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# The effects of exogenous factors and *post mortem* interventions to improve beef quality of Sudanese Baggara cattle


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BSc. (hons) Animal Production; MSc. Tropical Animal Production


Submitted in partial fulfilment of the requirements for the degree Ph.D. (Animal Science)


In the Department of Animal Science
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**Promotor: Professor E.C. Webb**

# SUMMARY

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This study investigated the effects of electrical stimulation (ES), age and weight at slaughter, breed type, post-freezing ageing, and the proteolytic extract of *Solanum dubium* (Gubbain) seeds on the carcass and meat quality of Sudanese Baggara cattle in Sudan. The study also assessed the microstructural and biochemical properties of Afrikaner x Bonsmara crossbred steer muscles treated with the Gubbain protease in South Africa. In order to achieve these goals, three trials were conducted. In the first trial, eighty Baggara cattle were selected according to breed type and age. The cattle (expermintal animals) were divided into two equal groups that were representative of the typical white Nyalawi breed type (n = 40) and typical red Mesairi breed type (n = 40). Each breed type was divided into two age groups: 20 bulls of about 5 to 5.5 years old, weighing between 280 and 310 kg; and 20 bulls of about 4 to 4.5 years old, weighing between 240 and 260 kg. The bulls were slaughtered and dressed according to the standard Halal method. Electrical stimulation (110 V for 30 seconds) was randomly applied to 50% of the carcasses in each group at 20 minutes *post mortem* (pm). The carcasses were stored in a chiller (2-4°C) within 45 minutes *post mortem*. Carcass pH and temperature were recorded at 0.17, 1, 3, 6, 9, 12, and 24 hours pm. Samples of *m. longissimus dorsi* (LD) were collected for meat analyses. Meat colour (L*, a*, b*, chroma, hue) was measured at 24 hours

pm, and then each sample was labelled and put in a plastic bag and frozen at -20°C until processing. The frozen samples were thawed and cut into two equal steaks. Half of the steaks were immediately analysed, while the rest were aged at 4°C for seven days and then analysed. The samples were analysed for instrumental colour, water-holding capacity (WHC), cooking loss (CL), Warner-Bratzler shear force (WBSF) values, and sensory quality. The second trial was for exploratory purposes, while the third trial was for detailed analyses. In the second trial, thirty LD were sampled at 24 hours pm from two age groups of Sudanese Baggara bulls. The ultimate pH and instrumental colour were determined at 24 hours pm. The beef LD samples were each cut into two steaks and randomised into two treatments injected with the Gubbain protease extract (10 per cent muscle weight) and left as a control. The steaks were incubated at 4$^{o}$C for 24 hours, after which they were analysed for pH, colour, CL, WBSF and sensory quality. The third trial was conducted on muscles from Afrikaner x Bonsmara crossbred steers to study the efficacy of the Gubbain protease extract injection in muscle tenderisation. Twelve *m. longissimus thoracis et lumborum* (LTL) steaks were sampled from both sides of several carcasses at 24 hours pm. Each steak was cut into two equal samples and then randomised for treatments (injection with Gubbain protease extract vs no injection). The steaks were incubated at 4$^{o}$C for 24 hours. They were then analysed for colour, sarcomere length (SL), myofibril fragment lengths (MFLs), WBSF, collagen solubility, muscle fibre types, quantification meat degradation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The results suggest that ES, animal age and weight affected meat quality attributes, while breed type had no significant effect. ES hastened the rate of pH decline significantly up to 24 hours pm. The ES of the Sudanese Baggara beef carcasses and the use of lighter and younger bulls showed higher L* values, lower a* values, higher hue values, and lower values for WBSF. The carcasses of the Mesairi breed type responded better to ES in terms of

tenderness than those of the Nyalawi breed type. The meat from electrically stimulated carcasses resulted in lower WHC with no effect on CL, compared with non-electrical stimulation (NES) carcasses. The WHC of the meat improved as age and weight at slaughter increased. No risk of cold shortening was observed in any of the treatment groups. Although post-freezing ageing showed a negative influence on meat colour stability, it resulted in lower shear force values, which suggest more tender beef. Panellists did not recognise any variations in meat sensory quality between the two age groups. In contrast, the differences in sensory tenderness and overall acceptability due to post mortem interventions (ES and enzymatic protease) were detected.

The meat injected with the Gubbain protease extract reduced the WBSF values by 62 to 65% compared with the non-injected samples. The Gubbain protease treatments increased b* values, cooking losses, and the sensory juiciness, tenderness, and overall acceptability of the beef. The injection with the protease extract showed better L* values compared with the control samples. Muscle samples treated with Gubbain protease had shorter MFLs (23.65 ± 3.22 vs 33.09 ± 2.33), probably due to the increased proteolytic activity, and also showed lower WBSF values (1.95 ± 0.70 vs 5.13 ± 1.01) than those samples from the control group. The muscles treated with the protease extract had a higher percentage of collagen solubility (36.30%) than the control muscles (18.40%). The muscle fibres from the treated samples with the Gubbain protease had more breaks and fractures than those from non-injected samples. The SDS-PAGE pattern of the LTL samples showed losses in the higher molecular weight fractions, accompanied by the appearance of many new lower molecular weight bands after treating the muscle with the Gubbain protease extract.

The study has shown that there is potential to improve the quality of beef of Sudanese Baggara cattle types if certain interventions (ES, slaughtering younger and lighter bulls, post-freezing ageing, and injection with Gubbain protease) are used. The study also paved the way for a novel and promising meat tenderiser for the beef industry.

# DECLARATION

I, Ahmed Dayain Abdalla Biraima declare that this thesis which I hereby submit for the degree Ph.D. (Animal Science) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed…….

Date…….

## Preface

This thesis has been prepared under the supervision of Prof. E.C. Webb in the Department of Animal and Wildlife Sciences, University of Pretoria. It consists of seven chapters that include an introduction, a literature review, and manuscripts published in or prepared for submission to peer-reviewed journals. Chapter One includes the general introduction, the study's purpose, the motivation for the study, and the hypotheses. Chapter Two reviews the literature on muscle and the meat quality of cattle as influenced by pre-slaughter factors (breed type, age, and weight of the animal) and *post mortem* interventions (ES, post-freezing ageing, and exogenous protease enzymes). Chapters Three, Four, Five, and Six are formatted according to the guidelines of the specific journals. Chapter Three is a scientific paper entitled "Effects of electrical stimulation and age at slaughter on carcass and meat quality of two Sudanese Baggara beef types", and was published in the *South African Journal of Animal Science*. In Chapter Four, a manuscript entitled "Influences of electrical stimulation, slaughter weight, and post-freezing ageing on beef quality and sensory attributes of Sudanese Baggara cattle" was written to be submitted to a journal. Chapter Five contains a manuscript prepared for submission to the *Meat Science* Elsevier journal entitled "Effects of age and protease extract of *Solanum dubium* seed on beef eating-quality of *longissimus* muscle from Sudanese Baggara cattle". Chapter Six also consists of a scientific manuscript entitled "Meat quality and the microstructural and biochemical properties of beef samples injected with protease extract from Gubbain (*Solanum dubium*) seed", which was prepared for submission to an Elsevier journal. The final chapter of the thesis contains the conclusion and recommendations of the research findings, and areas for further research.

The use of protease from the seeds of the Gubbain plant (*Solanum dubium*) as a meat tenderiser has been patented in Sudan. Some aspects of this research were peer-reviewed at the 50$^{th}$ South

African Society for Animal Science Congress (SASAS) and the 64th International Congress of Meat Science and Technology (ICoMST), and published as a research paper.

## Invention

A General Intellectual Property Registrar, Ministry of Justice, Sudan, has granted a patent for an invention entitled "Utilization of protease extracts from the *Solanum dubium* plant (Gubbain) as a meat tenderizer". The patent was granted to **Ahmed Dayain Abdalla Biraima** on 7 July 2019 under patent number 4114. It protects the commercial manufacture and use of proteases of the Gubbain plant as a meat tenderiser.

## Journal article

**Biraima, A.D.A**., Mohammed, A.M. & Webb, E.C., 2019. Effects of electrical stimulation and age at slaughter on carcass and meat quality of two Sudanese Baggara beef types. S. Afr. J. Anim. Sci. 49, 902-911. DOI: https://doi.org/10.4314/sajas.v49i5.14.

## Conference papers (full paper and presentation)

1. **Biraima, A.D.A.** & Webb, E.C., 2018. Tenderizing effects of protease extract from *Solanum dubium* (Gubbain) seed in *longissimus* muscle from Sudanese beef cattle. Paper presented at the 64th International Congress of Meat Science and Technology, Melbourne, Australia. Full paper available at: http://icomst-proceedings.helsinki.fi/index.php?year=2018.
2. **Biraima, A.D.A.**, Mohammed, A.M. & Webb, E.C., 2017. Effect of electrical stimulation on carcass and meat quality of different types of Sudanese Baggara cattle. 50th South African Society for Animal Science Congress, Port Elizabeth, Eastern Cape Province, South Africa. Abstract available at: https://www.sasas.co.za/wp-content/uploads/sites/14/2017/11/50th-SASAS-Congress-Book-of-Abstracts-Final_0.pdf.

# DEDICATION

*To my mother and father*

*To my loving wife and daughter; Yousra and Mayar*

*To my brothers, sisters, friends and fellow members*

# ACKNOWLEDGEMENTS

First, I thank the Almighty God for giving me the strength, patience and knowledge that enabled me to complete this project.

My utmost gratitude goes to my supervisor, Prof. E.C. Webb, for his support, encouragement, guidance and input throughout my studies.

I am indebted to the University of Pretoria (UP) and Ministry of Higher Education and Scientific Research, Sudan, for their financial support. I am also grateful to my employer University of Khartoum (UofK) for giving me a study leave to pursue my PhD degree.

I am extremely grateful to the Animal Production Research Centre (KUKU), Sudan for providing bulls and abattoir facilities. The support staffs at the Animal Production Research Centre in Sudan are acknowledged for their technical assistance.

