a decrease between day 2 and day 3. Thereafter, there were no noticeable changes observed for TBARS till the end of the storage period. For the control samples however, there was an increase observed in TBARS between day 0 and day 1 (Figure 4.2). Thereafter, there was a decrease in TBARS between day 1 and day 2. Afterwards, there was a gradual increase in TBARS between day 2 and day 5, followed by a sharp (p<0.05) peak at day 7. Towards the end of storage, there was a decrease again in TBARS in the control samples from day 7 to day 9.

4.3.4 Growth rates for TVC, LAB and total coliforms

There was a general increase observed for TVC, LAB and total coliforms for both the control and treatment samples over the storage period (Figures 4.3, 4.4 and 4.5 respectively). There was an average initial inhibition (at day 1) by the oil, which resulted in almost 1 log reduction in TVC and LAB (Figures 4.3 and 4.4).

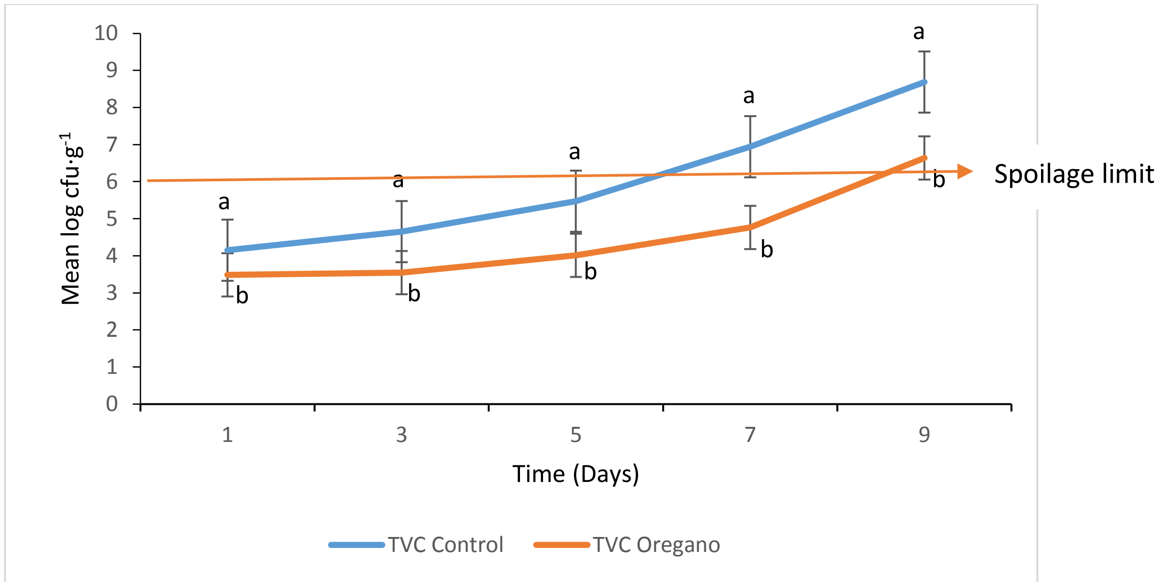

Figure 4.3: TVC growth in refrigerated black wildebeest meat under aerobic packaging and 1% oregano essential oil treatment

[a]/[b] Means with different letters show significant differences ($p<0.05$) between the oregano treatment and control groups

