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Effects of Wet and Dry Ageing on the Physical and Sensory Quality of Common Eland Meat

MASTER'S THESIS

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Declaration

I hereby declare that I have completed this thesis entitled **“Effects of Wet and Dry Ageing on the Physical and Sensory Quality of Common Eland Meat”** independently; all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references, and according to the citation rules of the FTA.

In Prague April 2021

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Glindys Virginia Luciano

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Abstract

The physical quality changes (pH, cooking loss, weep loss, CIE Lab colour, and WBSF) and the sensorial attributes of wet aged (vacuum packaged) and dry aged *longissimus lumborum* (LL) were evaluated for female (n = 6) common eland, during a 14-day *post-mortem* ageing period at 4°C. The dry aged LL muscle reached a maximum grilled shear-force of 57.6 N, while the wet aged LL muscle reached a maximum tenderness of grilled 63.3 N at Day 15. While values are typical for game meat, the meat could still be considered tough to the average consumer (> 49N). Weep loss increased throughout the ageing of the muscle, with the dry aged LL muscle having higher weeping loss. The LL muscle surface colour was less bright, but more red and yellow with ageing, for both wet aged and dry aged treatments, compared to the control. Additionally, descriptive sensory analysis demonstrated that dry aged LL muscle scored lower in abnormal aroma intensity and liver flavour compared to the wet aged LL muscle, which is a positive improvement in flavour for the consumer. The dry aged LL muscle scored higher in overall acceptability, even though the differences in tenderness between the two ageing techniques were not noted by the sensory panel. Thus, while ageing did improve the tenderness of the LL muscle, dry ageing showed favourable flavour development. However, the LL muscle shear force values were still above the threshold for the acceptable tenderness for the average consumer, and further processing techniques should be considered.

Key words: *Taurotragus oryx*; Game Meat; *Longissimus lumborum*; Meat Quality; Tenderness

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List of the abbreviations used in the thesis

BHT: Butylated hydroxytoluene

BF: *biceps femoris*

DFD: Dark, firm, and dry meat

DNPH: 2,4-Dinitrophenylhydrazine

DSA: Descriptory Sensory Analysis

GC-MS: Gas Chromatography - Mass Spectrometry

HPCL: High-performance liquid chromatography

HS-SPME: Headspace-solid phase microextraction

IAS: Institute of Animal Science

IMF: intramuscular fat content

KI: Kovats index

KOH: Potassium hydroxide

LL: *longissimus lumborum*

LSM: Least squares mean

LTL: *longissimus thoracis et lumborum*

MDA: Malondialdehyde

NIST: National Institute of Standards and Technology

PDMS: Polydimethylsiloxane

PUFA: polyunsaturated fatty acid

RI: Retention index

RT: Retention time

SD: Standard deviation

SEM: Standard errors of the mean

SOPs: Standard Operating Procedures

SPME: Solid phase microextraction pen

WBSF: Warner-Bratzler Shear Force

1. Introduction and Literature Review

1.1. Introduction

With a growing population, and demand for meat products, meat from game species (such as African antelope) may provide a potential resource to supplement this meat production, while also meeting modern consumer demands (Hoffman & Wiklund 2006). While the meat industry has had decades to develop meat products derived from domestic livestock, that is consistent in its nutritional properties, sensory quality, appearance, and safety (Troy & Kerry 2010) this is not the case for game meat products. The decades of quality and quantity development in regard to meat products from domestic livestock has enabled a mutual understanding between producers and consumers for what is acceptable and expected. However, the infancy of commercial game meat production, and research into its improvement of quality, presents an issue with regards to maintaining these acceptable attributes and expectations. Thus, there needs to be a sustainable and consistent supply of game meat, alongside the availability of high-quality products (Hoffman et al. 2005). While the common eland (*Taurotragus oryx*) has been identified as a suitable game species for game meat production (Lightfoot & Posselt 1977) little is known regarding optimising their meat quality for commercial consumption.

One such challenge that game meat faces is that, compared to domesticated livestock, their meat is considered tough, and it is known that consumers assess fresh meat-eating quality primarily by its tenderness (Grunert et al. 2004) Upon initial research, Needham et al. (2019a) and Needham et al. (2020a) found that the tenderness of fresh eland meat is unlikely to meet consumers expectations, considering their high shear force values. However, wet ageing and pelvic suspension of common eland carcasses has shown to improve tenderness, but dry ageing has not yet been investigated in common eland meat (Needham et al. 2020b). Dry-ageing has recently been revisited and revived in the meat industry, with flavour attributes supposedly benefitting from this process, yielding high-value premium products (Terjung et al. 2021).

Considering the potential of developing niche game meat products, dry ageing could be beneficial for flavour development in common eland meat, while improving tenderness. In this study we compared the effects of wet-ageing and dry-ageing on the physical and sensory quality of common eland meat.

1.2. Literature Review

1.2.1 Game Meat Production

In general, game farming systems are extensive in nature, and given the leanness of antelope game meat, the production of game meat in these settings not only fits the ethical requirements that consumers may have, but also the safety and health concerns that are raised in the context of intensive farming systems (Bartoň et al. 2014; Hoffman & Wiklund 2006). There are many reasons for the farming and meat production of game species, aside from the nutritional benefits, as a large proportion of land that is not suitable for the growth of crops is in fact suitable for ruminant production, as they can feed in various ways (Ramankutty et al. 2008). Also, the combination of farming an underutilized game species on land that is not optimal for the growth of crops for the production of meat simply makes sense from a sustainability context. Furthermore, current practises and hunting legislation in southern Africa support sustainable herd management and culling practices (van Schalkwyk & Hoffman, 2016). It is easy to see why even mixing game species with other forms of organic production is an attractive alternative to farmers. Additionally, game species can enhance and improve local biodiversity (Oberem & Oberem 2016).

Currently, the farming of game species exists in different part of the world. Game species that are currently farmed for game utilization include: blesbok (*Damaliscus pygargus phillipsi*), impala (*Aepyceros melampus*), gemsbok (*Oryx gazelle*), springbok (*Antidorcas marsupialis*), and the common eland (*Taurotagus oryx*) (Hoffman & Wiklund, 2006; van Schalkwyk & Hoffman, 2016). It has also been established that the common eland can be kept in cattle pens (Bothma 1996), in farm sizes also similar to cattle, as long as the animals are fed accordingly (Bartoň et al. 2014). Still, while farming of game species is a growing industry, particularly in South Africa, the limited research on the fresh meat

quality and processing of the meat is an obstacle for its commercialization (Hoffman & Wiklund 2006). This means that additional research into optimising game meat quality is crucial for the development of commercial game meat farming.

The economic aspects of game meat production are important, as profitability along with demand and supply dictate the success of game meat in the commercial market. Child et al. (2012) found that, in reality, game animals are more likely to generate higher net farm profit margins compared to domesticated livestock. Additionally, Berry (1986) noted that game species generate the highest net revenue per biomass weight, due to their multifaceted value in terms of ecotourism, hunting, and meat production. Currently, game meat is sold per kilogram since a grading carcass system has yet to be developed. It is for this reason that carcass yield is still the most important economic parameter for game meat production (Oberem & Oberem 2016). Fortunately, studies have shown that not only do antelope species produce leaner meat than domesticated species (Huntley 1971), but antelope species also tend to show higher dressing percentages than their livestock counterparts, which indicates a higher total meat yield (Van Zyl & Ferreira 2004).

The absence of a grading system for game meat production means that carcass yield is currently the most important economic parameter in production. Due to this, production systems are focused on producing animals with high carcass yields and dressing percentages (Needham et al. 2020c). In general, game meat is produced in extensive production systems, given the importance of carcass yield, farmers are interested in exploring more intensive systems. While there are some game species that are more suitable than others, knowledge of husbandry systems of various game species is needed. As previously mentioned, the common eland was identified as a suitable species for production as they adapt to intensive systems similarly to cattle.

1.2.2. Harvesting Practices & Microbial Safety of Game Meat

Harvesting of game meat is an important process that can significantly affect meat quality. In the case of game meat, hunters (so-called “biltong” hunters) and commercial culling teams are currently the primary means of

harvesting antelope species, using the free-bullet technique. The harvesting of ungulates and other game species can represent an added value for sustainably sourced meat, particularly in rural areas, and provide additional sources of income, including (but not limited to) ecotourism, trophy and recreational hunting, and live sales, all of which can have positive economic impact on local economies (Gaviglio et al. 2018). Good harvesting practices are important, such as immediate death, complete bleeding, immediate evisceration, and proper handling and transportation, to guarantee meat quality, preservation, and reduce levels of microbial contamination (Hoffman & Wiklund 2006).

The first step in ensuring this is to minimize ante-mortem stress, as meat pH and its post-mortem changes has a major impact on the resultant physical and sensorial meat quality, and microbial safety (Lawrie & Ledward 2006). Hoffman (2000) reported that animals that were stressed prior and during killing showed signs of ante-mortem stress, which includes high pH, low drip loss and darker meat—also known as DFD, which is common in African game species due to the harvesting methods (e.g., hunting) of the animal. Hoffman and Ferreria (2000) established that the decrease rate in pH in the first couple of hours post-mortem can be seen as an indicator that can provide useful information about the stress conditions of the animal before shooting and the efficiency of the exsanguination procedures. It is for this reason that the best practices for hunted animals are implemented, to obtain optimal acidification of muscles (Lonergan et al. 2010). Gill & Newton (1979) established that dry, firm, and dark (DFD) meat spoils at a faster rate, not necessarily due to high pH as reported by other researchers, but rather the absence of glycogen in meat with a high pH. Thus, the benefit of free-bullet harvesting is the omission of live animal transportation to abattoir facilities, which reduces the animal's stress.

Harvesting good practices and management differ depending on region and animal species. A study conducted specifically on harvesting practices in Italy by Viganò et al. (2019) found that in while there are quality parameters in place for the hygienic quality standards, good practices and management of the carcasses from the moment of shooting to the point of arrival at slaughterhouses are not always met when meat is harvested by hunters. Providing high-quality standards in wild game meat is crucial, as well as having an organized supply

chain, which can incentivize hunters to improve handling and management practices, while also creating a link between consumers and the local territory, which in turn can enhance the local natural resources and traditions (Viganò et al. 2019). Another example within another region, is that in order to improve the hygiene standards and commercial integration of game meat, commercial cropping (or culling) teams have been established in Southern Africa, who slaughter and process antelope carcass under strict and legislated conditions (van Schalkwyk & Hoffman 2016).