I am also grateful to the staff of the Agricultural Research Council-Animal Production Institute (ARC-API), Irene, Gauteng, South Africa, for research facilities. The personnel at ARC-Meat Science Department are thanked for their technical assistance. I would also like to thank my PhD colleague Dr Phetogo Monau for her assistance and support during difficult times of my study period.

I am extremely grateful to my mother and father for their love, prayers, caring and sacrifices for educating and preparing me for my future. A special thanks and love goes to my wife Yousra and our daughter Mayar for their love, prayers, understanding, and continuing support to complete this thesis successfully.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **AMSA** | American Meat Science Association |
| **ARC-AP** | Agricultural Research Council- Animal production |
| **ATP** | Adenosine triphosphate |
| **$Ca^{2+}$** | Calcium ion |
| **CL** | Cooking loss |
| **DAFF** | Department of Agriculture, Forestry and Fisheries |
| **DeoxyMb** | Deoxymyoglobin |
| **ES** | Electrical stimulation |
| **FAO** | Food and Agriculture Organization |
| **GDP** | Gross domestic product |
| **GLM** | General linear models |
| **GRAS** | Generally regarded as safe |
| **HVES** | High-voltage electrical stimulation |
| **IFT** | Institute of Food Technologists |
| **LD** | *m. longissimus dorsi* |
| **LL** | *m. longissimus lumborum* |
| **LT** | *m. longissimus thoracis* |
| **LTL** | *m. longissimus thoracis et lumborum* |
| **LVES** | Low-voltage electrical stimulation |
| **MARFR** | Ministry of Animal Resources, Fisheries and Rangeland |
| **MetMb** | Metmyoglobin |
| **MFI** | Myofibrillar fragmentation index |
| **MFL** | Myofibril fragment length |

| | |
|---|---|
| **MSA** | Meat Standards Australia |
| **MW** | Molecular weight |
| **NES** | Non-electrical stimulation |
| **OxyMb** | Oxymyoglobin |
| **$pH_{24}$** | Ultimate pH |
| **$pH_{48}$** | pH at 48 h *post mortem* |
| **SD** | Standard deviation |
| **SDS-PAGE** | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| **SF** | Shear force |
| **SL** | Sarcomere length |
| **SM** | *m. semimembranosus* |
| **SPSS** | Statistical Package for the Social Sciences |
| **ST** | *m. semitendinosus* |
| **SUP** | *m. supraspinatus* |
| **TRB** | *m. triceps brachii* |
| **UP** | University of Pretoria |
| **V** | Volts |
| **VIA** | Video image analysis |
| **WBSF** | Warner-Bratzler shear force |
| **WFP** | World Food Programme |
| **WHC** | Water holding capacity |

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# CHAPTER 1

## 1.1 GENERAL INTRODUCTION

Sudan is highly dependent on animal agriculture, with large cattle, sheep, goat, and camel populations of about 108.77 million animals (Ministry of Animal Resources, Fisheries and Rangeland (MARFR), 2018). The agricultural sector contributes about 30% of Sudan's gross domestic product (GDP), and the animal agricultural subsector, in turn, contributes about 61% of the agricultural GDP (Food and Agriculture Organization & World Food Programme (FAO & WFP), 2019). There are about 31 million head of cattle in Sudan (MARFR, 2018). Of these, the Baggara breeds are the main source of beef for the local and export markets. The indigenous Sudanese Baggara cattle are mainly found in the Darfur and Kordofan regions. Nomadic Baggara tribes own these cattle in an extensive rangeland production system (Alsiddig *et al*., 2010).

The nomadic Baggara tribes are always in search of feed and water, and their cattle are exposed to stressors such as herding, high temperatures, and a shortage of feed and water, especially during summer (Rahman, 2007). These stressors may suppress the animals' growth. However, the nomadic tribes keep their livestock/cattle mainly as a store of wealth (Fahey & Leonard, 2008). The cattle are thus sold and slaughtered when they are older (≥ four years old). All of these factors can yield carcasses that vary markedly in quality compared with those from other developing countries. Indeed, the examination of practices such as electrical stimulation or the use of exogenous proteolytic enzymes as novel tools in Sudan could make a new and meaningful contribution to better beef quality management. The adoption of these tools by the Sudanese beef industry could quickly and permanently improve beef quality.

## 1.2 Project theme

Meat science, focusing on the use of the electrical stimulation of beef carcasses and the application of exogenous proteolytic enzymes to improve meat quality (beef).

## 1.3 Aim of study

The overall objective of this thesis is to determine the effects of the ES technique, age and weight at slaughter, breed type, post-freezing ageing, and the proteolytic enzyme of *Solanum dubium* seeds on Sudanese Baggara beef muscle quality.

## 1.4 Specific objectives

1. To determine the effects of electrical stimulation and age at slaughter on the carcass and meat quality of two Sudanese Baggara beef cattle types.
2. To determine the influences of electrical stimulation, age and weight at slaughter, and post-freezing ageing on the beef quality and sensory attributes of Sudanese Baggara cattle.
3. To investigate the effects of the protease extract of *Solanum dubium* seed and age on the eating quality of *longissimus* muscle from Sudanese Baggara cattle.
4. To study the meat quality and microstructural and biochemical properties of beef samples injected with protease extract from Gubbain (*Solanum dubium*) seed.

## 1.5 Motivation

The Sudanese beef industry is in a unique situation when compared with other developing countries. Its production of beef depends mainly on its extensive rangelands. The indigenous Baggara cattle types produce carcasses that vary markedly in quality. Cattle are sold and slaughtered at mature ages. Nor is any intervention (electrical stimulation [ES], enzymatic) used to manage beef quality. In Sudan, nomadic tribes mainly own the beef cattle in an extensive rangeland production system (Alsiddig *et al*., 2010). Those tribes always migrate in search of feed and water. As mentioned earlier, the animals are thus exposed to a

number of stressors that can depress the cattle's growth, and they are traded and slaughtered at relatively mature ages. All of these factors can affect the quality of the beef and, in particular, its tenderness. However, tenderness is considered one of the most important quality attributes of beef and is preferred by consumers (Shackelford *et al*., 2001), while colour is the main factor observed when purchasing the product (Mancini & Hunt, 2005). The cause of variation in beef quality is complex, and depends on several factors such as species, breed type, age, body weight, gender, nutrition, and pre- and post-slaughter handling (Guerrero, 2013).

Previous studies have shown that *post mortem* interventions such as electrical stimulation (Agbeniga & Webb, 2014; Polidori *et al*., 2016; Agbeniga & Webb, 2018), ageing (Kim *et al*., 2013; Kim & Kim, 2017), and the use of exogenous protease enzymes (Ashie *et al*., 2002; Bekhit *et al*., 2014; Zhang *et al*., 2019) can improve the quality of beef. However, the electrical stimulation technique has never been implemented in the slaughter process of Sudanese beef cattle. Therefore, this study investigated the effects of the electrical stimulation technique on Sudanese Baggara cattle types as a novel tool to manage beef quality in Sudan better.

The exogenous plant proteases such as papain, bromelain, ficin, actinidin, and zingibain play an essential role in the meat tenderisation process by degrading the meat's structures (Bekhit *et al*., 2014). *Solanum dubium*, a recognised wild plant in Sudan known locally as Gubbein, has the protease enzyme. Seeds from this plant contain Dubiumin serine protease, which has a high proteolytic activity (Ahmed *et al*., 2009a; Ahmed *et al*., 2009b) and is safe for humans (Mohamed *et al*., 2016). To date the tenderising effects of *Solanum dubium* proteolytic enzyme on muscles are unknown. As the first experimental approach, the ability of *Solanum dubium* protease extracts to tenderise beef muscle was studied. The current study has

also determined the effects of the *Solanum dubium* protease enzyme on the microstructural and biochemical properties of beef muscle. The results can pave the way for a promising and novel source of a commercial meat tenderiser to improve meat quality.

The calpains proteolytic system plays a pivotal role in meat tenderisation during ageing (Frylinck *et al*., 2015; Koohmaraie & Geesink, 2006). Its activity has been reported to be stable for several months when meat is stored at -20°C (Kristensen *et al*., 2006). Therefore, ageing after freezing might have the potential to improve meat tenderness by allowing more proteolysis. This study investigated the effects of post-freezing ageing on the quality of beef from Sudanese Baggara cattle. This study also evaluated the influences of some factors, such as slaughter age and weight, and the differences between the two main breed types of Sudanese Baggara cattle (Nyalawi and Mesairi), on the meat characteristics. The results could contribute to new management policies that would improve the quality of Sudanese beef. On the other hand, the evaluation of the meat palatability of different breed types is important in determining the potential value of alternative genetic resources for profitable beef production.

## 1.6 Hypotheses

The tested null hypotheses were:

H0: Electrical stimulation, age at slaughter, and breed type have similar effects on the carcass and meat quality of Sudanese Baggara cattle.

H0: Post-freezing ageing and slaughter weight have similar effects on the beef quality and sensory attributes of Sudanese Baggara cattle.

H0: Beef muscle samples treated with the protease extract of the *Solanum dubium* seed have similar meat quality and microstructural and biochemical characteristics to those of untreated beef muscle samples.

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# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Introduction

Sudan is endowed with a large population of cattle that is estimated at 31 million heads (Ministry of Animal Resources, Fisheries and Rangeland (MARFR), 2018). Indigenous Baggara cattle are the main beef animal in Sudan providing beef for the local and export markets. However, Sudan's beef production depends mainly on extensive rangeland production systems that expose animals to stressors such as herding, lack of feed and water, high temperatures, and transporting on the hoof from rural areas to the local livestock markets in the main cities (Rahman, 2007; Alsiddig *et al*., 2010). In these extensive production systems, the yield of carcasses varies greatly in quality compared with those from other developing countries.