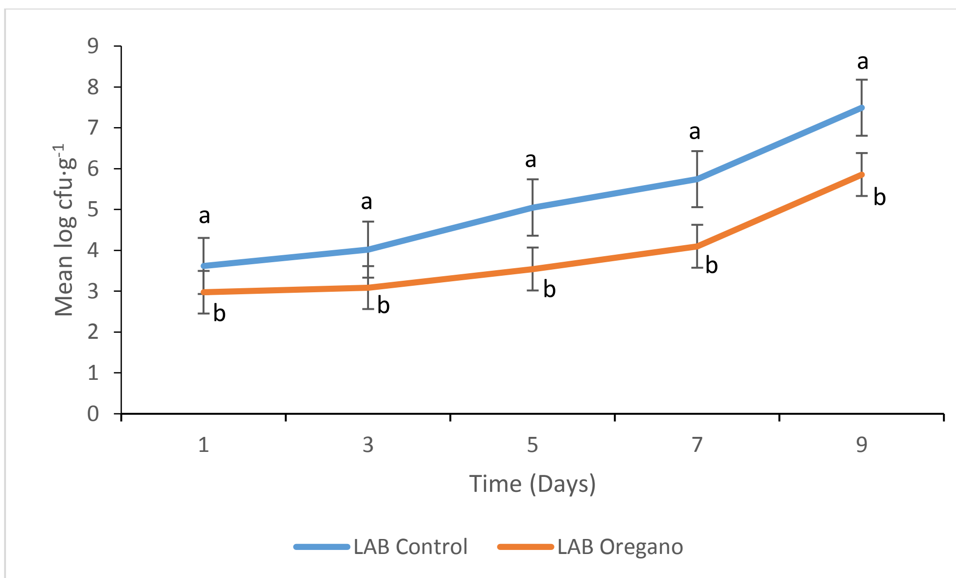

Figure 4.4: LAB growth in refrigerated black wildebeest meat aerobically stored (Treatment- 1% v/v oregano essential oil; Control- no oil)

[a]/[b] Means with different letters show significant differences ($p<0.05$) between oregano treatment and control groups

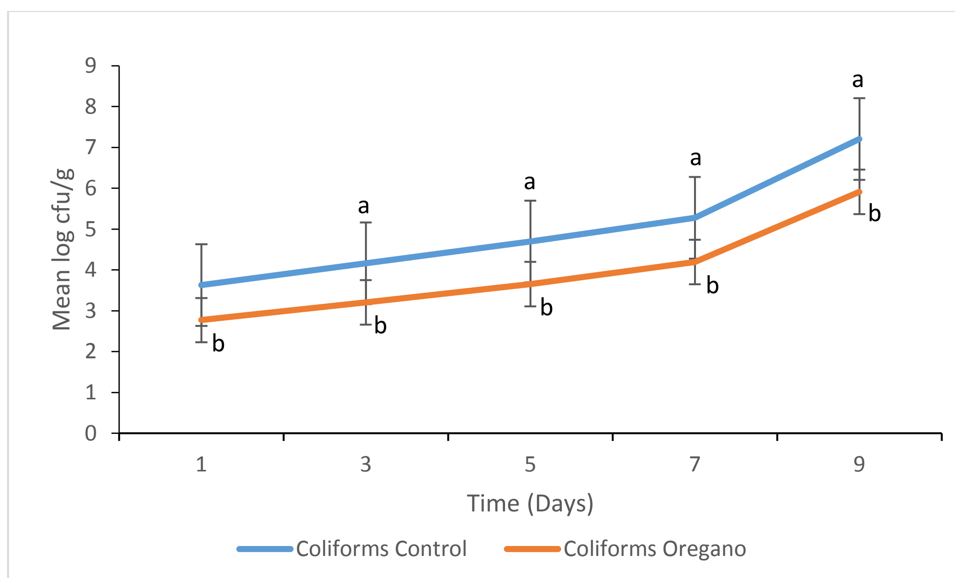

Figure 4.5: Total coliforms growth in refrigerated black wildebeest meat aerobically stored (Treatment- 1% v/v oregano essential oil; Control- no oil)

a/b Means with different letters show significant differences ($p<0.05$) between the oregano treatment and control groups

At approximately day 6 of storage, the counts for the control group for TVC and LAB reached 6 log cfu·g^{-1} (Figures 4.3 and 4.4 respectively). The treatment group however only reached that level at day 9. Total coliforms for both treatment groups just reached 6 log cfu·g^{-1} at the end of storage (Figure 4.5). For all the microorganisms, there was noticeable growth observed for both the oregano treatment and control groups between day 7 and day 9 (Figures 4.3, 4.4 and 4.5).

To compare the growth rates between the control and oregano treatment samples, linear regression was calculated for each microorganism over time (Table 4.2).

Table 4.2: Linear growth rates for TVC, LAB and Total coliforms cultured in black wildebeest meat.