Safety is imperative for consumers, and to meet expectations for both meat quality and safety according to the principles of hazard analysis and critical control points (HACCP), game meat harvesting, and processing has to adhere to these good practices (Casoli et al. 2005). For carcasses to be either accepted or denied depends on various factors, mainly the level of contamination that a carcass may have, as well as the bacterial species that are associated with it. Contamination includes surface contamination such as skin and hides on the animal carcass, at the point of shooting and evisceration (intestine and faeces) (Atanassova et al. 2008), and in the slaughterhouse's surfaces (Gill 2004). When animals are culled in the field, the animal are in contact the ground, which can be a source of contamination for the carcass, especially as cross contamination from the hides to the carcass can occur during the carcass dressing process (Gill 2004). However, it must be noted that keeping the skin on during the dressing and transportation process allows for the skin to act as a barrier against dirt and other sources of contamination; however, cross-contamination between carcasses during transport may be an issue, depending on packaging.

Regarding the overall safety of meat coming from game meat species, Gill (2007), noted that while there is the risk of food-borne pathogenic bacteria being present in products coming from wild game species, it is expected that the risks are small. Although, there has been reports of wildlife animals being carriers and transmitters of shiga toxin-producing *Escherichia coli* (STEC) strains (Miko et al. 2009). However, with necessary precautions, such sterilization of all equipment from the field and abattoir, carcass health inspection, maintenance of the cold chain, etc., contamination of the carcass at the point of slaughtering may be decreased. Still, as the commercial production of game species meat grows,

more research needs to be done on the role of food-borne diseases coming from wildlife game species, and their effects on the safety and shelf-life of game meat products originating from various regions under differing conditions.

1.2.3. Game Meat Nutritional and Physical Quality Traits

Meat quality, which includes physical, nutritional, microbial, and sensorial attributes, is affected by ante-mortem factors, such as species, nutrition, breed, sex, age, muscle type, and husbandry techniques, as well as by post-mortem factors, including pH, temperature, and ageing techniques (Kudrnáčová et al. 2018). The benefits of consuming meat from game species, from the nutritional point of view, are abundant. Game meat, which is classified as so-called “red” meat, has been found to be low in calories, as well as in cholesterol levels, it is a good source of protein, iron, zinc, vitamin B12, and of polyunsaturated fatty acid content (PUFA) (Bartoň et al. 2014).

Lastly, game meat can be low in fat, depending on factors such as age, sex, physiological condition (biological cycle), and harvesting condition (hunted vs farmed) (Ramazin et al. 2010). Furthermore, common eland meat *(Taurotragus oryx*), compared to beef, has lower levels of intramuscular fat with a higher proportion of PUFAs (Needham et al. 2019; Bartoň et al. 2014).

Moreover, while it has been established that red meat does indeed provide high quality proteins which could positively affect the incidences of malnutrition and aid food security in developing countries (McNeill & Van Elswyk 2012), consumers in developed countries do associate the consumption of red meat with high fat and cholesterol levels, deeming red meat to have high health risks (Wilcox et al. 2009). To motivate and support the commercialization of game meat, it is important to consider consumer opinions in this regard and educate them on the nutritional quality of game meat.

A relatively high degree of homogeneity and consistency has been achieved in meat quality when it comes to attributes such as nutrition, safety, appearance and sensory quality for domesticated species (Troy & Kerry 2010), due to the fact that the production of meat from domesticated species has reached economies of scales and has had decades to develop quality and

quantity; however, this has been harder to achieve in game meat production and products (Hoffman & Ferreria 2003). The reason for this is limited control in the entire process including ante-mortem and post-mortem factors, which greatly influence meat quality (Neethling et al. 2016). And while Van Schalkwyk and Hoffman (2016) developed standard operating procedures (SOPs) for the commercial harvesting of game species in Namibia, we are still in need of improvement of quality for the different game species under different production conditions.

Game meat can appear darker compared to domesticated livestock (Volpelli et al. 2003), but fortunately this does not appear as an issue to consumers as the dark colour of game meat is considered to be a typical attribute (Ramanzin et al. 2010). Needham et al. (2019) reported that, when compared to other game species, common eland meat was less dark and more closely resembled that of beef, which could be beneficial for consumer acceptance. The study of Needham et al. (2019) did note that the meat colour of the common eland was below the threshold value for consumer acceptability of venison meat according to the threshold values reported by Wiklund et al. (2001), inferring that game meat colour does matter to consumers, and needs to be taken into account when assessing meat quality. Furthermore, it should be noted that wild ungulates, such as the fallow deer, seem to be prone to pre-mortem stress, which is linked to higher pH values as well as a higher water holding capacity (Russo & Bentivoglio 2008). As mentioned earlier, ante-mortem stress is a concern, particularly for game meat quality. Such ante-mortem stress and pH changes can result in dark, firm, and dry meat (DFD), often seen in hunted African antelope, which is even darker in colour than usual, has poor tenderness, and decreased microbial stability (Shange et al. 2019).

Muscle fibre type composition affects meat quality (Kudrnáčová et al. 2018). The skeletal muscle of large mammals is composed of three fibre types, I, IIA and IIB, which derive their characteristics depending on the most abundant myofibrillar protein expressed in each fibre type (Brooke & Kaiser 1970). The metabolic properties of meat are affected by muscle fibre type and composition, as the muscle consequently post-mortem becomes meat (Gagaoua et al. 2016). Studies have found that in most farm animal species, muscle fibre characteristics

play a role in meat quality (Klont et al. 1998; Picard et al. 2002), but information regarding the muscle fibre type and its relation to meat quality in various game species is limited.

Tenderness is very important to consumers, as it is a parameter used to assess fresh meat quality, and as such, the optimization of tenderness is a focal point for game meat producers (Grunert et al. 2004). Given that toughness of meat is a challenge that is present in meat derived from game meat species, fibre typing can provide an explanation for the lack of tenderness in particular game meat species. For example, previous studies have found that muscles with IIB fibre type tenderize more rapidly during ageing (Dingboom & Weijs 2004; Klont et al. 1998). North et al. (2015), found that springbok reached tenderness after five days of ageing compared to beef at 14 days of ageing, this rapid tenderness in springbok was attributed to the fact that 50% of the *longissimus lumborum* muscle fibre type is IIB (Curry et al. 2012). While muscle fibre typing is not a focus point in this study, it still plays an important role in tenderness which we will touch later on in the review. Another important aspect that affects tenderness is that the structure of muscle fibres is supported by the layering of intramuscular connective tissue, the endomysium, the perimysium, and the epimysium. While the epimysium plays a small role in overall meat quality, since it is separated from the meat after cutting, the perimysium and endomysium play an important role in meat quality, weakening through the process of ageing meat (Zochowska-Kujawska et al. 2012).

1.2.4. Game Meat Flavour and Sensory Quality

In general, meat flavour is influenced by volatile aromatic compounds that stimulate the olfactory organs, which influence the sense of taste (Pegg & Shahidi 2004). The concentration of volatile aroma compounds in meat is important to determine the contribution in meat flavour (Lu et al. 2008). Additionally, free amino acids increase flavour development, due to the deterioration of proteins by peptides and aminopeptidases, which occurs in the ageing and tenderization process (Nishimura 1998). Keeping in mind that free-amino acids and peptides cause changes in meat flavours, it is important to consider the effect of ageing on the volatile aroma compounds, as well as on the

non-volatile compounds, that affect taste as well as the role of ageing, which can also affect flavour quality (Van Ba et al. 2014). However, without aroma, one or more of the primary taste sensations such as salty, sweet, sour, or bitter would dominate (Lawrie & Ledward 2006).

In meat, the flavour precursors can be divided into lipids and water-soluble components (Mottram 1998; Pegg & Shahidi 2004). Mottram (1998) established that sulphur-containing compounds are in fact crucial to the contribution of meat flavours, and when cooked, can significantly contribute to meat aroma. Additionally, lipids have been found to influence meat flavour, since volatile aroma compounds that are profiled in meat have been found to be predominantly derived from fat-derived compounds (Mottram 1998). Lipid degradation products, along with products of the Maillard reaction, produce a characteristic meat aroma (Wasserman & Spinelli 1972).

Since the fatty acid profile is important in the process of meat flavour development, this means that, depending on the fatty acid profile, meats from different species will have differing meat flavours. Additionally, intramuscular fat is important to cooked meat as it plays a role in flavour development (Wood et al. 2008) and tenderness (Webb & O'Neill 2008). It has been established that game meat differs from their domesticated livestock counterparts in terms of flavour (Neethling et al. 2016). However, Needham et al. (2019) reported that farmed eland meat, after receiving cattle diets (silage and concentrate feed), has been described to have highly favourable flavour and aroma attributes, similar to beef. In general, games species have higher meat PUFA percentages (Swanson & Penfield 1991), and Field (2004) found that South African game species have a stronger flavour compared to their domestic counterparts. This may be attributed to the highly variable natural vegetation in southern Africa that they consume, as described in impala from varying production systems with different nutrition (Needham et al. 2021).

Rødbotten et al. (2004) found that out of 15 game species evaluated in Norway, flavour attributes were in fact similar between species, but perceived differences remained. These species included reindeer meat and roe-deer meat, which were found to have the highest "gamey" flavour, overall, meat derived from

wild animals were found to have a liver-like flavour. These animals feed on pastures and forages, which has been linked to higher PUFA content in a number of games species (Wiklund et al. 2003). Similarly, Rincker et al. (2006) compared the sensory attributes of the *Longissimus dorsi* muscle in three different species: beef, reindeer, and caribou. The study found that while the reindeer and caribou meat had higher undesirable flavours, which were associated with the gamey-like and liver-like flavours, beef meat had a higher flavour intensity. Thus, it can be inferred that descriptors such as “gamey” or “liver” are used to describe off-putting or undesirable flavours among consumers. Intensity of game flavour, which is largely considered a negative attribute by most consumers, has been found to be reduced in beef when switched to a grain-fed diet (Brewer 2007).

Bureš et al. (2015) established that, when compared to Aberdeen Angus and Holstein cattle, meat from red deer and fallow deer had a higher overall gamey flavour and aroma. The authors attributed these species-specific flavours not only to the fatty acid profiles, but also to the PUFA content. Needham et al. (2019a) also had similar results, in which eland meat was found to be low in intramuscular fat and high in PUFAs compared to beef. Moreover, Needham et al. (2019b) found that the gamey flavours and aroma, which is undesirable to consumers, scored relatively low, which appears to be positive for consumer acceptance. Given that gamey flavours are considered undesirable, further research on the development and changes in common eland meat flavour (which is associated with desirable descriptors) while improving tenderness, is necessary.