In general, meat tenderness, colour, flavour, and juiciness have always been essential characteristics for meat consumers (Joo *et al*., 2013). The quality characteristics of meat are complex, since quality is greatly affected by many factors such as species, breed, gender, weight, age, diet, and pre-and post-slaughter handling (Guerrero *et al*., 2013). At *post mortem*, the quality of meat can be improved through three primary interventions: physical (e.g., electrical stimulation, mechanical tenderisation, ageing, freeze-thaw, and pressure treatments), chemical (e.g., injection, infusion, or marinating with sodium salts, calcium salts, and phosphate salts) and enzymatic (e.g., injection, infusion, or marinating with proteolytic exogenous enzymes) (Bekhit *et al*., 2014). This study focuses on the interventions of electrical stimulation, age at slaughter, breed type, post-freezing ageing, and injection with the proteolytic enzyme of *Solanum dubium* seeds to improve beef quality in Sudan.

## 2.2 Sudanese Baggara cattle

The indigenous Sudanese Baggara cattle belong to *Bos indicus* cattle, generally known as Zebu cattle, which represent the majority of cattle types in Africa (Omer *et al*., 2018). The nomadic Baggara tribes keep Baggara cattle in an extensive production system in which animals are herded on the open rangeland (Alsiddig *et al*., 2010). The name 'Baggara' was derived from an Arabic word meaning 'cattle herders'. The Baggara cattle are found in the grass acacia savannah belt between the western borders of Sudan and the White Nile (Alsiddig *et al*., 2010). The natural habitat of these cattle is characterised by seasonal variations in the quantity and quality of pasture, in addition to stressors such as high temperatures, diseases, and lack of water, particularly during summer (Rahman, 2007). Baggara keepers rear the animals mainly as a store of wealth, and they constantly migrate in search of water and feed (Fahey & Leonard, 2008). The cattle are thus sold and slaughtered at a relatively mature age. However, the indigenous Baggara cattle are a pillar of the Sudanese beef industry, since they are the main source of beef production for local and export markets (Alsiddig *et al*., 2010). They are often transported on the hoof for between 35 and 75 days to get them from the production areas to the terminal livestock markets around the main cities.

Baggara cattle breed types are identified and named as three breed types according to their ecological and geographic location and their tribal ownership – namely, Nyalawi, Mesairi, and Rizaigi. The primary colour of the Nyalawi breed type is white, while the main colour of the Mesairi and Rizaigi breed types is red (Alsiddig *et al*., 2010). The Nyalawi and Mesairi are the main breed types of Sudanese Baggara cattle (Omer *et al*., 2018). The Nyalawi breed type often had superior phenotypic beef characteristics (Alsiddig *et al*., 2010; Eltahir *et al*., 2018) and meat quality attributes (Biraima *et al*., 2014) over the Mesairi breed type.


**Figure 2.1 Nyalawi Baggara breed type (Eltahir *et al*., 2018).**

**Figure 2.2 Mesairi Baggara breed type (Eltahir *et al*., 2018).**

## 2.3 *Post mortem* changes and conversion of muscle to meat

Within the first 24 h *post mortem* (pm), several biochemical and physical changes occur in the muscles that result in the conversion of muscle to meat (Matarneh *et al*., 2017). When the animal dies, the skeletal muscles are still metabolically active and generating energy to maintain cellular homeostasis (Ferguson & Gerrard, 2014). As the oxygen disappears, anaerobic metabolism takes place to create energy via the conversion of glycogen to lactic acid. The lactic acid accumulates in the muscle, and it causes the muscle pH to drop gradually from around 7.2 in living tissue to 5.8 within 8 h pm, with an ultimate pH of approximately 5.6 reached at 24 h pm (Matarneh *et al*., 2017). The anaerobic metabolism generates less energy or Adenosine triphosphate (ATP) than aerobic metabolism (Engelking, 2015). Therefore, the

dissipation of energy is greater than its generation, which leads to muscle stiffness or rigor mortis (Latin for "stiffness of death") (Matarneh *et al*., 2017). During the *post mortem* period, as the anaerobic metabolism continues, the muscles gradually lose the ability to provide energy, and eventually the generation of energy or ATP fails (Warriss, 2000; Matarneh *et al*., 2017). When that happens, the muscle loses its extensibility due to the irreversible binding of actin filaments to myosin filaments, which results to the completion of rigor mortis (Warriss, 2000; Hwang *et al*., 2003). Figure 2.3 represents the relationship between the ATP concentrations and the onset of rigor mortis (Warriss, 2000). The proteolytic enzymes break down the integrity of the muscle structures during ageing and resolve the rigor mortis, resulting in more tender meat (Koohmaraie & Geesink, 2006). Before entering rigor, when the ATP level is high, the muscles' myofibrils are soft and relaxed (contract and relax), although the minimum shortening would occur if the muscles are exposed to temperatures between 14°C and 19°C (Locker & Hagyard, 1963). In contrast, muscles in the rigor stage are extremely tough because of shorter sarcomere and the formation of cross-bridges between actin and myosin molecules (Koohmaraie, 1996). However, meat post-rigor becomes more tender due to the action of released lysosomal protease enzymes on the degradation of myofibril proteins (Koohmaraie, 1996; Koohmaraie & Geesink, 2006).

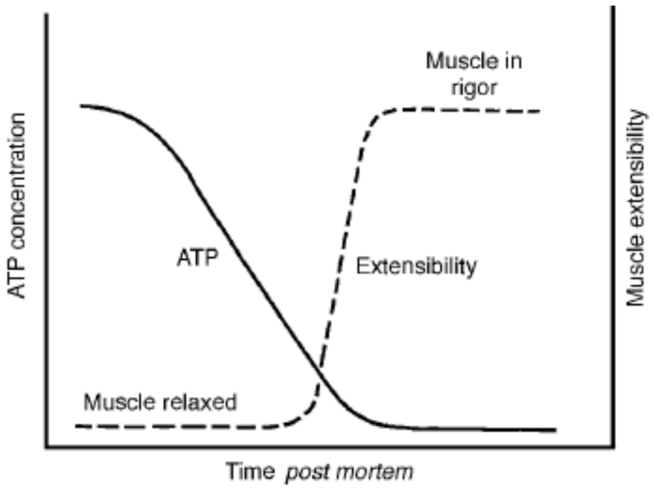


**Figure 2.3 The relationship between ATP concentration and the onset of rigor mortis (Warriss, 2000).**

The *post mortem* declines in carcass pH and temperature during rigor development are important for meat tenderness (Hannula & Puolanne, 2004). According to Pearson and Young (1989) and the review of Thompson (2002), cold shortening occurs if the muscle pH is above 6.0 with ATP still available for muscle contraction, and the muscle temperature falls below 12°C, while heat toughening occurs due to the combination of high temperatures (above 35°C) and low pH (below 6.0) in the muscle, causing the early exhaustion of proteolytic activity as a result of protease enzymes autolysis at high temperatures. According to the review of Thompson (2002), the temperature and pH relationship performed by the Meat Standards Australia (MSA) grading system is used to assess carcasses at risk of cold shortening (pH > 6 at temperature < 12°C) or heat shortening (pH < 6 at temperature > 35°C). Figure 2.4 illustrates the *post mortem* pH/temperature decline profiles implemented by the MSA (Thompson, 2002).

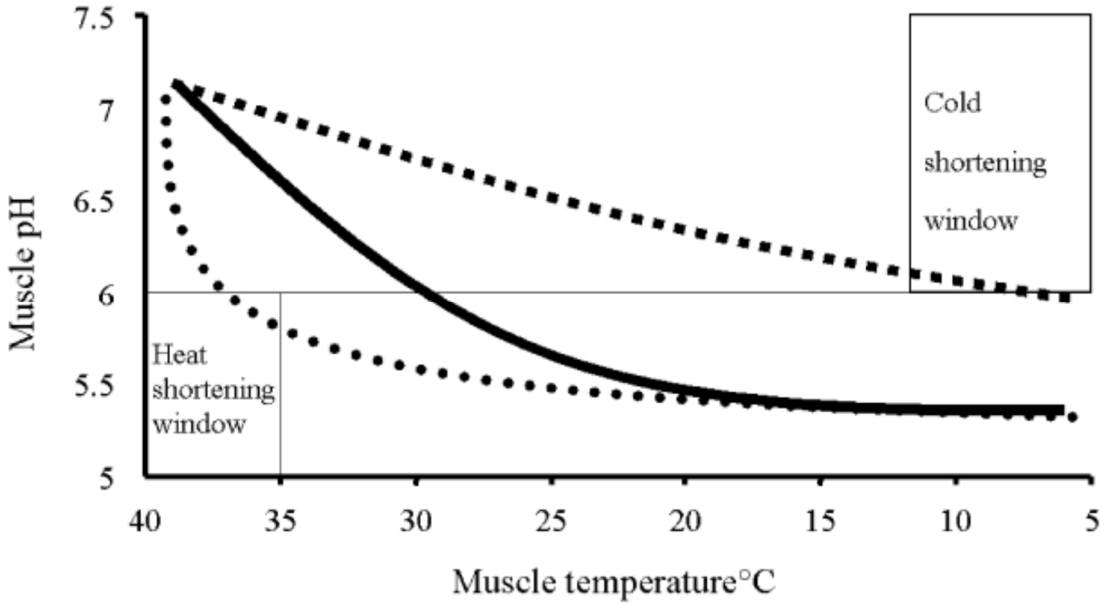


**Figure 2.4 The post mortem pH/temperature decline profiles used by the MSA to manage the drop in pH related to carcass temperature. The optimal rate of decline is indicated by the solid line, the cold shortening scenario by a dashed line, and the heat shortening scenario by a dotted line. Taken from Thompson (2002).**

The electrical stimulation (ES) of carcasses prevents the toughening of cold shortening by accelerating the onset of rigor mortis before the carcass temperature falls to values that induce cold shortening (Devine *et al*., 2014) – although, in certain situations such as overstimulation, the ES of carcasses can also cause muscle shortening or toughening at a high rigor temperature (Frylinck *et al*., 2015). This toughening is not only due to sarcomere shortening, but also due to early *post mortem* exhaustion of proteolytic activity (Kim *et al*., 2012). The toughening related to high rigor temperature probably happens in heavy beef carcasses (Geesink, 2017). The ES can also improve meat tenderness through the physical disruption of muscle structures or by accelerating the *post mortem* proteolysis (Hwang *et al*., 2003; Kadim *et al*., 2009). Other *post mortem* interventions such as ageing and using

exogenous protease enzymes could further enhance meat tenderness. Ageing allows more endogenous protease enzymes to increase the breakdown of muscle proteins (Kim & Kim, 2017). The use of exogenous protease enzymes is an additional way to hydrolyse meat proteins (Sullivan & Calkins, 2010). These *post mortem* interventions (electrical stimulation, ageing, and exogenous protease enzymes) could be used to manage beef quality in Sudan.