	TVC	Lactic acid bacteria	Total coliforms
Control	Y=0.56x+3.10	Y=0.46x+2.22	Y=0.39x+2.14
Oregano Treatment	Y=0.36x+2.62	Y=0.32x+2.76	Y=0.35x+2.99
p-value for the rate of change per day	0.0002	0.0013	0.1929

Y= TVC, Lactic acid bacteria or total coliforms; X= time in days

For TVC and LAB there was a significant difference between the growth rates of the control and treatment groups ($p<0.05$). Total coliforms however did not have different growth rates for the control and treatment groups.

4.3.5 Colour changes during aerobic storage

The effect of treatment on the colour (L*, a*, b* and C*) is shown in Table 4.3. In addition to the colour parameters, the percentages of metmyoglobin (MMb), Oxymyoglobin (OMb) and deoxymyoglobin (DMb) are also depicted in Table 4.3.

Table 4.3: Colour parameters and myoglobin states (mean ± SE[1]) for black wildebeest meat under refrigerated (2.6±0.6°C) aerobic storage over time (Treatment- 1% v·v^{-1} oregano essential oil; Control- no oil).

Colour parameter	**Treatment**	**Time (Days)**				
		1	3	5	7	9
L*	Control	30.1^{bx}±0.73	32.3^{ay}±1.69	31.8^{bx}±1.86	32.6^{ay}±2.02	32.8^{ay}±1.92
	Oregano	35.3^{az}±1.17	33.0^{ay}±1.58	32.9^{ay}±1.34	31.7^{ax}±1.49	31.6^{ax}±1.18
a*	Control	13.4^{az}±0.73	10.8^{ay}±0.45	9.4^{by}±0.57	9.4^{ay}±0.42	8.8^{ax}±0.30
	Oregano	12.2^{az}±0.41	11.0^{az}±0.37	10.2^{ay}±0.49	9.8^{ay}±0.74	8.7^{ax}±0.58
b*	Control	11.6^{ax}±0.98	10.9^{ax}±0.84	10.7^{ax}±1.05	10.9^{ax}±0.87	11.2^{ax}±0.86
	Oregano	12.6^{ay}±0.84	11.5^{ay}±0.73	11.0^{ax}±0.76	10.8^{ax}±0.97	10.3^{bx}±0.79
C*	Control	17.8^{az}±1.16	15.5^{ay}±0.83	14.3^{ax}±1.11	14.4^{ax}±1.06	14.4^{ax}±0.67
	Oregano	17.7^{az}±0.73	16.0^{ay}±0.71	15.1^{ay}±0.87	14.7^{ay}±0.92	13.5^{ax}±0.93
MMb[2] %	Control	38.4^{bx}±2.68	45.0^{ay}±1.53	51.0^{az}±0.15	50.8^{az}±1.36	52.6^{az}±5.54
	Oregano	43.7^{ax}±1.66	43.9^{ax}±1.45	47.8^{bx}±0.85	51.4^{az}±2.65	51.3^{az}±3.08
OMb[3] %	Control	45.9^{az}±1.12	40.6^{az}±0.92	34.3^{ay}±1.71	35.6^{ay}±1.56	32.1^{ax}±1.09
	Oregano	41.8^{bz}±1.49	40.8^{az}±0.95	35.4^{ax}±1.83	33.1^{ax}±1.69	31.9^{ax}±2.10
DMb[4] %	Control	16.0^{ax}±1.48	15.2^{ax}±1.03	16.7^{ax}±1.90	16.1^{ax}±3.44	14.8^{bx}±3.13
	Oregano	16.8^{ay}±1.91	15.1^{ay}±1.00	14.7^{bx}±1.50	13.7^{bx}±1.08	17.0^{ay}±2.74

[1]SE represents standard error

[2]MMb represents metmyoglobin

[3]OMb represents oxymyoglobin

[4]DMb represents deoxymyoglobin

$^{a}/^{b}$- different letters represent means with significant differences (p<0.05) between control and treatment

^{x,y,z}- different letters represent means with significant differences (p<0.05) over time