1.2.5. Role of fatty acids and volatile compounds in the sensory quality of meat

Lipids play a role in the development and influence of meat flavour and aroma, the reason being that the volatile compounds found in cooked meat are mainly composed on lipid-derived compounds (Mottram 1998). Just as tenderness and meat colour are important attributes to consumers, meat aroma is another important trait that not only affects the sensory quality of cooked meat but also acceptability of the product by the consumer (Calkin & Hodgen 2007). It

is important to note that meat aroma and flavour is thermally derived, as uncooked meat has little to no specific aroma or flavour, each type of cooked meat has its own unique flavour depending on the animal species (Shahidi et al. 2004). When cooking meat, there are reactions that take place, including the Maillard reaction, which is the interaction between sugars and amino acids, lipid degradation, caramelisation of carbohydrates and so on (Neethling et al. 2016). Other secondary effects can occur when cooking meat, but high temperatures need to be used in order to get to that point, which is not ordinary in day-to -ay cooking (Pegg & Shahidi 2004). Cooking methods play a role in the development of aromas and also in its sensory qualities as well, for example broiling will result in different aromas and flavours than grilling, due to the higher content of moisture and low surface temperature, a core temperature of 65°C is recommended if the focus is on flavour, whereas if overall sensory is preferred 75°C is recommended (Bejerholm & Aaslyng 2004).

Intramuscular fat is important in the aroma of cooked meat, as removal of triglycerides can result in changes of the aroma in cooked meat and the development of alcohols and aldehydes (Mottram & Edwards 1983). While it has also been established that inter- and intramuscular fat in meat contains low quantities of PUFA (structural phospholipids contain high PUFA) and have little to no influence on meat flavour, ultimately, the differences in the fatty acid profiles will have a role in the development of volatile aroma compounds due to the interaction of lipids with sugars and amino acids (Farmer & Mottram 1990). Additionally, lipids act as solvents for fat-soluble compounds, which become volatile when undergoing the cooking processes (Pegg & Shahidi 2004). Lastly, the oxidation of lipids produces aldehydes, alcohols, ketones and lactones, which means that lipid oxidation products also contribute to meat aroma development (Mottram 1998).

For the accurate analysis of the volatile compounds released from cooked meat, we have to identify the compounds involved and determine its impact, often by relating it to the sensory evaluation of the meat. While there are various methods with which to identify and analyse volatile compounds, Solid phase microextraction (SPME), which is an extraction technique for sample preparation, along with GC-MS, which is a combination of two techniques GC

and MS, has many advantages, including that it is simple to use, solvent-free, and cost-efficient (Bueno et al. 2019). SPME extraction is dependent on sample preparation, as well as the extraction conditions (Park et al. 2009). Wang et al. (2018) established that conditions of SPME extraction are important as changes in temperature, as well as time, influences the abundance and number of volatile compounds found. Kerth (2016) reported that cooking method could potentially affect the outcome for determining volatiles, but also the meat presentation, such as the way the meat is sampled, whether minced, thickness size, and/or pieces of difference sizes can impact the cooking effect, which means there could be modified heat exchange which affects the abundance and numbers of volatile compounds.

While the intramuscular fat content of game meat is considered to be rather low, there may be other volatile compounds that contribute to its unique flavour and aroma profile. Furthermore, processing techniques, especially those which affect lipid oxidation, may influence these compounds. Information regarding the volatile compounds present in game meat and game meat products is limited, and thus basic description of these are required, while keeping in mind the most appropriate evaluation techniques thereof.

1.2.6. Techniques in Wet Ageing and Dry Ageing: Opportunities for Game Meat Quality Improvement

There are particular factors that affect meat quality that can be improved through ageing, such as flavour, juiciness and tenderness (Kim et al. 2018). Tenderization of meat during ageing occurs post-mortem, due to post-rigor reduction in toughness, caused by the calpain protease system (Hopkins & Thompson 2002). More specifically, tenderization is caused by the proteolysis of the myofibrillar and cytoskeletal proteins, and changes in the connective tissues (Chriki et al. 2013). There are various ways in which the tenderness of meat can be improved, whether in the ante-mortem stage, through techniques such as immunocastration, which is considered an ethical alternative that has demonstrated favourable effects on the meat quality of livestock (Needham et al. 2017), or during the post-mortem process, through pelvic suspension (Needham

et al. 2020b), electrical stimulation, or meat processing techniques such as ageing, which should be furthered explored in game species.

With respect to the tenderization of meat after rigor-mortis, ageing can be used to improve meat quality. There are various methods for meat tenderization through ageing, the most basic techniques involving vacuum/wet, dry, or permeable bag dry ageing. In the meat industry vacuum-ageing (or wet-ageing) is used most often, due to lower ageing losses and convenience during storage and transport (Warren & Kastner 1992). Before the development of the wet vacuum/sealing techniques that were developed in the 1970s, dry-ageing in a cooler was the most typical method for the fresh preservation and value-adding process of meat; what we know now as wet or vacuum ageing became more widespread with the development of vacuum packaging (Dashdorj et al. 2016).

Thus, wet ageing is a relatively new process, where the meat is protected from spoiling and drying out when stored in a cooling environment with temperature control, at -1 to 2°C, lasting anywhere from 3 to 83 days (Kim et al. 2018). Since wet ageing is the most frequently used packaging method in the meat industry, there are benefits, including significant reductions in product weight loss and trim loss, weight being a factor that is of the upmost economic importance for both livestock and game meat (Terjung et al. 2021; Kim et al. 2018). Furthermore, less space is required during ageing in vacuum packaging, and there is a higher adaptability and reduction in microbial factors, as cooling helps with extending the shelf-life of the product, without significantly sacrificing sensorial attributes (Kim et al. 2018). Lastly, while it is the case that wet ageing can improve palatability traits of meat, the development of undesirable flavour attributes such as, bloody, metallic, and sour, can occur (Warren & Kastner 1992), which are not accepted by consumers. Particularly, temperature and ageing time are the most critical factors in controlling the meat characteristics (Kim et al. 2018).

On the other hand, dry ageing is known to enhance flavour attributes and palatability of meat, giving the meat a unique flavour profile, which has described such as, beefy, nutty, roasted, buttery, and sweet (Kim et al. 2016). The major reasons for dry-ageing meat, particularly beef, is solely for the enhancement of

palatability—mainly focused on attributes such as flavours and tenderness (Kim et al. 2016). Dry ageing has been practiced in small meat purveyors for upscale butcher shops, local meat processors and gourmet restaurants (Savell 2008). Currently, dry ageing of primal and sub-primal cuts take place without the protective packaging (Savell 2008). Which can present with additional challenges particularly in the food of microbiological safety.

While ageing the primal and sub-primal cuts is more common, hanging the whole carcass side in a cooler for 10 to 35 days, otherwise known as conventional carcass dry ageing, is still practiced in small local meat processors as an added value to bring in more consumers (Jeremiah & Gibson 2003) Meat palatability must contain attributes such as flavour, juiciness and of course tenderness, as these are factors that are crucial to consumers (Smith et al. 2008). Additionally, the same parameters that consumers evaluate beef, consumers evaluate game meat with these same attributes, thus expectations are the same. Interestingly, about ninety-five percent of consumers discriminate the quality of beef based on its tenderness (Boleman et al. 1997).

While dry-ageing has been found to have desirable flavour development, which is acceptable to consumers, there are disadvantages to dry-ageing, including losses due to drying (Smith et al. 2014), and associated trimming, which is due to crust formation due to the ageing process, which is related to higher productions costs and thus to higher retail prices (Witte et al. 2020). The losses due to drying as well as trimming is particularly important when evaluating economic returns, and the price of dry aged meat products generally reflect the compensation there of, making it a premium product.

A study conducted by Smith et al. (2014) found that wet aged ribeyes produced greater percentages of ribeye fillets, ribeye cap steaks, and lean trimmings compared to dry aged, additionally, the wet aged ribeyes also yielded a higher percentage of fat trimmings, purge, and heavy connective tissue and bone when compared to the dry aged beef. Overall, wet aged ribeyes had a greater total saleable yield than dry aged ribeyes, with 1.5 more saleable products. The findings were similar to those found by Laster et al. (2008) and Smith et al. (2008) also had similar finding in the difference in yield between wet

aged and dry aged beef short loins. Overall, the processing of dry aged sub-primal cuts needed to be priced higher compared to the wet aged sub-primal cuts. Further research needs to be conducted on consumer willingness to pay for dry aged meat products.

On the other hand, Smith et al. (2014) found that the extreme dry ageing of beef carcasses also resulted in the extreme drying of the *M. Spinalis thoracis* and *M. gluteobiceps*, and undesirable flavours, which were described as putrid and musty. Moreover, it has been established that beef aged over 28 days can potentially result in the formation of undesirable changes and tastes associated with bitterness and sourness (Neethling 2016). Thus, the outcomes of several studies have provided inconsistent results, with others showing no detectable dry-ageing impacts on palatability attributes of beef (Laster et al. 2008 & Smith et al. 2008). Studies have also concluded that wet ageing can actually result in the equivalent palatability attributes of beef muscle as their dry aged counterparts, with the main difference being the shrink and trimming losses that are associated with dry ageing (Parrish et al. 1991). These various contradictory findings raise the question of the efficiency and profitability for producers to produce dry aged meat.

While there is a lot of research on the ageing process of beef, there are limited studies on the effects of meat ageing from game species. As noted in Neethling (2016), research that actively investigates the effects of ageing and ageing time on the sensory quality on various game meats is limited. Compared to domesticated livestock, it appears that game meat does differ in its optimal ageing period for tenderness (North & Hoffman 2015), being much shorter in springbok (~ 7 days) compared to that reported for beef. Reindeer *Longissimus* muscles were found to have higher levels of proteolytic enzyme activity, which is known to reduce toughness of the muscle post-mortem, and lower inhibitor levels, compared to beef muscles (Barnier et al. 1999). In the case of ostrich meat, Marks et al. (1997) established that when ostrich meat was aged for seven days, the development of the intensity of flavour was greater when compared to beef cuts. Thus, it is clear that ageing protocols should be investigated for each game species individually and cannot simply be extrapolated from those used for beef or lamb.