## 2.4 Meat quality attributes

According to consumers' perceptions, meat quality attributes can be categorised into three types: appearance and technological characteristics (e.g., colour, texture, purge or drip loss, and water holding capacity); eating quality (e.g., flavour, juiciness, and tenderness); and reliance quality traits (e.g., nutrition, safety, ethics, origin, and brand name) (Joo *et al*., 2013). The colour, tenderness, flavour, and juiciness of meat are regarded as critical attributes that affect the quality of beef and thus consumer satisfaction (Mancini & Hunt, 2005; Cho *et al*., 2010).

### 2.4.1 Meat colour

Meat colour is the first significant attribute that influences consumer decisions when purchasing meat (Mancini & Hunt, 2005). The colour of meat depends on the light-scattering properties of meat in addition to its myoglobin content and its chemical nature (Matarneh *et al*., 2017; Hughes *et al*., 2018). The three primary forms of myoglobin are deoxymyoglobin (DeoxyMb), metmyoglobin (MetMb), and oxymyoglobin (OxyMb). DeoxyMb refers to the colour of fresh-cut meat, which is dark purplish-red or purplish-pink. When meat is allowed to bloom (oxygen exposure) the DeoxyMb converts to OxyMb, giving a bright red or pink colour (Mancini & Hunt, 2005; Matarneh *et al*., 2017). Many factors are involved in the formation of MetMb from DeoxyMb or OxyMb, such as meat pH, temperature, reducing enzyme activities, and – in some cases – microbial growth (Mancini & Hunt, 2005). The iron in the DeoxyMb

and OxyMb forms is in the ferrous state ($Fe^{2+}$); when this is oxidised to ferric iron ($Fe^{3+}$) it becomes MetMb and gives meat an undesirable brown colour (discolouration) (Warriss, 2000; Mancini & Hunt, 2005). The visible inter-conversion of myoglobin redox forms on the surface of meat is summarised in Figure 2.5 (Mancini & Hunt, 2005).

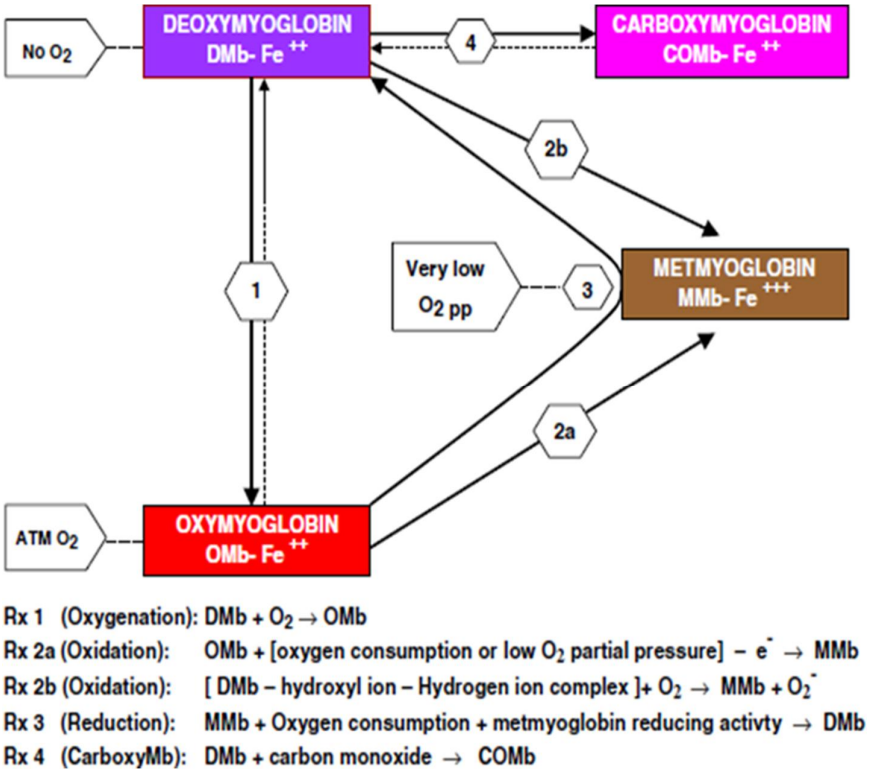


**Figure 2.5 Summary of the visible inter-conversion of myoglobin redox forms on the surface of meat (Mancini & Hunt, 2005).**

Several factors may influence the colour of meat, such as the ES of the carcass, the storage or ageing of the meat, and the animal's species, breed, and age and weight at slaughter, as discussed below.

### 2.4.2 Meat tenderness

Tenderness is the quality attribute most preferred by beef consumers, and inconsistent tenderness is an important issue in cattle (Bhat *et al*., 2018b). In general, the meat tenderisation

process is regarded as the controlled denaturation and proteolysis of muscle structure and protein that results in the weakening of the meat texture (Bekhit *et al*., 2017). The tenderness of meat is influenced by both pre-slaughter factors such as species, breed, nutrition, weight and age of the animal, and ante-mortem handling, and by *post mortem* factors such as ES, ageing, and *post mortem* conditions (Warner *et al*., 2017). During the *post mortem* period, beef tenderness can be significantly improved through three primary interventions – physical, chemical, and enzymatic – as mentioned above. The role of *post mortem* interventions (e.g., ES, post-freezing ageing, and exogenous protease enzymes) and pre-slaughter factors (e.g., breed, age, and weight) in meat tenderness will be discussed in this chapter.

### 2.4.3 Water-holding capacity and cooking loss

Water-holding capacity (WHC) is defined as the ability of meat and meat products to hold water during the application of external pressure (e.g., pressing, cutting, and grinding) as well during storage, transport, processing, and cooking (Hamm, 1986; Pearce *et al*., 2011). The visual characteristics of fresh meat depend largely on its WHC, which in turn affects the consumer's willingness to purchase the meat (Warner, 2017). Meat and meat products with poor WHC often lose weight due to the high losses of water as drip and purge, which may influence the cooking yield and the sensory traits of the final product (Warner, 2017). Most of the water in the muscle is found in the cell structures, involving the extra- and intra-myofibrillar spaces; therefore, the changing intracellular structure may affect the loss of water. When muscles shrink during rigor development, water is mobilised into extra-myofibrillar spaces where it is easily lost as a drip (Pearce *et al*., 2011). Rapid pH fall and high pre-rigor temperatures cause protein denaturation, which leads to poor WHC and higher drip loss (Kim *et al*., 2014).

Cooking loss (CL) refers to the decrease in the weight of meat and meat products during the cooking process (Honikel, 1998). Cooking is a heating process of meat at sufficiently high temperatures that leads to protein denaturation and makes it edible by the consumer (Garcia-Segovia *et al*., 2006). The denaturation of proteins results in structural muscle changes that lower the WHC of meat, leading to cooking losses (Honikel, 2009).

### 2.4.4 Sensory evaluation

The sensory evaluation of meat is a scientific approach that is used to evoke, measure, analyse, and interpret the reaction of humans (consumers and semi-trained or trained sensory panels) to meat sensory attributes (e.g., aroma, colour, flavour, juiciness, texture or tenderness, and overall acceptability) as perceived by the senses of sight, touch, smell, hearing, and taste (Institute of Food Technologists (IFT), 2007). The American Meat Science Association (AMSA, 2015) published "research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat" that provide detailed information for sensory evaluation. In the meat industry, sensory evaluation is critical, as it provides information and helps in developing the products economically by reducing risk in decisions related to product development and strategies to meet consumer demands (Sharif *et al*., 2017).

## 2.5 Factors affecting meat quality attributes

Meat quality attributes are very complex and are influenced both by pre-slaughter factors such as species, breed, sex, nutrition, weight and age of the animal, and *ante-mortem* handling, and by *post mortem* factors such as ES, ageing, and *post mortem* conditions (Guerrero *et al*., 2013; Bekhit *et al*., 2014; Warner *et al*., 2017). This section discusses the beef quality attributes influenced by pre-slaughter factors (breed and age and weight of the animal) and *post mortem* factors (ES, post-freezing ageing, and exogenous protease enzymes).

### 2.5.1 Breed

Cattle breeds or genotypes affect meat quality, and thus considerable attention is given to improving beef quality through genetic selection. As the cattle breeds differ in their carcass characteristics because of variations in animal physiology, meat probably varies in quality depending on the animal breed (Hocquette *et al*., 2005). It is well known that *Boss indicus* cattle produce tougher beef than do *Boss taurus* breeds (Whipple *et al*., 1990). The lower tenderness is associated with a lower rate of *post mortem* tenderisation due to higher calpastatin (calpain-inhibitor) activity in the muscles of *Boss indicus* cattle (Strydom, 2008). Modika *et al*. (2015) reported variations between cattle breeds (Angus, Brahman, Bonsmara, Charolais, and Nguni) in meat colour (visual and instrumental colour measurements) and tenderness. However, other researchers reported that breed does not have a major influence on beef quality characteristics (Du Plessis & Hoffman, 2007; Saccà *et al*., 2018).

In Sudan, the diversity of its agro-ecological zones, climate, and cultural conditions has resulted in different Baggara beef breed types (e.g., Nyalawi and Mesairi). The Nyalawi breed type often had superior phenotypic beef characteristics (Alsiddig *et al*., 2010; Eltahir *et al*., 2018) and meat quality traits (Biraima *et al*., 2014) over the Mesairi breed type.

### 2.5.2 Age and weight at slaughter

Animal age and slaughter weight affect carcass and beef quality characteristics by influencing the muscle structure properties and meat physiology (Sañudo *et al*., 2004). Age and weight at slaughter are often analysed together because the increase in animal weight depends on its age, except when nutrition is manipulated or the animal has been exposed to periods of restricted nutrition (Guerrero *et al*., 2013). Consumers prefer beef from younger and lighter animals because it is thought that younger animals produce good quality meat in term of tenderness, juiciness, and flavour than older, heavier animals (Vergara *et al*., 1999).