For L*, there was a slight increase observed throughout the 9 day storage period for the control group (Table 4.3). The oregano treatment group on the other hand experienced a significant decrease (p<0.05) in L* over the storage period. From visual inspection, the oregano treatment samples appeared lighter at the beginning of storage but got increasingly darker as time progressed. Both groups (treatment and control) had an increase in a* over time (Table 4.3). There were no noticeable changes in b* for the control group whereas there was a more pronounced decrease noted for the oregano treatment group (Table 4.3). For both groups, there was a decrease in Chroma (C*) over time. Metmyoglobin percentage increased significantly over time for both groups (Table 4.3). OMb percentage on the other hand decreased for both groups over time. DMb percentage for the control group did not change over time whereas there was a noticeable decrease in the DMb of the treatment group between days 5 and 7. Thereafter the DMb percentage increased to values similar to the initial

percentage. Based on visual inspection of the meat, the darker colour of the treatment samples rendered it unattractive; overall, the control samples appeared more attractive than those treated with oregano essential oil.

4.4 Discussion

Plant essential oils which contain high levels of phenolic compounds have been shown to possess antimicrobial properties (Baratta *et al*., 1998). Sivropoulou *et al*. (1996) reported that the main components in the oregano oils they tested were carvacrol, thymol, gamma terpinene and p-cymene. However, there were significant differences in the compositions of the oils analysed in their study. In the current study, the oil was composed of mostly carvacrol, followed by thymol (Table 4.1). The variability between essential oils also leads to differences in antimicrobial and antioxidant properties. The variation can be explained by differences in climate, soil and other environmental factors (Hulankova *et al*., 2013). Therefore, testing of the oil used in this study was necessary, to specify that the results obtained are for this particular oil (and its composition) and results may be different when a different oregano essential oil extract is used.

There was a significant lowering of pH initially in the treatment group compared to the control group with the addition of oregano essential oil (Figure 4.1). Thereafter, the pH increased gradually for both groups but remained lower in the treatment group until the end of storage (Figure 4.1). For the control group, a change in pH from approximately 6 to approximately 6.15 was observed between day 5 and 7 whereas in the treatment group a change in pH (5.9 to 6.1) was only observed between day 7 and 9 (Figure 4.1). The pH of oregano essential oil in this study was found to be 5.4 (results not shown). However, Ozcan *et al*. (2008) reported oregano essential oil to have a pH of 6. The addition of the oil to the meat in the current study could have led to the initial decrease in pH. Thereafter, the increase in pH observed was most likely as a result of microbial metabolism in combination with glycolysis. As meat begins to spoil, microorganisms attack the free amino acids, liberating ammonia and amines which cause an increase in pH due to their alkalinity (Karabagias *et al*., 2011). Also, the increase in pH could potentially encourage the growth of other microorganisms which are inhibited at lower pH thereby accelerating spoilage.

Lipid oxidation is a major cause of quality deterioration in meat (Love & Pearson, 1971; Ladikos & Lougovois, 1990). The oxidation reaction is complex and involves the reaction of fatty acids with molecular oxygen (Ladikos & Lougovois, 1990). The process follows a radical chain reaction, which leads to the degradation of lipids and the development of rancidity (Ladikos & Lougovois, 1990). Also, the resulting loss in quality occurs as the oxidised lipids interact with other tissue constituents (Love & Pearson, 1971). In plant extracts such as oregano essential oil, which have been shown to possess

antioxidant properties, the antioxidative properties has been attributed to the presence of phenols and polyphenols (Velasco & Williams, 2011). Velasco & Williams (2011) also reported that the antioxidant properties of oregano essential oil increased mainly due to an increase in carvacrol and thymol. In this study, TBARS in the treatment group was significantly lower than that of the control group at the beginning of storage (Figure 4.2). Thereafter, in the treatment group TBARS remained stable under 10 mg MDA·kg^{-1} meat throughout the storage period. Ariza-Nieto *et al*. (2012) reported that carvacrol and thymol exhibit antioxidant properties by reacting with lipid and hydroxyl radicals and changing them to stable products. That could explain the relative stability of the TBARS levels in the oregano treatment group. In the control group however, instability was noted. This could be attributed to an uninhibited free radical reaction, promoted by pro-oxidants such as heme-iron. The instability of the resulting oxidation-reaction products (MDA) could explain why there was an increase initially then a decrease noted at the end of storage (Figure 4.2). Chouliara *et al*. (2005) observed a similar trend in bream fillets and this was attributed to the possible decomposition of MDA as storage progressed.