Currently, only two wet ageing studies on eland meat have been conducted, the first of which aged the *longissimus thoracis lumborum* and *bicep femoris* muscles for 35 days, and the results showed that while the shear force values decreased, they still exceeded those values which consumers would deem tender (Needham et al. 2020a). However, a combination of pelvic suspension with a wet aged treatment showed that eland meat reached its maximum tenderness at 7 days of ageing, with values of tenderness deemed acceptable to consumers (Needham et al. 2020b). However, a study comparing the effects on wet-ageing and dry-ageing of common eland meat still has not been conducted.

As previously mentioned, dry aged meat can potentially develop acceptable flavours and can provide incentive for the development of high-value game meat products. While game meat can prove beneficial for the sustainable improvement of food security in developing areas, the relevancy for the meat industry lies in high-value products for the high-end consumer. The commercialization of game meat and development of high-value game meat products remains relatively underdeveloped, and investigation into such processing techniques of game meat is needed to assist the game meat market. The development of premium-valued products through techniques such as ageing, could positively influence the value of game animals. Thus, further research into these post-mortem processing techniques, such as dry ageing, is required in game meat, with the basic investigation into its effects on game meat quality and sensory acceptance being necessary.

In conclusion, game meat is viewed as a healthy alternative compared to other red meats; however, much remains undefined with regards to the optimal post-mortem processing thereof, and the development of high-value game meat products. African antelope species are also currently underutilized, while being maintained under controlled game farming conditions, and thus may be further incorporated into the local commercial meat industry for additional economic and nutritional gain. However, there are many challenges that the production of game meat faces, including meat quality issues such as flavour and tenderness, which can be improved with meat ageing techniques. Thus, further research is needed to optimize game meat processing and explore the potential of different ageing

techniques in improving the sensory quality of popular antelope meat species, such as common eland. Additionally, to have a stable consumer base, there needs to be an understanding of what is expected from producers and consumers regarding game meat and game meat products.

2. Aims of the Thesis

This thesis thus aimed to compare the effects of wet versus dry ageing of common eland meat on its physical, microbial, and sensory quality. More specifically, the objectives were:

- To evaluate the effect of ageing when compared to the control (fresh meat without ageing) on the physical characteristics of common eland meat.
- To describe the potential losses and benefits of the two ageing techniques.
- To compare the effects of dry and wet ageing on the instrumental physical meat quality of common eland meat.
- To assess the effects of dry and wet ageing on the chemical composition of eland meat.
- To assess the effects of dry and wet ageing on the microbiological quality of eland meat.
- To compare the effects of dry and wet ageing on the sensorial attributes of common eland meat, as assessed by a trained panel.

2.1. Research questions

1. Does ageing, in general, affect the overall quality of farmed eland meat?

2. How will dry ageing, when to compared to wet ageing, affect the physical, chemical, microbiological, and sensory meat quality?

2.2 Hypotheses

H_0: Ageing will not affect the physical quality of common eland meat.

H_1: Ageing will affect the physical quality of common eland meat.

H_0: Wet-ageing and dry-ageing will not differ in their effect on the sensorial attributes or physical quality of common eland meat.

H_1: Wet-ageing and dry-ageing will differ in their effect on the sensorial attributes and physical quality of common eland meat.

3. Materials and Methods

The experiment was conducted at the Common Eland Research Facilities of the Czech University of Life Sciences Prague, at Lány (50°7'41.704"N, 13°57'31.370"E) in the Central Bohemia region, Czech Republic (accreditation no. 63479_2016-MZE-17214; ethical clearance no. CZU 20/19). The sub-adult female eland for the experiment were randomly selected from a herd of 50 farmed common eland, during routine culling operations.

3.1. Animal Husbandry and Slaughtering

The experiment was carried out on six sub-adult female common eland. All animals were slaughtered on site at the research facility, within a squeeze-chute to which they were habituated to, thus minimizing pre-mortem stress. Slaughtering was performed using a captive bolt, rendering the animal unconscious. Immediate exsanguination of the animal using a thoracic stick followed, and bleeding of the animal was performed while the carcass was suspended. After the slaughter was completed, the internal organs were removed and placed in a bag labelled accordingly and transported together with the carcasses (with skin on) in a refrigerated truck to the research slaughterhouse of the Institute of Animal Sciences (IAS). The carcasses were processed in the assigned slaughter order and placed into a cool room for 24 hours (± 4°C), while suspended by the Achilles tendon.

3.2. Sample Collection and Ageing Protocol

After the carcasses chilling, the *longissimus thoracis et lumborum* (LTL) muscles were removed from each side of the carcasses and placed onto a sterilized cutting board, and the *longissimus lumborum* (LL) region of the muscle was separated from the LTL, using a sterilized knife. Each muscle was randomly assigned to either wet or dry ageing, per animal. Approximately 150 g of each eland LL (left and right) was collected into sterile plastic bags for microbiological analysis (Control/d1). Thereafter, muscle pH was measured using an inoLab pH 730 meter (WTW, Weilheim, Germany) in each muscle. Approximately 250 g of

each LL was taken to determine the chemical composition and lipid oxidation, according to the methodology described by Bartoň et al. (2014). Two steaks of approximately 2 cm thick were cut from each muscle for physical meat quality analysis, described further below. A third steak was cut for volatile fatty acid analysis, and frozen at -20°C until analysis.

The remaining LL muscle was weighed, and then either vacuum-packaged (wet ageing), or left unpackaged (dry aged), and placed into a controlled chamber for 14 days, at a temperature setting of 2°C, with humidity levels of 80 % and the air exchange in the chamber was 15% per hour. The ageing time length was chosen based on the previous research by Needham et al. (2020a, 2020b). After the ageing period (D15), the muscles were weighed to determine moisture loss, and placed onto a sterile cutting board. The outer edge of the muscle was cut off, and samples were taken for microbiological analyses again (150 g); however, the crust and the inside of the dry aged muscles were collected separately, avoiding cross contamination. The dry aged muscles were then photographed, together with a ruler scale, to determine the muscle area and crust penetration. A steak was cut for volatile fatty acid analysis, and frozen at -20°C. The physical measurements were then repeated, and samples were taken for chemical analyses and lipid oxidation analysis.

3.3. Physical, Chemical and Lipid Oxidation Analyses

3.3.1. Physical Analyses

The pH (pH24) values were obtained using a pH and temperature probe (inoLab pH 730 set, WTW, Weilheim, Germany). Of the two steaks cut, both on D1 and D15 for each muscle, the first steak was bloomed for 45 minutes (Needham et al. 2019) and were then measured for CIE Lab surface colour with a portable spectrophotometer (CM-700d, Konica Minolta, Osaka, Japan; aperture diameter: 8 mm; illuminant: D65; observer angle: 10° and specular component: 0% UV). Six colour measurements were taken at random places on the samples and were then averaged for each of the L*, a* and b* values. The second steak was used for determining the cooking loss percentage, by comparing the weights before and after cooking (Honikel, 1998). Steaks were

weighed, then placed in plastic bags and into a water bath at 80°C until the steak reach at internal temperature of 75°C (AD14TH, AmaDigit, Kreuzwertheim, Germany). The temperature of the steak was measured using a thermometer probe, which was placed right at the centre of the steak. After the steak was cooled down to room temperature and weighed, six cubes measuring 1 × 1 × 2 cm were cut out of each sample, which were then used for shear force determination (WBSF). The average peak shear force was determine using the Universal Texture Analyzer 3365 (Instron, Canton, MA, USA) fitted with a standard Warner-Bratzler blade at a speed of 100 mm/min. The peak force (N) to shear each sample was then recorded and averaged per sample.

3.3.2. Proximate Chemical Composition

The LL samples (approximately 200 g) intended for chemical analyses were homogenized in a food blender and frozen at −20 °C. Dry matter content was determined by oven drying at 105 °C to a constant weight. Dried samples were pulverized using a Grindomix GM200 knife mill (Retsch, Haan, Germany) and analysed for crude protein (Kjeltec 2400, FOSS Tecator AB, Höganäs, Sweden), crude fat by extraction with petroleum ether (Soxtec Avanti 2055, FOSS Tecator AB, Höganäs, Sweden), and crude ash by incineration at 550 °C in an electric furnace for 24 hours.

3.3.3. Quantification of Lipid Oxidation

Malondialdehyde (MDA) concentrations in the LL muscles were determined using high-performance liquid chromatography (HPLC). Eland muscle samples were saponified with 1 ml of 1 M Potassium hydroxide (KOH) and 10 µl of 0.02 M Butylated hydroxytoluene (BHT) in methanol. This mixture was then placed in an enclosed tube and then placed in a water bath at 60°C for 1 hour, while continuously shaking, in the dark. Afterwards, the solution was cooled down and then acidified using concentrated HCI to ~pH 2. The sample was centrifuged at 15,000 × g for 5 min, at approximately 5°C. To 200 µl of the supernatant, 300 µl of 0.1 M HCl and 50 µl of the 2,4-Dinitrophenylhydrazine (DNPH) solution were added. The resulting mixture was vigorously mixed and kept at 50°C for 1 hour in the dark. At the end of the derivatisation procedure, if

necessary, the sample was allowed to cool down and then once again centrifuged at 15,000 × g for 5 min at approximately 5°C. The solution was then placed in a vial, where 10-45 µl of the solution was taken, and injected into for chromatographic analysis according to Czauderna et al. (2011).

3.4. Crust Penetration and Surface Area Loss

The images were calibrated using the Fiji-ImageJ open access software (Schindelin et al. 2012; Rasband 2018), according to the ruler scale presented within each photograph. Using the software, ten measurements were taken per sample on each photograph, these measurements were then averaged to obtain the average depth crust formed on each of the dry aged eland meat (D15). This was done by drawing a line from the edge/tip of visible crust on the meat sample vertically to the outer edge of the crust.

3.5. Microbiology

To evaluate the influence of the ageing treatments on the microbiota composition of eland meat, samples of the LL muscle were analysed according to the adjusted methodology described by Li et al. (2013). During transport, the samples were stored on ice and aseptically grinded on the day of collection. Meat samples were taken from the inner portion of the meat, to exclude surface contact areas. For the dry aged crust, the whole crust was included in its analysis. The samples were homogenized, and 1 g of each sample was diluted in 9 ml of peptone water, prepared by adding 10 g of bacteriological peptone (Oxoid, Brno, Czech Republic) and 5 g of sodium chloride (Penta, Prague, Czech Republic) to 1 l of distilled water, and then vortexed. These initial samples were used to create a ten-fold serial dilution of every meat sample, up to 10^{-5} dilution. Every dilution sample was then shaken in a water-bath for 20 minutes at 37°C, and then vortexed. Subsequently, 50 µl of the sample dilution was spread-plated on a Petri dish with the appropriate solid medium (Oxoid, Brno, Czech Republic) in triplicate. To determine the total aerobic bacteria counts, samples were incubated on a Plate Count Agar for 72 hours at 30°C. The *Enterobacteriaceae* were cultivated 24 hours at 37°C on MacConkey Agar. The enumeration of lactic

acid bacteria followed culturing samples for 5 days at 25°C on MRS Agar. Yeast and moulds were cultivated using plates with Sabouraud Dextrose Agar with Chloramphenicol for 7 days at 25°C.