In general, beef from older animals tends to be a darker (lower L* values) and redder (higher a*) colour than meat from younger, lighter animals. The darker redder meat might be the result of a higher myoglobin content, since meat colour depends on the myoglobin content (Ponnampalam *et al*., 2017; Matarneh *et al*., 2017). Indeed, the concentration of muscle myoglobin increases as the animal ages (Humada *et al*., 2014). Many studies have shown that younger, lighter animals give beef with a brighter and better red colour relative to the meat obtained from older, heavier animals (Du Plessis & Hoffman, 2007; De Palo *et al.*, 2013; Marti *et al*., 2013; Czyzak-Runowska *et al*., 2017; Marenčić *et al*., 2018). However, other studies (Bureš & Bartoň, 2012; Kopuzlu *et al*., 2018) have reported that older animals could provide beef with a lighter colour than that from younger cattle. That may be due to the greater thickness of subcutaneous fat in an older animal, which is often positively correlated with meat lightness (Bruce *et al*., 2004), while Preziuso and Russo (2004) reported no influence of slaughter age on meat lightness. Du Plessis and Hoffman (2007) studied the effect of slaughter age (18 and 30 months of age) on the carcass and meat characteristics of beef. Their results showed no influence of slaughter age on cooking loss percentage and drip loss, while steers slaughtered at 18 months old yielded more tender meat than animals slaughtered at 30 months old. Studies by Czyzak-Runowska *et al*. (2017) also indicated that slaughter age does not affect the cooking loss percentage and WHC. Still, younger bulls produce beef with lower shear force (SF) values and higher scores for sensory evaluation traits.

### 2.5.3 Electrical stimulation of beef carcasses

The electrical stimulation (ES) technique is one of the major *post mortem* interventions in the meat industry. It involves applying an electrical current to animal carcasses, causing muscle contractions (Adeyemi & Sazili, 2014). According to Bekhit *et al*. (2017), the idea of passing electricity through muscles has been known since the 1600s, when Swammerdam

discovered that frog muscles contracted when electrically stimulated. In 1749 Benjamin Franklin found that the ES of a turkey muscle tenderised the meat (Lawrie & Ledward, 2006). However, significant experimental work with ES on carcasses started only in the 1950s (Bekhit *et al*., 2017). In the 1960s, the New Zealand meat industry implemented rapid and high freezing rates for carcasses intended for the export market for quicker preservation and better microbiological condition, as well as an effective processing system. The fast and high freezing process caused an extreme cold shortening, and yielded tougher meat (Bekhit *et al*., 2017). After that, the ES was investigated to prevent the toughening of cold shortening by accelerating the onset of rigor mortis before the carcass temperature fell to values that induced cold shortening (Petch, 2001). The ES was seriously commercialised in 1973 (Lawrie & Ledward, 2006), and it was first adopted in New Zealand and then in Australia, and has since become a standard process in abattoirs (Bekhit *et al*., 2017).

The ES technique has been widely used in the meat industry to improve the meat quality attributes of livestock and, in particular, its tenderness and colour (Nazli *et al*., 2010; Kahraman *et al*., 2011; Cetin *et al*., 2012; Mombeni *et al*., 2013; Agbeniga & Webb, 2014; Pouliot *et al*., 2014). The ES of carcasses improves the meat quality attributes through three mechanisms: (1) preventing the toughening of cold shortening by accelerating the onset of rigor mortis before the carcass temperature falls to values that induce cold shortening; (2) physical disruption of muscle structures; and (3) accelerating the *post mortem* proteolysis (Hwang *et al*., 2003; Kadim *et al*., 2009). However, many researchers have shown that ES may improve meat tenderness without the occurrence of cold shortening (Babiker & Lawrie, 1983; Hwang *et al*., 2003). The ES accelerates the *post mortem* proteolysis by allowing the endogenous protease enzymes to start earlier (early tenderisation process) at higher carcass temperatures, and results in more tender meat (Strydom *et al*., 2005; Simmons *et al*., 2008). The early combination of low carcass

pH and high temperature causes the denaturation of muscle protein structures and thus increases the light-scattering properties of meat, resulting in better meat quality (Kim *et al*., 2014; Hughes *et al*., 2018). However, the rapid fall of pH due to ES may result in a greater loss of water in beef with a poor WHC (Li *et al*., 2006; Strydom & Frylinck, 2014; Agbeniga & Webb, 2014), which can affect the cooking yield and the sensory traits of the final product (Warner, 2017).

As reviewed by Adeyemi and Sazili (2014), two main types of ES are studied in most of the literature: low-voltage electrical stimulation (LVES) (voltage less than 100 V), and high-voltage electrical stimulation (HVES) (voltage higher than 100 V). In general, LVES is used at about 10 to 20 min *post mortem*, while in cases of a long delay time between bleeding and stimulation (up to 60 min *post mortem*) HVES is applied. However, the HVES and LVES techniques produce effective stimulation with both advantages and disadvantages; the ideal method depends on the cost, animal type, existing slaughter floor, and throughput.

Table 2.1 summarises the effect of ES on beef quality characteristics, as reported in the literature. Obviously, the use of ES in the beef industry improves the quality attributes of meat such as tenderness, colour, and palatability; however, some researchers have reported negative impacts on some quality features such as drip loss, WHC, and cooking loss.

**Table 2.1 Summary of some reports that have assessed the effect of electrical stimulation on beef quality characteristics**

| Sample/breed | ES treatment | Outcomes | References |
|---|---|---|---|
| Meuse-Rhine-Ijssel breed bull | Immediately after bleeding (5-10 min *post mortem*)<br>ES: 85 V, 14 Hz for 60 sec<br>ES: 300V, 50Hz for 90 sec | pH: Lower pH up to 8 h *post mortem* in both ES groups than in control<br>Colour: Higher L*, a* and b* values in both ES groups than in control<br>Shear force: Significant lower SF values in both ES groups than in control<br>Sarcomere length: Significantly shorter in control than in ES groups<br>Sensory tenderness: Significantly higher scores in both ES groups than in control | Eikelenboom *et al*. (1985) |
| *M. longissimus thoracis* (LT) of cattle (steers and heifers) | At 45 min *post mortem*<br>ES: 470 V, 60 Hz for 60 sec | pH: Lower pH up to 6 days *post mortem*<br>Colour: Brighter and redder meat colour up to 6 days *post mortem*<br>Shear force: Significantly lower values<br>Consumer ratings (flavour, juiciness, tenderness, and overall palatability): Significantly higher | Aalhus *et al*. (1992) |
| *M. longissimus* of Chinese Yellow crossbred bulls | Immediately after bleeding<br>ES: 24 V, 50 Hz for 30 sec | pH: Faster pH decline up to 3 h *post mortem* in ES carcasses than in control<br>Temperature: No significant effect on carcass temperature decline<br>Water-holding capacity (WHC): ES muscle has a more significant loss in WHC than control muscle<br>Cooking loss: ES muscle has significantly higher cooking loss than control muscle<br>Shear force: Significantly lower | Li *et al*. (2006) |
| *M. longissimus dorsi* (LD) of Angus and/or Hereford steers | Immediately after bleeding<br>ES: 21 V, 60 Hz for 20 sec | pH: The carcass pH of ES group was significantly lower than the non-electrically stimulated group (NES) up to 3 h *post mortem*<br>Drip loss: No significant influence<br>Cooking loss: No significant influence<br>Sensory colour and firmness: No significant effect<br>Shear force: ES carcasses showed lower shear force values (P =0.06) throughout the 28-day ageing period than NES carcasses | Kim *et al*. (2007) |

**Table 2.1 (Continued) Summary of some reports that have assessed the effect of electrical stimulation on beef quality characteristics**

| Sample/breed | ES treatment | Outcomes | References |
|---|---|---|---|
| LT of Omani beef | At 20 min *post mortem*<br>ES: 90 V, 14 Hz for 60 sec | pH: Faster rate of pH decline up to 12 h *post mortem*<br>Colour: Increased lightness (L*) and no effect on redness (a*) and yellowness (b*)<br>Sarcomere length: Significantly shorter in NES carcasses than in ES carcasses<br>Myofibrillar fragmentation index (MFI): ES muscle showed a higher percentage in MFI than NES muscle<br>Expressed juice: No significant effect<br>Cooking loss: No significant effect<br>Shear force: Significantly lower | Kadim *et al*. (2009) |
| LD and *m. semimembranosus* (SM) of cattle (≥ 3 years old) | At 45 min *post mortem*<br>ES: 500 V, 50 Hz for 60, or 120 sec<br>ES: 800 V, 50 Hz for 60, or 120 sec | pH: All ES-treated carcasses showed a significantly lower pH at 24 h *post mortem* than NES carcasses<br>Colour: ES treatment did not influence L* values; ES groups showed lower a* values than NES cattle<br>Sensory parameters (appearance, colour, thickness, and flavour): ES groups have higher sensory scores than the NES group<br>Shear force: Significantly lower in ES groups than in the NES group<br>800 V treatments were superior to 500 V, and 120 sec was better than 60 sec | Nazli *et al*. (2010) |
| Holstein bulls | Immediately after bleeding<br>ES: 800 V for 25 sec | pH: Faster rate of pH decline up to 24 h *post mortem*<br>Colour: Good stability and better red colour at 7 days *post mortem*<br>Evaporative loss: ES increased the evaporative losses<br>Tenderness: ES improved the meat tenderness | Mombeni *et al*. (2013) |