Botsoglou *et al*. (2003) reported a significant oxidation reduction (three-fold) in aerobically stored turkey meat preserved with 200 mg·kg^{-1} oregano essential oil. Also, Chouliara *et al*. (2007) recorded a very low degree of oxidation in chopped chicken meat treated with 1% oregano essential oil and kept under aerobic conditions. However, the aforementioned studies had TBARS values of below 1.5mg MDA·kg^{-1} meat at the stated conditions. According to Insausti *et al*. (2001) the threshold of detection of off-flavours and odours for humans is 5 mg MDA·kg^{-1} of meat. Both groups in the current study exceeded this limit at the beginning of the study. Similar to this study, Neethling *et al*. (2014) reported initial TBARS for aerobically stored tuna as above 10 mg MDA·kg^{-1} of meat. Leygonie *et al*. (2012) suggested that lipid oxidation continues during frozen storage, therefore it is highly probable that lipid oxidation was still occurring during the one year of frozen storage of the meat used in this study. Neethling *et al*. (2014) proposed that as water freezes, this leads to an increase in substrate concentration which may initiate the primary phase of lipid oxidation. Upon thawing, the increase in solutes could cause acceleration in the secondary phase of lipid oxidation, whose by-products are measured by the TBARS method (Leygonie *et al*., 2012). Also, freezing can damage cell membrane structure in the muscle thus leading to release of pro-oxidants upon thawing (Leygonie *et al*., 2012). Ferric heme is one of the major pro-oxidants in muscle tissue (Love & Pearson, 1971); the high myoglobin and iron content of game meat potentially leads to accelerated lipid oxidation.

The importance of microbial spoilage lies in the issue of food safety and quality. Legislation stipulates the safe limits for most pathogenic and spoilage microorganisms for most foods. However, for game meats there are no direct stipulations and the values for red meat in general are used. For the purpose

of this discussion, reference for limits required for red meat will be made. Meat pH is known to influence microbial spoilage; with higher pH leading to quicker spoilage (Chapter 3). In the control samples, there was a difference between growth of TVC; DFD meat attained 6 log cfu·g^{-1} at day 5 whereas Normal meat reached that level at day 7 (results not shown). This trend is similar to what was observed pertaining to DFD and Normal meat in the shelf-life study (Chapter 3). Initial pH readings however did not alter the effects of the oregano treatment in this current study and are thus not discussed further.

In the current study, an initial inhibition of total viable counts (TVC), lactic acid bacteria (LAB) and total coliforms was observed with the addition of oregano oil (Figures 4.3, 4.4 and 4.5). Chouliara *et al*. (2007) however reported no difference in microbiological loads between the control and treatment groups at day 0, implying that there was no initial inhibition observed in their study. Skandamis & Nychas (2001) stated that TVC in their study of minced meat treated with 1% oregano essential oil, had reached maximum levels at day 6 of storage. TVC was mostly comprised of *Pseudomonas*, followed by *B.thermosphacta* and lactic acid bacteria (Skandamis & Nychas, 2001); the composition of TVC was not determined in the current study. Chouliara *et al*. (2007) however reported having observed no growth in TVC for chicken breasts treated with 1% oregano essential oil from day 0 to day 3 of refrigerated aerobic storage. Thereafter, Chouliara *et al*. (2007) observed an increase in the TVC which reached 6.83 log cfu/g at day 20 of refrigerated aerobic storage.

Contrary to this study, Chouliara *et al*. (2007) reported no difference between the control and treatment groups initially for lactic acid bacteria. Furthermore in their study, there was a 2 log reduction which was observed between days 0 and 2; thereafter growth occurred gradually and reached 7 log cfu·g^{-1} at the end of 25 days of storage. Skandamis & Nychas (2001) proposed that inhibition due to oregano essential oil addition can occur through reduction of the initial microbial load and also the reduction of the growth rate. It has been suggested that depending on the specific composition of the essential oil used, the level of inhibition and mechanism observed will be different. In the current study, both a decrease in initial load and growth rate was observed for TVC and LAB (Table 4.2) with the use of the oil.