3.6. Volatile Compounds Analysis (GC-MS)

3.6.1 Sample Preparation and Extraction

Three animals were randomly selected and the volatile compounds for each animal for both ageing treatments were evaluated (n=9). Samples were collected on D1 (control) and D15 (wet aged and dry aged), they were then grilled until the inside of the muscle reached a temperature of 70°C (AD14TH, AmaDigit, Kreuzwertheim, Germany), after which the samples were homogenized, and 2 grams of each sample was placed into a 4 mL clear glass vial and sealed with a hole cap and PTFE-faced silicone septa. Each glass vial was then labelled according to the animal, day, and treatment. The samples were then packed into a clear sealable bag and placed in the freezer at -18°C until the time of analysis. A modified methodology based on Moran et al. (2021) was used for the analyses of volatile compounds.

Before the analysis took place, the samples were taken out of the refrigerator and placed in room temperature to thaw. Prior to extraction, a preheated water bath was prepared at 70°C and each sample was then equilibrated within the water bath for 15 minutes, after which the vial was then subjected to Headspace-solid phase microextraction (HS-SPME). The SPME device, a silica fibre coated with 100 µm thick polydimethylsiloxane (PDMS) film was placed inside the headspace of the sample for 15 minutes. Then it was inserted into the injector, where the substances were desorbed. The fibre was left in the injection system until further extraction. The SPME fibre was reconditioned at 250°C before using to eliminate contamination from any previous usage. This blank measurement was performed for a total of 1 hour. Only one fibre was used throughout the entire extraction process. This method was repeated for each sample.

3.6.2. GC-MS Analysis

The GC-MS analysis was processed on the Agilent 7890B/5977a GC/MSD System (Agilent Technologies, Santa Clara, California, USA) equipped with an autosampler Agilent 7693, a HP-5 column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent19091s-433). The carrier gas helium had a flow rate of 1 ml/min. The injecting temperature was set at 250°C and maintained during the whole chromatography run, which was set to 55 minutes. The GC injector port operated in splitless mode with a 0.75 mm i.d. liner. The optimized GC oven temperature was programmed to increase from 40 °C (1 min) to 160 °C at 4 °C/min. MassHunter Workstation Software Qualitative Analysis Version B.07.00 was utilized to analyse the mass spectra. The software program was then set to obtain the peak areas by integration. Identification of compounds was based on a comparison of the retention indices (RIs) against the mass spectra with the National Institute of Standards and Technology library version 2.2 (NIST, USA). The confirmation of identification of components was based on the comparison of Kovats Index (KI) values, which were calculated by using the retention times of n-Alkanes series ranging from C7 to C40 (Sigma-Aldrich). The area under each identified peak was calculated, and the percentage contribution of each volatile compound was determined relative to the total area of all compounds identified in the sample. Substances that were not possible to be confirmed by comparison of KI was due to their unavailable retention time data.

3.7. Sensory Evaluation

The 2 cm-thick steaks cut for sensory analysis after ageing were weighed and cooked on a fiamma double glass-ceramic plate grill. For dry aged samples, the edges and crusts were removed. The plate grill was first pre-heated at 200°C and then a steak was placed and cooked until the internal temperature reached 70°C. The internal temperature was taken by inserting a temperature probe (AD14TH, AmaDigit, Kreuzwertheim, Germany) into the centre of the meat. Afterwards, the cooked steaks were taken off the plate, and cooking loss was determined, this was done by comparing the weight of the steaks before they were grilled and after they were grilled. This was followed by obtaining six

samples to determine the WBSF, as described earlier. For preparing the sensory evaluation samples, 2 × 2 × 2 cm cubes were cut out of the steaks, omitting the edges which had contact with the grill, and then placed into glass containers (with lids) that were preheated, and maintained at 50°C for approximately 1 hour, until the sensory evaluation. Additionally, the containers were marked with randomized codes to ensure unbiases.

The sensory evaluation was performed by ten trained panellists (ISO 8586-1 1993) who were instructed to sit in individual booths, that were marked with a number, and under red lighting (ISO 8589 1988). The panellists were provided with an evaluation form on which the panellists were asked to give their evaluation of the sensory attributes of the common eland meat samples that underwent either a wet ageing or dry ageing treatment, according to the descriptors used in Bartoň et al. (2014). However, additional descriptors were also added, as it would allow panellist to clearly identify more specific sensorial attributes linked to dry ageing (Table 1). Each panellist was provided with two samples (wet aged vs. dry aged) from the same animal for the purpose of comparison, as demonstrated in Table 1. As the glass containers with the samples had randomized coding, the panellists were not aware of this comparison, and six sets of two samples were presented. Overall, the descriptory sensory analysis (DSA) included 12 samples from six animals. The panellists received the samples in a randomized fashion. Panellists were asked to open their container and take the time to first evaluate the /aroma, followed by the assessment of the texture and flavour, which were assessed on a continuous scale from 0 to 100. Lastly, the panel was provided with bread and water to cleanse their palette between samples.

Table 1. Description and scale of the sensory attributes (aroma, flavour, and texture) used to evaluate the *longissimus lumborum* from female common eland (*Taurotragus oryx*).

Attribute	Evaluation	Scale
Beef Aroma Intensity Game Aroma Intensity	Before eating sample	0 = very low 100 = very high
Tenderness	After first two or three chews	0 = very tough 100 = very tender
Juiciness	After first five or ten chews	0 = very low 100 = very high
Fineness		0 = very coarse 100 = very fine
Chewability	After at least fifteen chews	0 = difficult to chew 100 = easily chewable
Beef Flavour Intensity Game Flavour Intensity Abnormal Flavour Intensity Liver Flavour Sour Flavour Nutty Flavour Roasted Flavour	After first five or ten chews	0 = very low 100 = very high
Overall Acceptance	After completion of evaluation	0 = not acceptable 100 = highly acceptable

3.8. Statistical Analysis

The data was analysed in SAS (Version 9.4, SAS Institute Inc., United States). Data were evaluated using mixed linear models, and the parameters were estimated by the REML method using the MIXED procedure. For the physiochemical and microbiological data, the statistical model utilized the random effect of animal, and the fixed effect of ageing treatment (control, wet or dry ageing). As the design of the sensory data was to compare the effect of wet vs dry ageing within one animal, the assessor was also included as a random effect, together with the animal, and the ageing treatment (wet vs dry ageing) being a fixed effect in the model. A significance level of 5% was used throughout.

The data in Table 3 are presented as least squares means (LSM) and standard errors of the mean (SEM). Differences between groups were compared

using Tukey's range test. Due to the sub-sample size of the animals for the volatile compounds analyses, no statistics were performed on the data.

4. Results

4.1. Physical Characteristics

Results for physical characteristics are shown on Table 2. Meat pH for the wet and dry treatments of the LL muscle differed ($P < 0.001$); the pH increased from D1 to D15 for the wet treatment, similarly, the pH of the dry aged meat increased from D1 to D15. The lightness of colour of the muscle under the wet and dry treatment also differed (L*; $P = 0.001$), with the dry ageing meat being darker on D15 compared to the wet treatment on D15. The dry aged LL muscle appeared redder (a*; $P = 0.029$) compared to the wet aged muscle on D15, which did not differ from that of the control. There was no apparent difference in the yellowness of the LL muscle under either treatment (b*; $P = 0.165$). The WBSF of the cooked meat ($P = 0.189$) and WBSF of the grilled meat did not differ between the ageing treatments, as well as compared to the control ($P = 0.415$). Muscle cooking loss differed between treatments ($P < 0.001$), with there being a higher cooking loss for the wet aged muscle than the dry aged muscle, both of which were lower than the cooking loss on D1 (control). Grilling loss percentage did not differ between wet aged and dry aged LL muscles ($P < 0.184$). Weep loss did differ ($P < 0.0001$), with the dry aged muscle having a higher weight loss on D15 compared to the wet aged muscle on D15.

Table 2. Physical characteristics of eland *longissimus lumborum* muscles before (control, day 1 post-mortem) and after wet or dry ageing, for 14 days.

Parameter	Treatment			*SEM*	*P*-value
	Control	Wet	Dry		
pH	5.62[a]	5.64[a]	5.72[b]	0.06	**<0.001**
L* (lightness)	40.9[a]	38.9[b]	37.6[b]	1.66	**0.001**
a* (redness)	11.2[b]	12.3[ab]	12.5[a]	0.91	**0.029**
b* (yellowness)	12.2	12.9	12.9	1.09	0.165
WBSF (N) cooked meat	77.6	90.6	85.9	7.04	0.189
WBSF (N) grilled meat	-	63.3	57.6	5.99	0.415
Cooking loss (%)	31.4[a]	20.4[b]	17.1[c]	0.80	**<0.001**
Grilling loss (%)	-	19.8	16.9	2.11	0.184
Weep loss (%)	-	3.0 [b]	22.1[a]	0.61	**<0.001**

[a,b,c] Means with different superscripts within the same row differ significantly *(P < 0.05)*

WBSF: Warner-Bratzler shear force

4.2 Chemical Composition

The chemical composition of the LL muscle after different ageing treatments, and the control (D1), are depicted in Table 3. The moisture content between the two ageing treatments differed ($P < 0.001$), with the wet aged muscle having a higher moisture content compared with the dry aged muscle; however, a higher moisture content was found in the control muscle compared to the dry aged muscle only. Crude protein content differed ($P < 0.001$), with the dry aged muscles having a higher crude protein value compared to the wet aged LL muscles and control samples. Similarly, intramuscular fat differed ($P = 0.002$), with the dry aged LL muscles having higher values than the wet aged and control muscles; however, the control muscles had higher values compared to wet aged. The ash content also differed between the two treatments ($P < 0.001$), with dry aged LL muscles having a higher ash content compared to the wet aged. Additionally, there was an overall increase in ash content from D1 to D15 in both the wet aged and dry aged LL muscles, compared to D1 (control). The

malondialdehyde (MDA) levels differed in both LL muscle ageing treatments (*P* = 0.047), with the dry aged LL muscles having higher levels of MDA (higher degree of lipid oxidation) compared to the wet aged LL muscles and the control.