**Table 2.1 (Continued) Summary of some reports that have assessed the effect of electrical stimulation on beef quality characteristics**

| Sample/breed | ES treatment | Outcomes | References |
|---|---|---|---|
| LD of beef steers | Shortly after slaughter (animals had lost about 90% of their blood)<br>ES: 810 V for 60 sec | pH: Significant increase in carcass pH decline due to ES up to 6 h *post mortem*<br>Temperature: No significant influence on carcass temperature decline<br>Drip loss: ES muscles have more significant losses than NES muscles<br>Cooking loss: ES muscles have more significant losses than NES muscles<br>Shear force: ES group showed lower shear force values than the NES group | Agbeniga & Webb (2014) |
| *M. longissimus lumborum* (LL) of Bonsmara steers | Immediately after bleeding<br>ES: 150 V, 17 Hz for 15, 45, or 90 sec | pH: ES carcasses have significantly lower pH than NES carcasses for up to 3 h *post mortem*<br>Colour: The 45 and 90 sec ES carcasses showed a brighter red colour at 2 days *post mortem*<br>Sarcomere length: NES muscles have shorter sarcomere lengths than 45 sec and 90 sec, while 15 sec has shorter sarcomere lengths than 90 sec<br>WHC: ES muscles have a more significant loss of water than NES muscles<br>Shear force: Significantly lower in ES muscles than in NES at 2 days *post mortem* (90 sec higher than 15 sec and 45 sec) | Strydom & Frylinck (2014) |

ES = electrical stimulation; NES = non-electrical stimulation

### 2.5.4 Post-freezing ageing

After the completion of rigor mortis, the endogenous proteolytic enzymes will start to tenderise the muscle and progress during ageing (storage) under certain conditions (Bekhit *et al*., 2017). During *post mortem* ageing, the lysosomal protease enzymes hydrolyse the structural proteins of the muscle, giving more tender meat (Koohmaraie & Geesink, 2006). It is believed that endogenous calpain, cathepsin, proteasome, and caspase protease enzymes contribute to the meat tenderisation process (Kemp & Parr, 2012). However, while the calpains proteolytic system is responsible for the main tenderisation process during ageing (Frylinck *et al*., 2015; Koohmaraie & Geesink, 2006), other protease enzymes have been suggested as also being involved in the process (Bekhit *et al.*, 2017). Kristensen *et al*. (2006) have shown that the calpain enzyme is stable for several months when meat is frozen at □20°C. Several studies have found that meat subjected to ageing after freezing shows an improved tenderness as a result of calpain activity (Whipple & Koohmaraie, 1992; Grayson *et al*., 2014; Kim & Kim, 2017).

Grayson *et al*. (2014) studied the tenderising effects of the post-freezing ageing process in beef *m. longissimus lumborum* (LL) and *m. semitendinosus* (ST). In both LL and ST, the ageing after freezing up to 12 and 14 days showed a higher rate of proteolysis, which resulted in lower shear force values compared with ageing up to two days prior to freezing. Studies by Kim and Kim (2017) reported that beef muscles aged after freezing (frozen at -28°C for two weeks, then thawed/aged at 2°C for three weeks) have significant losses through purge compared with muscles aged before freezing (aged up to three weeks at 2°C, frozen for two weeks, then thawed) and frozen/thawed only. Both aged muscle samples (prior and post-freezing ageing) showed significantly lower shear force values than just frozen samples. Aroeira *et al*. (2016) also indicated that post-freezing ageing intervention increases the

proteolysis, and may contribute to beef tenderness up to seven days of ageing, but it is dependent on the breed of animal. More recently, Aroeira *et al*. (2020) reported that beef *Longissimus thoracic* (LT) samples subjected to post-freezing ageing have higher proteolysis than conventionally (unfrozen) aged samples up to 14 days of ageing. From the literature mentioned above, it is clear that post-freezing ageing intervention may be useful in improving beef's tenderness, and so might be a further option in managing the beef industry in Sudan.

### 2.5.5 Exogenous proteases

In general, the endogenous proteolytic systems hydrolyse muscle proteins during ageing, and result in more tender meat, as already mentioned above. However, this process does not over-tenderise meat because the endogenous proteases are limited and undergo self-autolysis (Goll *et al*., 2003). As reviewed by Morton *et al*. (2019), the endogenous protease enzymes during the *post mortem* ageing do not hydrolyse the collagen protein. Therefore, the use of exogenous enzymes is needed to break down the connective tissue, resulting in more tender meat. Many researchers have shown that the use of exogenous proteolytic enzymes hydrolyses the muscle protein structures effectively and improves meat tenderness (Ashie *et al*., 2002; Ionescu *et al*., 2008; Han *et al*., 2009; Sullivan & Calkins, 2010; Liu *et al*., 2011; Istrati *et al*., 2012; Rawdkuen & Benjakul, 2012; Bekhit *et al*., 2014). Therefore, exogenous proteases derived from plants or microbes can be added to the muscles when additional tenderisation is needed (Bekhit *et al*., 2014; Arshad *et al*., 2016).

The use of exogenous proteases in food applications must be safe for human consumption. Therefore, the United States Department of Agriculture has approved many sources of proteases as 'generally regarded as safe' (GRAS). The five recognised GRAS proteases for use in the meat industry are papain, ficin, bromelain, Aspergillus oryzae protease,

and Bacillus subtilis protease (Sullivan & Calkins, 2010). Table 2.2 summarises the five proteases classified as GRAS (Sullivan & Calkins, 2010).

**Table 2.2 Summary of the proteases classified as generally regarded as safe (adapted from Sullivan & Calkins, 2010)**

| Enzyme | Type | Source | Protease class |
|---|---|---|---|
| Papain | Vegetable | Papaya | Cysteine |
| Bromelain | Vegetable | Pineapple | Cysteine |
| Ficin | Vegetable | Figs | Cysteine |
| Bacillus Protease | Bacterial | *Bacillus subtilis* | Serine |
| Aspartic Protease | Fungal | *Aspergillus oryzae* | Aspartic |

The exogenous proteolytic enzymes have the capability of hydrolysing myofibrillar and connective tissue (collagen) proteins; however, the rate of hydrolysis depends on many factors such as type of enzyme, pH, temperature, and ageing time (Arshad *et al*., 2016). Table 2.3 shows the pH, temperature, and strength of degradation of some proteases (Sullivan & Calkins, 2010).

Several researchers have reported other potential plant protease enzymes for improving tenderness, such as actinidin family cysteine proteases from kiwifruit (Liu *et al*., 2011), zingibain family cysteine proteases from ginger rhizome (Ha *et al*., 2013) and dubiumin family serine proteases from the Gubbein plant (*Solanum dubium*) (Ahmed *et al*., 2009a). Some exogenous protease enzymes often have a negative impact on meat quality; for example, papain may cause a mushy texture and off-flavours (Bhat *et al*., 2018a); also, high concentrations of some non-plant proteases may cause a bitter flavour (Qihe *et al*., 2006).

**Table 2.3 Summary of the pH, temperature, and strength of degradation of some protease enzymes (adapted from Sullivan & Calkins, 2010)**

| Enzyme | Active pH | Optimal pH | Active temperature | Optimal temperature | Hydrolysis of myofibrillar proteins | Hydrolysis of collagen |
|---|---|---|---|---|---|---|
| Papain | 4.0–9.0 | 4.0–6.0 | 50–80 | 65–75 | Excellent | Moderate |
| Bromelain | 4.0–7.0 | 5.0–6.0 | 50–80 | 65–75 | Moderate | Excellent |
| Ficin | 5.0–9.0 | 7.0 | 45–75 | 60–70 | Moderate | Excellent |
| Bacillus | 5.0–9.0 | 7.0 | 50–65 | 55–60 | Moderate | Poor |
| Aspartic | 2.5–7.0 | <6.5 | 40–60 | 55–60 | Poor | Excellent |

Gubbein (*Solanum dubium*) seeds contain dubiumin serine protease, which is a good source of an exogenous proteolytic enzyme for food applications (Ahmed *et al*., 2009a). The Gubbein plant is found in Sudan, and its seeds are safe for human consumption (Mohamed *et al*., 2016). The Gubbein protease enzyme is stable and active at a pH between 3.0 and 12 and temperatures between 20°C and 90°C (Ahmed *et al*., 2009a,b). Sudanese dairy farmers have long used seeds or proteolytic enzymes from this plant as a traditional method to coagulate milk for making cheese. The *Solanum dubium* protease enzyme has also traditionally been used to remove hair from animal hides (Ahmed *et al*., 2009b). However, research about this protease has concentrated mainly on its use in dairy technology (Yousif *et al*., 1996; Ahmed *et al*., 2009a,b; Talib *et al*., 2009; Abdalla *et al*., 2010; El-Owni *et al*., 2011; Kheir *et al*., 2011; Talib *et al*., 2011). A summary of beef characteristics affected by exogenous protease is presented in Table 2.4.

**Table 2.4 Summary of beef characteristics affected by exogenous proteases, as reported in the literature**

| Muscle sample | Treatment | Outcomes | References |
|---|---|---|---|
| Beef shoulder | Elastase from *Bacillus sp.* EL31410 | Treated muscles showed a remarkable decrease in hardness, higher scores for sensory tenderness than untreated muscles.<br>Myofibrillar structures were more substantially degraded at 48 h of ageing in elastase-treated samples than in untreated samples. | Qihe *et al*. (2006) |
| Beef muscles | Papain and bromelin proteases | Injection with the papain and bromelin protease enzymes showed a physical disruption of muscle and connective tissue, resulted in a high solubility of structural proteins and increased meat tenderness. | Ionescu *et al*. (2008) |
| *M. triceps brachii* (TRB) and *m. supraspinatus* (SUP) | Papain, bromelain, ficin, ginger rhizome, *Bacillus subtilis* protease, and two *Aspergillus oryzae* proteases | All treated samples showed a significant improvement in sensory and instrumental measures of tenderness, but the highest tenderness was observed with papain.<br>Bromelain hydrolysed connective tissue (collagen) more than myofibrillar proteins.<br>Ficin showed a more balanced hydrolysis of both contractile and connective tissue proteins.<br>Ginger showed more off-flavour than all other treatments.<br>Microbial proteases tended to break down myofibrillar proteins more than connective tissue, and resulted in good sensory scores. | Sullivan & Calkins (2010) |
| Beef thigh from adult cows (more than 9 years old). | Commercial papain and bromelain (2 mg protease injection /100 g meat) as well as natural sources of pineapple and papaya fruits | The use of proteases showed a significant improvement in meat tenderness. but had a negative impact on water-holding capacity. | Istrati *et al*. (2012) |