SANS (2007) stipulated that the maximum permitted levels for TVC at the end of storage for whole muscle red meat is 6 log cfu·g^{-1}. According to this stipulation, the control group had an average maximum of 6 days shelf-life whereas the treatment group had a shelf-life of 9 days. Therefore, based on TVC levels, the treatment lengthened the shelf-life of black wildebeest meat by 3 days.

Although the treatment had an effect of lowering the initial counts, there was no significant effect on the growth rate for total coliforms (Table 4.2, Figure 4.5). A possible explanation can be found on the

mechanisms of action proposed for the main constituents of oregano essential oil; carvacrol and thymol. The hydroxyl groups and their location on these molecules render them lipophilic (Velasco & Williams, 2011). Therefore, carvacrol and thymol are capable of disrupting cell membrane structure. Ultee *et al*. (2002) found that carvacrol destabilised the cell membrane in bacteria thus reducing the membrane potential. Consequently, this leads to a disruption in the movement of ions, ATP and nutrients across the membrane possibly leading to cell death (Velasco & Williams, 2011). Ultee *et al*. (2002) also reported an increase in cell membrane fluidity and a further increase in the leakage of ions when carvacrol was applied to *Bacillus cereus* cells. The majority of total coliforms are gram negative microorganisms; they have an external lipopolysaccharide layer over the peptidoglycan cell wall (Burt, 2004). The outer membrane could potentially act as a barrier to essential oil components, resulting in more resistance in gram negative in comparison to gram positive organisms (Burt, 2004). For all the microorganisms tested, there was a growth spurt observed between days 7 and 9. In the treatment group, this is possibly due to exhaustion of the essential oil added.

Sensory results for studies with 1% oregano essential oil vary; Chouliara *et al*. (2007) found an objectionable odour and taste at 1% in chicken meat whereas Skandamis & Nychas (2001) reported no adverse effects at the same level. Karabagias *et al*. (2011) suggested that differences in sensory results could be as a result of the different types of meat used. As mentioned previously, the essential oils differ in their composition and that could also lead to flavour differences even in samples treated with the same concentration of different oils.

Freezing and thawing the meat prior to chilled storage could also accelerate spoilage. Leygonie *et al*., (2012) proposed that freezing damages the muscle fibre structure, thereby increasing the ease of microorganism penetration upon thawing. Furthermore, upon thawing, the muscles would release drip which is rich in nutrients, thus facilitating the growth of microorganisms as the meat thaws and after thawing. Based on those findings, it can be implied that black wildebeest meat in the current study could have potentially had a longer shelf-life if it was not first subjected to freezing.

Apart from lipid oxidation and microbial quality and safety, meat colour is also an important factor in the determining shelf-life. Consumers make a purchasing decision based mostly on the colour of meat (Mancini & Hunt, 2005). Although it is mostly for consumer appeal, colour can also be an indicator of the underlying changes occurring in the meat. DFD meat is darker and this phenomenon was discussed (Chapter 3). Initially, the treatment group was lighter than the control group irrespective of meat pH (Table 4.3). Initial lightness could be due to the method of application of the oil; some of the myoglobin was lost thus resulting in a lighter colour. Additionally, the increased wetness on the surface of the treatment group may have resulted in increased reflectance thereby making the meat lighter

(Faustman, 1994). Thereafter, there was a significant darkening in the treatment group whereas the control group increased in lightness. Redness (a*) decreased in both treatment groups but b* did not change in the control group whereas it decreased in the oregano treatment group (Table 4.3). C* decreased over time for both groups and there was no difference at the end of storage. Comparison between studies is not an easy task as there are no studies on the shelf-life characteristics of black wildebeest meat under similar conditions; in fact no studies on the colour stability of this game species has been reported. However, certain trends based on the principles of colour change in meat will be discussed.