Table 3. Chemical composition of the eland *longissimus lumborum* muscle before (control, day 1 post-mortem) and after wet or dry ageing, for 14 days.

Parameter	Treatment			*SEM*	*P*-value
	Control	Wet	Dry		
Moisture (%)	74.75[a]	74.34[a]	67.40[b]	0.364	**<0.001**
Crude protein (%)	22.89[a]	23.55[a]	29.98[b]	0.281	**<0.001**
Intramuscular fat (%)	0.42[a]	0.32[b]	0.50[a]	0.093	**0.002**
Ash (%)	1.03[c]	1.10[b]	1.49[a]	0.013	**<0.001**
MDA (mg/kg)	0.35[b]	0.73[b]	1.64[a]	0.244	**0.047**

[a,b,c] Means with different superscripts within the same row differ significantly *(P < 0.05)*

MDA: malondialdehyde (lipid oxidation)

4.3 Crust Penetration and Surface Area Loss

The dry aged LL muscles' (n=6) overall surface area, wet surface area, and dry surface area were measured and depicted in Table 4. The average percentage loss per muscle was 16.7%, with one dry aged LL muscle sample having the maximum percentage loss of ~21%, while the minimum percentage loss recorded was 13%. There was low variability in the penetration depth of the crust, which means that crust formation and penetration were similar across all samples.

Table 4. Descriptive analysis of crust penetration and surface area loss of dry aged common eland meat on D15 post-mortem.

Parameter	Mean	Minimum	Maximum	Standard Deviation
Overall Surface Area (cm^2)	44.05	35.87	51.30	6.15
Wet Surface Area (cm^2)	36.62	29.06	41.97	4.89
Dry Surface Area (cm^2)	7.43	5.71	10.67	1.86
Percentage Loss (%)	16.78	13.08	20.79	2.75
Depth Crust Average (cm^2)	0.32	0.26	0.39	0.06

4.4 Microbial Analysis

The microbial composition of the *longissimus lumborum* muscle of eland meat is shown in Table 5. The total bacteria count (TBC) between the different ageing treatments, and when compared to the control, did not have any significant differences ($P = 0.296$). The Enterobacteriaceae (EAB) count differed ($P < 0.001$) with between treatments, with dry aged meat having a lower count compared to wet aged meat, but a higher count compared to the dry crust. Lactic acid bacteria (LAB) count was significantly different ($P = 0.021$; the LAB count was similar for the control and wet aged treatment, but the values differ significantly from dry aged meat. The LAB count from the dry crust was significantly higher compared to the dry aged meat. Yeast levels between treatments did not differ ($P = 0.939$), but mould levels did differ between treatments ($P = 0.004$), with levels decreasing compared to the control. Wet aged meat had lower mould counts compared to dry aged meat, while on the other hand, the dry crust had lower mould development compared to the control treatment and internal meat samples of both wet and dry ageing treatments.

Table 5. Total bacteria count (TBC), Enterobacteriaceae (EAB), lactic acid bacteria (LAB), yeast and mould counts before (control, day 1 post-mortem) and after wet or dry ageing, for 14 days.

Parameter (log CFU/g)	Treatment				*SEM*	*P*-Value
	Control	Wet	Dry	Crust		
TBC	5.46	5.60	4.37	5.18	0.517	0.296
EAB	4.58[a]	2.87[b]	0.29[c]	0.01[c]	0.394	**<0.001**
LAB	3.18[ab]	3.61[ab]	1.99[b]	3.97[a]	0.605	**0.021**
Yeast	4.39	4.13	4.26	4.41	0.476	0.939
Mould	4.22[a]	1.42[b]	2.00[b]	0.53[b]	0.654	**0.004**

[a,b,c] Values with different superscripts differ at $P < 0.05$.

Unit: log CFU/g, but data of < log 1.0 was adjusted to 0.01

(TBC) total bacteria count

(EAB) Enterobacteriaceae

(LAB) lactic acid bacteria

4.5 GC-MS Analysis of Volatile Compounds

In the GC-MS analysis, a total of 11 compounds of 3 different classes (aldehyde, alcohol, and hydrocarbon) were identified in the eland meat samples. The chemical composition is demonstrated in Table 6. Hexanal and nonanal were the primary compounds identified. In the control, hexanal (73.5%) was the main constituent, followed by another aldehyde, nonanal (9.47%), and the alcohol, 1-octen-3-ol (4.44%). Similarly, in the wet aged treatment, hexanal (64.19%) was the main compound with the highest percentage contribution, followed by benzaldehyde (11.29%) and nonanal (10.07%). The percentage contribution of benzaldehyde in the wet aged LL muscle differed from the control (2.17%) and the dry aged meat (3.32%), as the percentage contribution was lower in both the control and dry aged meat compared to the wet aged LL muscle. Within the dry aged LL muscle, hexanal (73.43%) was the main component with the highest percentage contribution, followed by nonanal (6.19%); the other compounds' percentage contributions were similar. However, the dry aged treatment differed with the control and wet aged treatment in that the alcohol, 2-

Ethylhexan-1-ol (3.17%), only appeared in the dry aged treatment but was not detected in the control or wet aged treatments.

Table 6. Volatile compounds identified using gas chromatography-mass spectrometry in cooked eland meat, before (control, day 1 post-mortem) and after wet or dry ageing, for 14 days (day 15). Values are presented as a percentage of the contribution of each volatile compound relative to the total compounds identified in the sample.

Compound	RT (mins)	RI		Relative Contribution of Compound (%)		
		Obs	Pubs	Control	Wet	Dry
Pentan-1-ol	6.68	773	779	1.44	1.24	2.80
Hexanal	7.43	801	800	73.50	64.19	73.43
m-Xylene	10.01	872	872	0.21	0.09	0.33
Heptanal	11.14	904	899	3.04	2.98	2.73
Benzaldehyde	13.75	976	978	2.17	11.29	3.32
1-octen-3-ol	14.32	991	978	4.44	4.97	3.45
Octanal	14.84	1006	1001	2.29	3.85	2.82
2-Ethylhexan-1-ol	16.02	1041	1045	[c]	[c]	3.17
2-Decen-1-ol	17.42	1082	[d]	1.41	0.92	1.20
Nonanal	18.31	1109	1098	9.47	10.07	6.19
Pentadecanal	33.93	1724	1717	2.02	0.41	0.54

RT: retention time; RI: retention index

[a] retention index is calculated from retention times and based on C7-C40 alkanes

[b] Data taken from NIST (2018)

[c] Compound not detected in the sample

[d] Literature data not available

4.6 Sensory Evaluation

The effect of the two ageing treatments on the sensory characteristics of the LL muscle of eland meat were evaluated by trained panellists after 14 days of ageing, and the results are summarized in Table 7. Beef aroma intensity did not differ between the ageing treatments (P =0.465). Additionally, game aroma intensity (P = 0.214), tenderness (P = 0.673), juiciness (P= 0.280), fineness (P = 0.763), chewiness (P = 0.939), beef flavour intensity (P = 0.549), game flavour

intensity (*P* = 0.811), abnormal flavour intensity (*P* = 0.109), sour flavour (*P* = 0.415), nutty flavour (*P* = 0.529), roasted flavour (0.114), and overall acceptance (*P* = 0.153) did not differ between treatments. However, abnormal aroma intensity (*P* = 0.013) and liver flavour (*P* = 0.014) did differ, with wet aged meat having a higher abnormal aroma and liver flavour compared to dry aged meat.

Table 7. The effect of different ageing on the sensory characteristics of eland meat (*longissimus lumborum*) as evaluated by a trained panel after 15 days of ageing, scored on a 0 to 100 continuous line scale.

Descriptor	Ageing		*SEM*	*P-value*
	Wet	**Dry**		
Beef aroma intensity	43.8	46.1	5.02	0.465
Game aroma intensity	46.0	42.6	4.37	0.214
Abnormal aroma intensity	**29.6**	**22.2**	**6.08**	**0.013**
Tenderness	58.0	59.5	5.65	0.673
Juiciness	53.3	57.6	4.62	0.280
Fineness	55.4	54.2	4.50	0.763
Chewiness	55.5	55.8	5.23	0.939
Beef flavour intensity	50.9	49.3	4.51	0.549
Game flavour intensity	36.9	36.3	4.76	0.811
Abnormal flavour intensity	19.7	16.5	4.73	0.109
Liver flavour	**29.1**	**22.0**	3.99	**0.014**
Sour flavour	34.2	31.5	5.94	0.415
Nutty flavour	18.2	19.5	4.45	0.529
Roasted flavour	28.5	24.5	5.14	0.114
Overall acceptance	47.9	52.8	4.27	0.153

5. Discussion

Meat quality is affected by pH, which can influence an array of parameters, such as shelf-stable life, which includes factors such as colour and microbial stability. In this study, it was observed that pH values increased from D1 to D15 for both wet aged and dry aged LL muscle; the rise of pH values was also observed in Shange et al. (2019), the study focused on the physiochemical and microbiological changes of back wildebeest under two treatments; high pH muscle and normal pH muscle, as well as in the work of Rodriguez-Calleja et al. (2005) where the focus was on pH levels on rabbit meat and its microbiological effects. When amino acids breakdown, they release other basic compounds that increases the alkalinity, thus increasing pH (Gill & Newton 1979). It must be noted that the by-products of this process are responsible for spoilage symptoms, including off-odours and off-flavours (Braggins 1996). Additionally, bacteria growth is not necessarily affected by a variation of pH between 5.5 and 7 (Gill & Newton 1979).

In the present study, the pH levels were always under 6, meaning that for game meat, these are normal pH levels that could potentially benefit from a longer shelf-life. Braggins (1996) reported that with an increase in ultimate pH values there was an increase in the development of undesirable flavours and odours, as well as a decrease in the concentration of aromatic compounds such as aldehydes. However, in this study the pH value of dry aged meat was higher than the control and wet aged meat, but the percentage of aldehyde compounds, was lower (89.04%) compared to the control (92.50%) and wet aged (92.79%) muscle, while no direct conclusions can be drawn due to the limited subset of data—this is an interesting observation. Van Ba et al. (2013) found that volatile aroma compounds developed desirable attributed of meat at a pHu of 5.5 compared to a pH of 6.2. It is possible that because the pH level of the dry aged meat was higher compared to the other treatments, this could explain the lower concentration of aldehydes.