**Table 2.4 (Continued) Summary of beef characteristics affected by exogenous proteases, as reported in the literature**

| Muscle sample | Treatment | Outcomes | References |
|---|---|---|---|
| *M. longissimus dorsi* (LD) of beef | Purified actinidin enzyme from kiwifruit | Shear force decreased by 10% when LD muscles treated at 37 °C for 2 h.<br>Actinidin had a significant increase in nitrogen solubility index and WHC when muscles incubated with 0.9 unit enzyme/g muscle.<br>SDS-PAGE pattern showed the presence of many new low molecular weight bands (<10 kDa) when muscles treated with different levels of actinidin for 30 or 60 min. | Aminlari *et al*. (2009) |
| Beef connective tissue and topside myofibril extracts | Commercial papain,bromelain, actinidin, and zingibain protease | Highest degradation in myofibrillar beef proteins occurred with actinidin protease, while highest hydrolysis in meat connective tissue proteins occurred with zingibain protease. | Ha *et al*. (2012) |
| Beef connective tissue and topside myofibril extracts | Kiwifruit and asparagus enzyme extracts | Both protease extracts hydrolysed the beef myofibrillar and connective tissue proteins; however, the protease extract from kiwifruit appears to be most effective in hydrolysing myofibrillar and connective tissue proteins. | Ha *et al*. (2013) |
| Beef round cuts | Bromelain powder from pineapple crowns | Bromelain had an increase in hardness, WHC, moisture content, and a*value, and a decrease in pH, cooking loss, and L* and b*values. | Nadzirah *et al*. (2016) |
| *M. Longissimus lumborum* (LL) of bulls | Papain (0.1% enzyme solution) | Papain caused significant changes in the microstructure of muscle, resulting in a substantial decrease in shear force and textural parameters. | Barekat & Soltanizadeh (2017) |

## 2.6 Chapter summary

Beef production in Sudan depends on extensive production systems that produce carcasses that differ significantly in quality from those from other developing countries (Rahman, 2007; Alsiddig *et al*., 2010). Therefore, there is a critical need for research into interventions and strategies that may make a new and meaningful contribution to better beef quality management.

This chapter reviewed the literature on the muscle and meat quality of cattle as influenced by pre-slaughter factors (breed, age, and weight of the animal) and *post mortem* factors (ES, post-freezing ageing, and exogenous protease enzymes). Pre-slaughter factors such as the breed and age and weight of the animal are important determinants of beef quality differences (Guerrero *et al*., 2013). Therefore, the assessment of such factors may help to provide valuable information for producers to produce beef of a higher quality.

Although the application of the ES technique has been widely used in the meat industry to improve the quality of livestock meat, it is not yet used in Sudan. ES enhances the quality of beef through three mechanisms: (1) preventing the toughening of cold shortening by accelerating the onset of rigor mortis before the carcass temperature falls to values that induce cold shortening; (2) physical disruption of muscle structures; (3) and accelerating the *post mortem* proteolysis (Hwang *et al*., 2003; Kadim *et al*., 2009). However, many studies have reported that ES may improve meat tenderness without the occurrence of cold shortening (Babiker & Lawrie, 1983; Hwang *et al*., 2003). Several studies have reported that beef subjected to ageing after freezing showed an improvement in its tenderness because of calpain activity (Whipple & Koohmaraie, 1992; Grayson *et al*., 2014; Aroeira *et al*., 2016; Kim & Kim, 2017; Aroeira *et al*., 2020). However, the proteolysis during *post-mortem* ageing does not hydrolyse the collagen. Therefore, the use of exogenous enzymes is needed to break down the connective tissue (collagen) and result in more tender meat (Morton *et al*., 2019).

Several studies have shown that exogenous proteases improve beef tenderness by degrading both myofibrillar and connective tissue proteins (Ionescu *et al*., 2008; Sullivan & Calkins, 2010; Ha

*et al*., 2013; Barekat & Soltanizadeh, 2017). The protease extract from the seed of the Gubbein plant (*Solanum dubium*) would be a great potential source of exogenous protease for meat tenderisation. *Post mortem* interventions such as ES, post-freezing ageing, and exogenous proteases appeared to be effective at improving the quality of beef.

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# CHAPTER 3

## Effects of electrical stimulation and age at slaughter on carcass and meat quality of two Sudanese Baggara beef types

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## 3.1 Abstract

This study aimed to evaluate the influences of electrical stimulation (ES), age at slaughter, and breed type on muscle pH, the decline in carcass temperature, and meat quality attributes of Sudanese indigenous Baggara cattle. Eighty bulls from two types of Sudanese Baggara cattle – Nyalawi (n = 40) and Mesairi (n = 40) – were allocated to two age groups – namely, group 1 (240-260 kg) and group 2 (280-310 kg). Electrical stimulation was applied for 30 seconds at 20 minutes *post mortem* to 20 randomly selected carcasses from each breed type and compared with 20 carcasses from each type that were not electrically stimulated (NES). Samples of the *Longissimus dorsi* muscle were collected for meat analyses. Breed type showed no significant influence on meat quality characteristics, while ES and age at slaughter did. Electrical stimulation accelerated the carcass pH decline significantly up to 24 hours *post mortem*. Meat from electrically stimulated carcasses and younger animals resulted in higher L* values, lower a* values, higher hue values, and better tenderness. Older Mesairi animals had darker meat than their younger counterparts. Electrical

stimulation reduced water-holding capacity (WHC), although it had no influence on cooking loss (CL). Meat from older cattle showed better WHC compared with meat from younger animals. The ES treatment decreased the variations in meat tenderness between the younger and older bulls. It is concluded that the use of ES and younger bulls produced more tender meat with better colour. Therefore, these practices should be adopted in Sudan to ensure better beef quality management.

**Keywords:** Electrical stimulation, *Longissimus dorsi* muscle, meat characteristics, Mesairi, Nyalawi, Sudan

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## 3.2 Introduction

Sudanese indigenous Baggara cattle provide a major source for the domestic and export beef markets. They are generally owned by nomadic Baggara tribes, who always migrate in search of feed and water (Alsiddig *et al*., 2010). Baggara cattle are maintained on natural grassland, which is characterized by seasonal fluctuations in the quantity and quality of feed in addition to stresses from high environmental temperature and movement on hoof (Rahman, 2007). These cattle are often herded for 35 to 75 days to the local livestock markets, and are generally slaughtered at relatively mature ages (≥ 4 years old). All these factors could affect meat quality negatively, especially meat tenderness. In Sudan, castration of cattle is not practised for beef production, but it is used when the bulls are used for draft purposes.

Beef consumers regard tenderness as a very important quality characteristic (Miller *et al*., 2001; Verbeke *et al*., 2010), while colour is the main factor that is observed when purchasing the product (Mancini & Hunt, 2005). The cause of variation in meat quality attributes is complex and depends on factors such as species, breed type, age, bodyweight, gender, nutrition, and pre- and post-slaughter handling (Guerrero *et al.*, 2013).

Electrical stimulation of carcasses is one of the major practices that is used for improving meat quality (Adeyemi & Sazili, 2014). Electrical stimulation causes muscles to contract, resulting in a high anaerobic glycolytic rate, and hastens pH decline. This leads to early onset of rigor mortis before the carcass temperature drops to values that cause cold shortening and toughening (Simmons *et al*., 2008; Devine *et al*., 2014). Electrical stimulation provides an additional means of increasing tenderness through changes in muscle fibre structures either by physical disruption or by increasing the activity of protease enzymes (Hwang *et al*., 2003; Kadim *et al*., 2009). Electrical stimulation of carcasses has been used to improve tenderness and colour in beef (McKenna *et al*., 2003; Nazli *et al*., 2010; Mombeni *et al*., 2013; Agbeniga & Webb, 2014), lamb (Cetin *et al*., 2012; Pouliot *et al*., 2014), goat (Biswas *et al*., 2007; Cetin *et al*., 2012; Pophiwa *et al*., 2016), chicken (Kahraman *et al*., 2011), and pigs (Channon *et al*., 2003).

However, ES may have negative impacts on WHC in beef by hastening pH decline (Li *et al*., 2006; Agbeniga & Webb, 2018). The current focus of the meat industry is to produce a product of good quality and consistent supply. Currently, in Sudan, ES is not used at beef abattoirs. Nor is any technology employed at abattoirs to improve beef quality. Therefore, there is a need to investigate new techniques that may help to achieve a more desirable beef quality. The purpose of this study was to determine the effects of ES, age at slaughter, and breed type on muscle pH, the decline in carcass temperature, and meat quality attributes of Sudanese indigenous Baggara cattle.

## 3.3 Materials and methods

This research project was approved by the Animal Ethical Committee of the University of Pretoria, South Africa (approval number: EC076-17). The study was carried out at the Animal Production Research Centre (KUKU) in Sudan. A total of 80 Baggara cattle were selected according to breed type and age. They were divided into two equal groups that were representative of the typical white Nyalawi breed type (n = 40) and typical red Mesairi breed type (n = 40). Each breed type was divided into two age groups, namely 20 bulls of about 5–5.5 years old, weighing between 280 and

310 kg, and 20 bulls of about 4–4.5 years old, weighing between 240 and 260 kg. All animals were fed the same diet and slaughtered at the adjacent abattoir of the Animal Production Research Centre. Cattle were fasted and offered only ad libitum water for 12 hours before slaughter. Animals were slughtered and dressed according to the standard Halal method. This method of slaughtering lawful animals has several conditions to be fulfilled. A very sharp knife should be used to cut the throat and major blood vessels in the neck but leaving the spinal cord intact. During slaughtering itself, the specific phrase in the Arabic language "Bismillah, Allahu Akbar", which means "In the name of Allah, Allah is the greatest", should be pronounced by the slaughter-man, and the animal should be alive (Abdullah *et al*., 2011).