Contrary to this study, Hulankova *et al*. (2013) reported a slight increase in L* in minced beef treated with 0.2% oregano essential oil and no significant change in the control. Also, an increase in redness (a*) was reported for the treatment group. However, similar to this study, Hulankova *et al*., (2013) also reported a decrease in C* and b* in minced beef over time. On the other hand, Unal *et al*., (2014) observed increases in L* values in the treatment and attributed this to the protective effect of the essential oil on colour lightness. Chouliara *et al*. (2007) however reported a decrease in L* in chicken breast treated with 0.1% oregano essential oil although at 1% concentration of the oil, they reported an increase in L*. Alhijazeen (2014) also mentioned that oregano essential oil stabilized L* during storage for the treatment group in chicken. Game meat however has a higher myoglobin content than most red meat; therefore the assumption that more essential oil compounds would be needed to stabilize colour was made. Although the concentration used in the current study was high enough to inhibit microbial growth and limit lipid oxidation, it may not have been enough to cause a stabilization in the colour. The temporal decrease in C* and a* reported in numerous studies (Chouliara *et al*., 2007; Kerry *et al*., 2000; Unal *et al*., 2014) was similar to the current study and was attributed to the oxidation of myoglobin to metmyoglobin over time, resulting in decreased redness. This is further confirmed by the metmyoglobin percentage, which increased throughout storage for both the treatment and the control groups (Table 4.3). It is also noteworthy that metmyoglobin percentage was significantly lower in the oregano treatment group from day 1 to day 5 (Table 4.3).

Claus (2006) reported that when myoglobin levels are between 30-40%, browning becomes visible in beef. Based on that classification, both treatment groups had experienced significant browning (as seen through visual evaluation of the samples) at the beginning of storage. The myoglobin existed mostly as metmyoglobin and oxymyoglobin throughout the trial. The levels of deoxymyoglobin were below 20% throughout the study. Deoxymyoglobin formation is favoured in the absence of oxygen; in this study the meat was stored aerobically. Although oxymyoglobin percentage was relatively high throughout storage, there was a temporal decrease observed in both the oregano treatment and control groups. The instability of myoglobin throughout storage could be due to its damage during

freezing, which makes myoglobin more prone to oxidation (Leygonie *et al*., 2012). Furthermore, Leygonie *et al*. (2012) outlined a theory of metmyoglobin reducing activity which was initially presented by Livingston & Brown (1981). The theory states that muscle enzyme activity in fresh muscle is higher than in freeze/thawed muscle. Also, protein, lipid and pigment oxidation are interlinked in meat (Unal *et al*., 2014). Therefore, lipid oxidation which is initiated during freezing could produce pro-oxidants which consequently oxidize iron and lead to myoglobin denaturation (Unal *et al*., 2014). This can possibly explain the overall colour instability observed in this study.

4.5 Conclusions

The preservation of black wildebeest meat by 1% oregano oil lengthened the microbial shelf-life by 3 days under aerobic storage. However, the lipid oxidation and colour results showed that the meat was unacceptable although it is argued that freezing the meat prior to experimentation may have caused this instability. Alternatively, the high pH of the meat may have played a more significant role in the shelf-life stability than was originally thought; this aspect (interaction between essential oil and acidity of meat) warrants more research. Nonetheless, from the results obtained in this study there is potential of using oregano oil to effectively improve game meat safety even though a general conclusion on the overall effects of oregano essential oil on the meat quality cannot be made in this case. Therefore, further studies on fresh game meat to observe how the myoglobin stability and lipid oxidation would be affected with the addition of oregano oil are warranted. Furthermore, sensory studies should also be run together with each batch of oregano essential oil used, to specify at which levels it can be applied; although this type of experiment is challenging as the use of sensory panels is time consuming and there is always the challenge to ensure that the meat being evaluated by the panel is microbiologically safe. In conclusion, there is potential to use oregano essential oil as a preservative for black wildebeest meat although more research is needed.

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Chapter 5: General conclusions and recommendations

Game meat is capable of providing a partial solution to the global food crisis (Chapter 1). A potential niche for game meat was identified; it can be marketed as a healthier alternative to conventional red meat (Chapter 2). As the South African game meat industry continues to grow, there is need for more research pertaining to safety and hygiene within the abattoirs. The aim would be to determine the typical microbial loads and ultimately develop standards specifically for game meat hygiene. Growth of the industry also requires that it is not marred with any negative publicity pertaining to food safety issues or zoonosis (Chapter 1). Also, it would be important to find if there are any effects of region and season on the microbial profile of game meat.