Microbial spoilage can be present in aged meat and is undesirable as it can pose health issues. There are microbial spoilage limits that have been used by Shange et al. (2018) in game meat, to understand what counts are acceptable

and not acceptable in terms of safety. Currently, bacterial counts of 7 log cfu/g are considered unacceptable to the current consumer for consumption. In the present study, the LL muscles under the various treatments were under 7 log cfu/g for total bacteria count (TBC), which means that the control, along with the aged meats, would be considered acceptable for consumption. The TBC was higher in the control and wet aged meat, which can be due to the fact that the wet aged meat was packaged, as well as both the control and wet aged meat had a higher moisture content compared to dry aged meat. While packaging can effectively be a barrier for many forms of contamination, the packaging can also create a thriving environment for anaerobic bacteria.

This is further explained by the fact that LAB was higher in wet aged meat compared to the control and dry aged meat. Similar results were reported by Parrish et al. (2001), in which LAB is dominant in this kind of environment. Yeast levels did not different across treatments in the present study, but yeast counts can be decreased in dry ageing by ageing in a bag compared to traditional dry ageing (Ahnström et al. 2006). Mould growth was higher in the control, which means that is it likely that there was the presence of cross-contamination or transfer of mould spore to the carcass. Another contributor could be due to the differences in moisture content compared to the dry aged and wet aged meat, and while the moisture content did not differ between the control and wet aged meat, the wet LL muscle was aged in a vacuum package, which again, acts as a barrier.

Moreover, the presence of *Enterobacteriaceae* in the control indicates that there was initial contamination that could be related to the harvesting process, which typically occurs during the dressing/slaughtering processes (Gouws et al. 2017; Humphrey & Jørgensen 2006). Furthermore, potential lacerations of the gastrointestinal tract and contact with the carcass skin contributes to the microbiological contamination of the muscle (Gill 2007). It is for this reason that proper protocol of harvesting and dressing procedures are followed, as well as ensure the hygiene of workers and slaughterhouse to decrease the potential of microbial contamination. Further research on techniques to minimize contamination of muscle during the dressing of the animal should be investigated under less-controlled field harvesting conditions for game species.

Moisture content was lower for dry aged meat compared to the control and wet aged meat, as well as the weep loss. This is simply due to the fact that dry aged meat has higher muscle weight loss, as during the ageing process the muscle is essentially dried out, without any barrier to prevent moisture loss. While dry ageing does lead to a development of positive sensorial attributes, it is also linked to higher losses compared to wet aged meat. According to the results of the crust penetration, ~20% of overall surface area of the LL muscle was lost after ageing. Given that currently game meat's most important economic parameter is yield, this is an important aspect that producers as well as commercial consumers need to take into consideration. Lastly, further research on alternative uses of the trimmings lost as the crust should be investigated, such as adding it to other processed products to improve their flavour. Xue et al. (2021) reported that using beef crusts as an additive in the production of beef patties did not have any negative effects on lipid oxidation, moreover, adding the dry aged beef crust was found to enhance the flavour of the beef patties.

The colour stability of meat plays an important role in a consumer's decision in meat selection (Needling et al. 2016). In South African game meat, it is typical to have a meat colour range from dark red to red brown in colour. These colour differences have been attributed to the fact that wild ungulates, such as eland, are more active compared to their livestock counterparts (Hoffman 2001), and thus meat myoglobin content is higher in game species, for example, the myoglobin content was reported to be of 7.25 to 7.50 mg/g for impala (Hoffman et al. 2005). During ageing of the eland meat in this study, the L* (brightness/lightness) decreased, while a* (redness) and b* (yellowness) of the meat surface (after blooming) increased over the ageing period. Lightness of colour decreased with ageing because of changes in protein structures, allowing for a higher dispersion of light (Joseph & Connely 1970). However, the overall colour of the meat still had levels similar to what is generally expected of game meat (L* > 33, a* > 13, b* ~10, C* > 17 and Hab > 36; Shange et al. 2019), meaning that ageing did not have a significant impact on the colour stability of the meat. The low IMF content that is found in game species also plays a role in the darkness of its colour, by giving the meat a darker appearance, which is typically undesirable by consumers (Hoffman & Wiklund 2006). While colour is

important to the consumer, the redness and brightness of the muscles in this study would still be considered acceptable to the consumer (Shange et al. 2019).

The WBSF is an indicator that gives insight to the toughness or tenderness of meat. Currently, consumers consider meat with a shear force of <42.87 N as tender (Destefanis et al. 2008). The shear force values of grilled LL muscle were similar to the results of Needham et al. (2020a), where the BF muscle reached a maximum tenderness of 57.5 to 67.5 N, and the LTL muscle reached a maximum tenderness of 57.0 to 67.5 N. However, broiled LL muscles had high shear force values, which would deem the meat to be unacceptable to the consumer, but when looking at the shear force after grilling, more favourable values were obtained. The application of heat can either increase tenderness or decrease it depending on two main facts: time and temperature (Ismail et al. 2019). Cooking methods impact not only the juiciness and tenderness, but also the volatile aroma of the meat (Kerth 2016; Shahidi 2004; Bejerholm & Aaslyng 2003). While cooking method was not a point of focus for this study, it is still an area for further research on how different cooking methods, at different temperatures and durations, can further improve tenderness of game meat.

When meat is cooked, there are modifications of the connective tissue as well as of the myofibrillar proteins (Ismail et al. 2019), additionally the application of heat allows for the dissolvement of collagen which means the softening of the muscle, or increase in tenderness — on the other hand, heat can also be responsible for denaturing myofibrillar proteins which can decrease tenderness (Walsh et al. 2010). Overall, shear force values for both grilled meat and broiled meat of the LL muscle would indicate that for the average consumer, the LL muscle would still be considered tough after both vacuum/wet ageing and dry ageing, therefore concluding that additional strategies to improve tenderness. However, when considering the sensory evaluation, the sensory panel did not determine any differences in tenderness between the ageing treatments and scored the samples on the “more tender” side of the line scale.

There are various ageing techniques that exist to enhance the quality of meat, and currently, vacuum-ageing, or wet ageing, is the most popular method used within the commercial industry, due to lower ageing losses and

convenience during storage and transport (Warren & Kastner 1992). On the other hand, dry ageing has been found to enhance desirable flavour development; however, there are additional ageing losses due to a higher loss of moisture (Smith et al. 2014), as already mentioned, moreover, cooking loss values are lower for dry aged meat compared to wet aged meat. In general, temperature is the main factor impacting cooking loss in meat Modzelewska-Kapituła et al. 2012), as well as the period of time the meat is cooked for (Silva et al. 2015).

Furthermore, ante-mortem and post-mortem factors directly and indirectly influence the tenderness of meat (Koohmaraie et al. 2002). Hawkins et al. (1987) reported that over 50% of the tenderness outcome is dependent on muscle traits. Intramuscular fat content also plays a role in meat tenderness (Webb & O'Neill 2008), with higher IMF content having higher tenderness levels. Needham et al. (2019) and Bartoň et al. (2014) have established that eland meat has low levels of intramuscular fat, and given that eland meat is considered tough, this can provide further supports the linkage between tenderness and IMF. Game species are known to have lower IMF levels due to higher activity levels as these are wild/free-range animals. Moreover, the levels of IMF content and moisture having an important relation to one another (Cho et al. 2010), the results of this study agree with Han et al. (1996), that higher IMF levels account for a lower cooking loss and vice-versa.

Meat flavour is important to consumers and it is a combination of tastes and aromas (James & Calkins 2008). Volatile compounds are responsible for determining the aroma and flavours of cooked meat, and a myriad of compounds are responsible for this, as aroma and flavours cannot be attributed to just a single compound or class of compounds (Pegg & Shahidi 2004). In this study, wet aged meat scored higher for liver flavour compared to dry aged meat; liver-like flavour has been attributed to gamey flavour (Neethling et al. 2016). Gamey flavour functions as the main contributing factor to flavour the various game species, but the lack of specific descriptors makes it difficult to compare between different studies (Needling et al. 2016). Gamey flavour, along with abnormal aroma, could also be attributed to lipid oxidation, as the degradation of lipids can release off-odours and flavours. While the levels of MDA were higher for dry aged meat compared to the control and wet aged meat, it is also known that dry aged

meat benefits from the development of unique flavour. Higher MDA levels are also expected in the dry aged meat, as the dry aged meat was not packaged, and thus exposed to oxygen. On the other hand, wet aged meat does not necessarily benefit from the development of deeper more complex flavours as does dry aged meat, and thus the levels of MDA could have negatively impacted the sensory quality of the wet aged meat, which can provide an explanation of to higher abnormal odour compared to dry aged meat.

The development of particular gamey flavours could also be attributed to the diets of games species. Particularly, South African species, with the availability of different types of terrain in the region have access to different types of feed (Van Der Merwe et al. 2013) which could influence the flavour of game meat, especially compared to domesticated livestock. The reason that diet can influence the flavour of meat is because diet has been found to influence fatty acid profiles in ruminants (Wood et al. 2008), which as it has been established fatty acids influence not only meat flavour but also aroma. Additionally, the wet aged LL muscle had more intense abnormal aroma intensity compared to dry aged meat, this can be attributed to the fact that dry aged meat tends to develop different flavours and aromas that are favourable to the consumer. Overall, there were no differences found in the overall acceptance of either wet aged or dry aged meat, despite the more favourable scores for liver flavour and abnormal aroma intensity in dry aged eland meat.

6. Conclusions & Recommendations

Ageing under the current techniques had a negligible influence on eland meat tenderness, and the meat could still be considered as tough to the average consumer. Thus, additional processing techniques would need to be further implemented to improve tenderness quality, such as pelvic suspension or alternative ageing techniques and times. Overall, there was a preference for dry aged meat amongst the sensory panel, as it scored higher in overall preference and scored lower in abnormal aroma intensity and liver flavour, both of which are negative attributes from the consumer’s perspective. Thus, dry ageing indeed showed better flavour and aroma development than wet ageing, but overall

acceptance was not significantly affected. Dry aged meat showed higher weep loss compared to the control and wet aged meat, as well as ~20 % crust loss, making it an expensive product. Overall, ageing did not negatively influence the colour of eland meat and produced microbially-safe meat. Further research on meat internal cooking temperatures and times should be conducted, to investigate appropriate cooking methods for game meat, considering the differences obtained for the shear force in the broiled versus grilled preparation methods. Additionally, a combination of ageing techniques should be further investigated, as well as alternative technologies, such as dry-bag ageing, to further improve the tenderness of eland meat.