In the first section of this study (Chapter 3), the microbial quality of black wildebeest (*Connochaetes gnou*) meat with pH ranging from 5.54 to 6.49 was determined. It was found that DFD (pH >6) meat had higher growth rates for the microorganisms tested than Normal (pH <6) meat from the same species under chilled (4.2±0.8°C) vacuum storage. As a result, the meat would spoil at a faster rate which indicates that the marketing and supply chain of such meat has to be developed to ensure that it reaches the consumer while it is still of a good quality. However, it is suggested that with adherence to hygiene principles (efficient standard operating procedures) during the harvesting, this could lead to meat with very low initial loads of microorganisms thereby possibly lengthening the shelf-life. Also, although some studies mentioned that vacuum packaging is unsuitable for DFD meat due to greening; in this study this was not observed. Therefore, vacuum packaging can be suggested during distribution of game meat even though it is DFD, although this would depend on the identities of the microorganisms initially present in the meat. It is also noteworthy that spoilage depends on a variety of factors including initial microbial load, packaging and storage temperature. Therefore results differ across studies, based on the different conditions used.

It is a common assumption that the main reason for DFD meat spoilage is the high ultimate pH values. However some findings stated that adding glucose to meat lengthens the shelf-life. Therefore, another important consideration is that meat differs in the concentrations of biochemical compounds such as glucose and lactate, which would ultimately influence the rate at which meat spoils. It is recommended that on studies involving DFD meat and microbial spoilage, the concentrations of these substrates throughout the shelf-life studies be quantified. In this study however, it should be noted that the meat was frozen prior to experimentation and this may have led to a relatively quicker attainment of high microbial numbers regardless of the ultimate pH. Therefore, it would be suggested that in microbial quality and shelf-life studies, fresh meat should be used. The initial microbial loads

were also in different ranges for both DFD and Normal meat. This may have affected the growth patterns of the microorganism and it would be suggested that the meat undergo a pre-treatment to decontaminate and attempt to normalise the initial microbial counts. Further research can be done on the decontamination techniques that will be suitable for game meat. The differences in initial microbial loads also may indicate inconsistencies during processing of the meat, which can be controlled through adherence to hygiene.

Due to the differences in pH in the black wildebeest meat, a question arose as to whether the ultimate pH could possibly influence the efficacy of a preservative applied (oregano essential oil). Oregano essential oil was noted to be one of the most potent, antimicrobial plant extracts (Chapter 2). This is in relation to the identification of game meat as an organic food; it would be beneficial to apply natural preservatives to lengthen shelf-life of game meat and processed products. It was found that pH did not influence the ability of the preservative to hinder microbial growth (at 2.6±0.6^{o}C). Also, the treatment successfully lengthened the shelf-life of black wildebeest (for both the DFD and Normal meat) by 3 days under aerobic packaging (based on TVC levels). However, a definite conclusion could not be made based on the lipid oxidation and colour of the meat, as it was already unsatisfactory at the beginning of the shelf-life study. Again a recommendation is being made to test the efficacy of oregano essential oil under different conditions in extending the shelf-life of fresh black wildebeest meat. Also, with the use of natural preservatives it would be advisable to include sensory analysis of the fresh meat although this is a complex process due to training of the panels and also ensuring microbial safety prior to the analysis. Another point of consideration is that with plant extracts, the concentrations of the active compounds differs with each new batch, depending on a number of factors such as environmental conditions. Therefore, the results obtained in this study are specific for oregano essential oil used and may not apply to another oil of a different composition. Further research needs to be conducted on the use of natural preservatives in meat, under different sets of conditions to broaden knowledge in this field.

In conclusion, this study successfully determined the microbial quality of DFD and Normal black wildebeest meat under chilled, vacuum storage. The microbial quality and shelf-life can be tested under a myriad of conditions to determine when the meat begins to spoil. Although no definite conclusions can be made on the general effects of oregano essential oil (1% $v \cdot v^{-1}$) on the shelf-life based on other quality parameters (lipid oxidation and colour), the essential oil effectively inhibited all microorganisms tested.