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8. Appendices

APPENDIX I: Sensory evaluation questionnaire provided to the panellists to score the wet and dry aged eland meat, using the continuous line scale method.

protokol senzorického hodnocení "eland 2020"	box num:	set num:
kód hodnotitele / assessor:	dne: 27.08.2020	

Intenzita vůně hovězího m. (beef aroma intensity)

velmi nízká	velmi vysoká

Intenzita vůně zvěřiny (game meat aroma intensity)

velmi nízká	velmi vysoká

Intenzita abnormální vůně (abnomal odour intensity)

velmi nízká	velmi vysoká

Křehkost (tenderness)

velmi nízká	velmi vysoká

Šťavnatost (juiciness)

velmi nízká	velmi vysoká

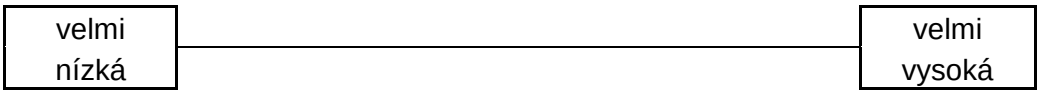
Vláknitost (fineness)

velmi nízká	velmi vysoká

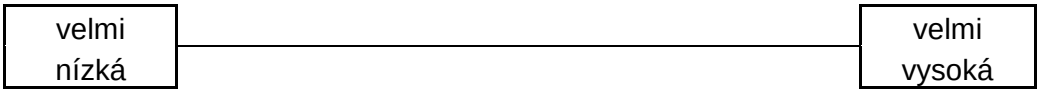
Žvýkatelnost (Chewability)

velmi nízká	velmi vysoká

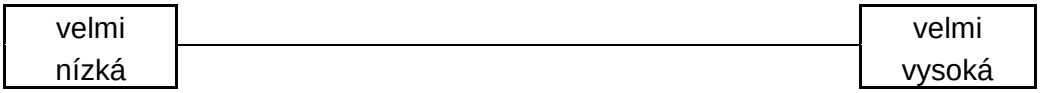
Intenzita chuti hovězího m (beef flavour intensity)



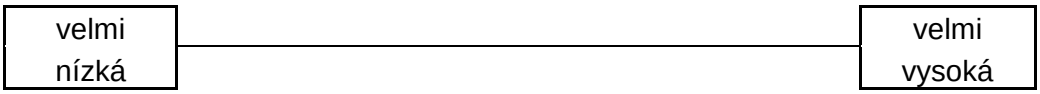
Intenzita chuti zvěřiny (game meat flavour intensity)



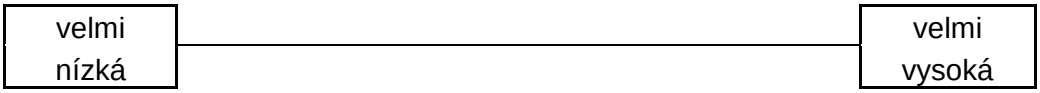
Intenzita abnormální chuti (abnormal flavour intensity)



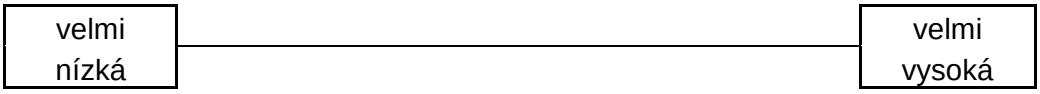
Chuť jater (liver flavour)



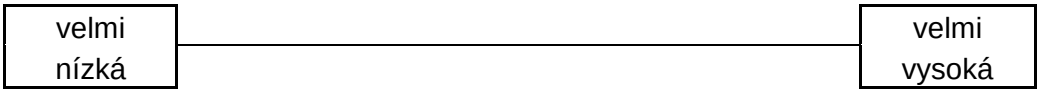
Chuť kyselá (sour flavour)



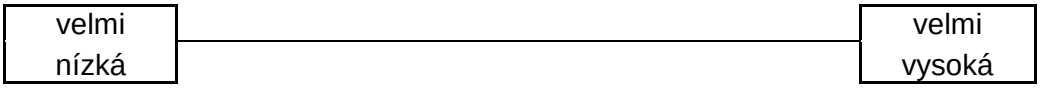
Chuť oříšková (nutty flavour)



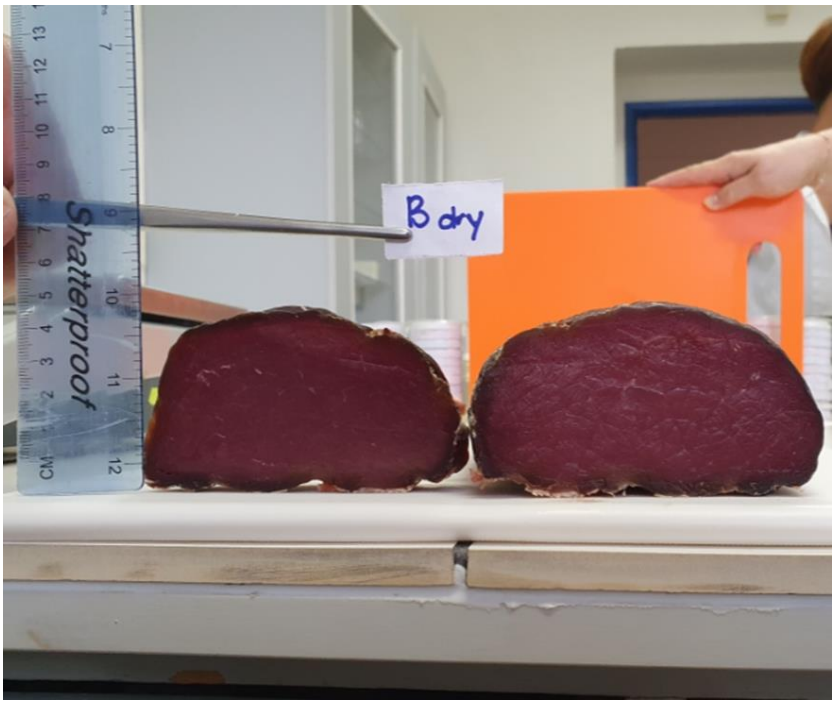
Chuť pečeného masa (roasted flavour)



Celková přijatelnost (overall acceptance)

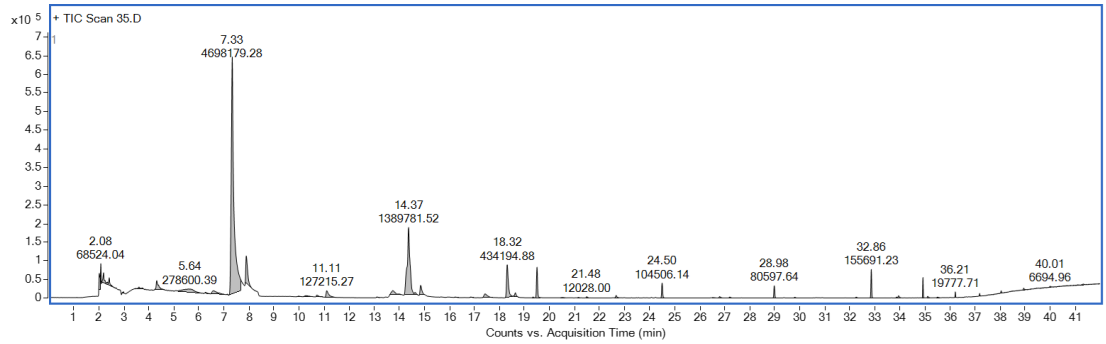


APPENDIX II: Images of LL muscle overall surface, wet surface, and crust penetration depth measured, using ImageJ software.

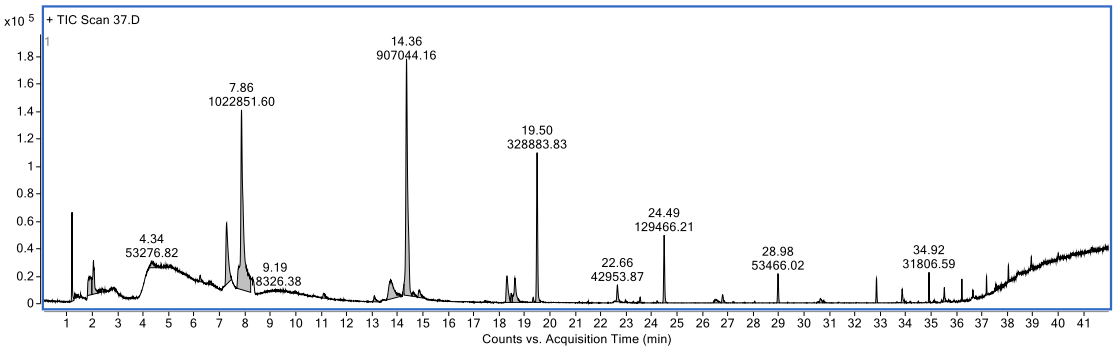


APPENDIX III: Examples of chromatograms from the GC-MS Analysis of eland *longissimus lumborum* meat volatiles.

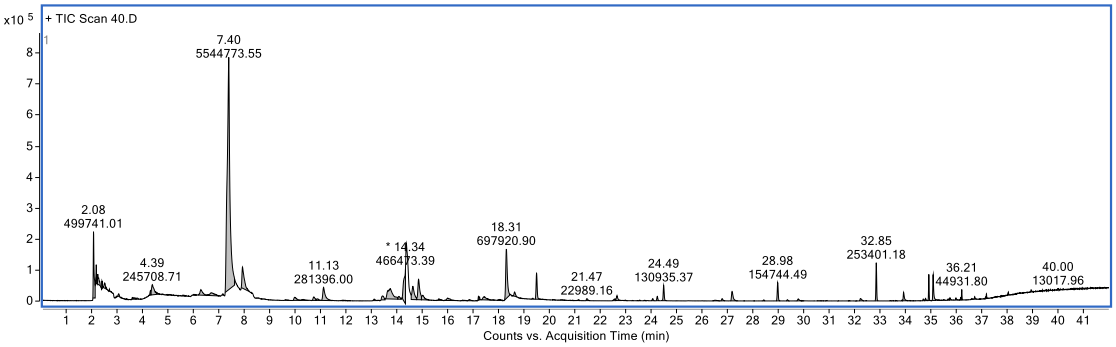
Animal C on D1 (Control)



Animal 231 D15 Wet Aged



Animal C D15 Dry Aged



Animal 231 D15 Wet Aged