One half of the carcasses in each group were randomly selected and electrically stimulated for 30 seconds at 20 minutes *post mortem* using an electrical stimulator (110 V) (Jarvis, model ECS-1, South Africa). The ambient temperature of the abattoir was 25–30 °C. Carcasses were moved to a chiller (2–4 °C) at approximately 45 min *post mortem* and chilled for 24 hours before removing muscle samples. Muscle pH and temperature were recorded with a portable pH/temperature meter (Hanna Instruments, code- HI99163) that is specially designed for the meat processing industry. The values of muscle pH and temperature were measured at a point between the $10^{th}$ and $11^{th}$ ribs on the *Longissimus dorsi* muscle. They were measured at 10 min (0.17 hours), 3 hours, 6 hours, 9 hours, 12 hours, and 24 hours *post mortem*.

*Longissimus dorsi* muscle samples were cut between the $9^{th}$ and $12^{th}$ ribs of the left side of each carcass at 24 hours post slaughter for meat quality analysis. Muscle samples were left to bloom at 25 °C (room temperature) for 20 min. Meat colour was taken with a Hunter Lab ColorFlex EZ (Model 45/0 LAV, Hunter Laboratory Associates, Inc., Reston, Virginia, USA) using illuminant D65 at 10° standard observer to determine L* (lightness), a* (redness), and b* (yellowness) values. Chroma (intensity of the red colour/saturation index) and hue angle (meat discoloration) were

determined using these formulas: chroma = $(a^{*2}+b^{*2})^{1/2}$, and hue angle = $\tan^{-1}$ (b*/a*) (Hunt *et al*., 1991). The ColorFlex EZ was calibrated immediately before readings against black and white tiles according to the guidelines of the manufacturer. The mean of three random readings was used for statistical analysis. Each sample was labelled and put in a plastic bag and frozen at − 20 °C until processing.

The frozen beef samples were thawed at 4 °C for 36 hours. Water-holding capacity was determined following the procedure of Babiker & Lawrie (1983), which was based mainly on the evaluation of the amount of water pressed out of the meat under standard conditions. A 0.5 g minced meat sample was placed on humidified Whatman filter paper (No.4), stored in a desiccator over-saturated potassium chloride solution and pressed between two plexiglass plates at 25 kilograms (kg) weight for 2 minutes. The area of the meat was defined with a ballpoint pen, and the paper was dried. Meat and loose water areas (in $cm^2$) were determined with a planimeter to calculate the WHC ratio by the equation "WHC ratio = (loose water area – meat film area) /meat film area". A large WHC ratio indicates a high watery condition of the meat or a diminution in the WHC of muscle. For cooking loss (CL), each sample was weighed and put into a plastic bag and cooked in a water bath at 80 ºC, while the internal temperature of meat samples reached 70 ºC. The cooked samples were chilled overnight at 4 °C. The samples were later blotted dry and weighed. Cooking loss (CL) was calculated as a percentage of the initial weight by the equation "cooking loss (CL) % = ((weight loss after cooking)/ initial sample weight) x 100" (Honikel, 1998).

Beef tenderness was measured by mean of a shear force (SF) test, with a Warner-Bratzler instrument (G-R Elec. Mfg. Co. Manhattan, Kansas 606502). The test was done on the same samples used for cooking loss measurements. A preliminary investigation was done to decide the best thickness and enough length of the slice of the meat sample to be clamped and sheared by the instrument. Then, rectangular meat samples (cross-section, 1 x 1.5 cm), 10 cm long were removed

from the cooked muscles. The single peak force value expressed in kg required to cut the meat samples by shearing perpendicular to the muscle fibres was recorded (Szczesniak, 1963). A mean of 2 single peak force values per sample was recorded for statistical analysis.

Data were analysed as a 2 X 2 X 2 factorial design using SPSS 11.5 for Windows (2003, SPSS version 11.5, SPSS Inc., Chicago, IL, USA). The model used in the analyses of the dependent variables (pH, temperature, and meat quality parameters) contained the fixed effects of ES, age at slaughter, and breed type. Appropriate interactions were also estimated, although these were not significant in most cases. Main effect means were thus presented. The results of significant interactions are provided in the text where appropriate. Means of significant interaction were separated by Tukey's test at a 5% significance level using the Statistix 8.0 for Windows (Analytical Software, Tallahassee, FL, USA). Data were expressed as means ± standard deviation (SD).

### 3.4 Results and discussion

Breed type had no influence on meat quality traits ($P$ >0.05). Generally, the meat quality traits were not affected by the interaction of breed type × age at slaughter × ES. Electrical stimulation treatment hastened the carcass pH decline ($P$ <0.001) up to 24 hours *post mortem*. The stimulated carcasses had lower pH values at 1, 3, 6, 9, 12, and 24 hours post mortem compared with the NES carcasses (Figure 3.1). Mombeni *et al*. (2013) reported results that were similar to what was observed in the present study in terms of the effect of ES on the decline in beef carcass pH up to 24 hours *post mortem*. In other species, Cetin *et al*. (2012) found that the stimulated carcasses of lamb and goat with different levels of voltage (50, 100, and 250 V) resulted in a significant pH decrease up to 24 hours post mortem compared with non-stimulated ones. However, other studies reported the significant effect of ES on post-mortem pH decline up to 6 hours *post mortem* (Agbeniga & Webb, 2014; Polidori *et al*., 2016; Pophiwa *et al*., 2016; Agbeniga & Webb, 2018).

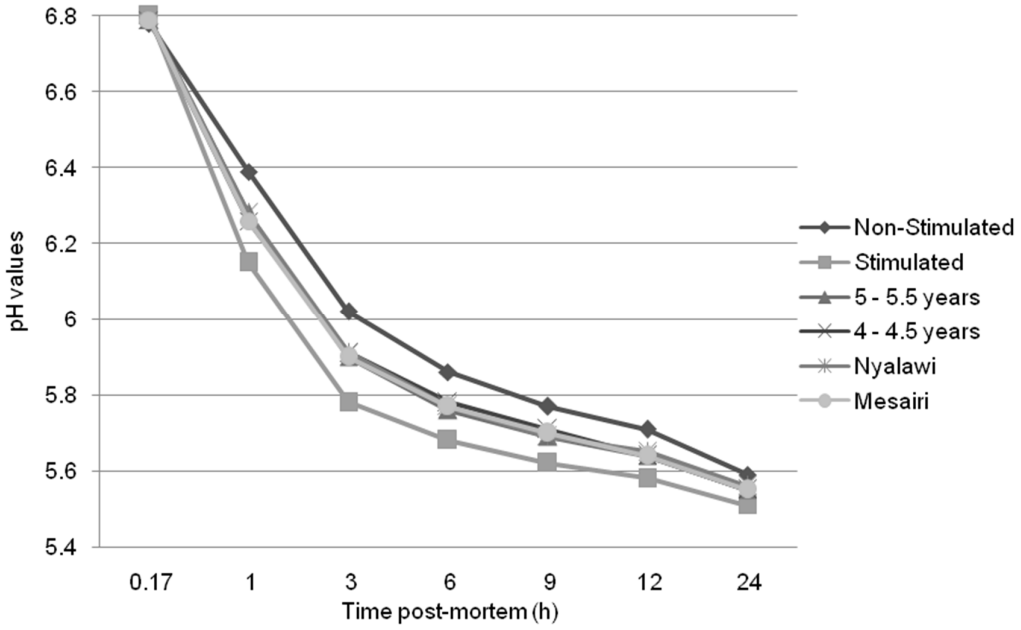


**Figure 3.1 Graphical illustration of the effects of electrical stimulation, age at slaughter, and breed type on pH decline of Sudanese beef carcasses**

The observed effects of ES on the decline in carcass pH up to 24 hours post mortem may be due to factors that influence the amount of glycogen stored before slaughter. In general, the degree of acidification of *post mortem* muscle depends on the muscle glycogen concentrations (Pösö & Puolanne, 2005). The results of O'Neill *et al*. (2018) indicate that a short fasting period before slaughter may increase the amount of available muscle glycogen. These authors also found that a short fasting period ante-mortem had the lowest *post mortem* muscle pH values for Brahman cattle at 2, 3, and 9 hours *post mortem* and Nguni cattle at 6, 9, and 24 hours *post mortem*. In this study, the feed was withdrawn for 12 hours before slaughter, which could result in high muscle glycogen concentration and explain the prolonging effects of ES on pH up to 24 hours *post mortem*.

The other possible reason for the extended effects of ES on muscle pH could be the lower stress during the ante-mortem period. It is well known that exposure of beef cattle to ante-mortem stress causes low muscle glycogen stores, which eventually results in meat with a high ultimate pH

(Njisane & Muchenje, 2017). Kadim *et al*. (2010) reported that ES affected the ultimate pH of the non-transported (less stressed) Dofari goat breed. The authors stated that within the non-transported group the stimulated carcasses had lower significant ultimate pH (5.61) at 24 hours post mortem than the non-stimulated ones (5.75), while the pH at 24 hours in the transported (more stressed) groups was not affected ($P$ >0.05) by ES. The animals in the present study were not exposed to transportation stress and few other stressors because the feedlot is on the same premises as the abattoir. Neither breed type nor age at slaughter had any influence on muscle pH decline ($P$ >0.05) (Figure 3.1). Similar findings were reported by Schutt *et al*. (2009), Kadim *et al*. (2009; 2010), and Czyżak-Runowska *et al*. (2017).

The present study indicates that ES (110 V) had no significant effect on the carcass temperature decline of Sudanese indigenous Baggara bulls. At 1 hour *post mortem*, the average temperature value of stimulated carcasses was slightly higher (32.43 ± 1.64) than NES ones (31.89 ± 1.61), but not significant ($P$ >0.05) (Table 3.1). Similarity, Li *et al*. (2006) reported that the rate of carcass temperature decline was not affected by low voltage electrical stimulation in Chinese Yellow crossbred bulls. Agbeniga & Webb (2014) also reported that carcass temperature decline was not influenced by ES. Breed type did not influence the rates of carcass temperature decline ($P$ >0.05), while age at slaughter was significantly affected by the carcass temperature decline (Table 3.1). Carcasses from animals of 4–4.5 years old that weighed 240–260 kg chilled faster than those from animals of 5–5.5 years old that weighed 280 to 310 kg, and the subsequent carcass temperature values were significantly different at 3 hours, 12 hours, and 24 hours *post mortem* (Table 3.1). Obviously, the carcasses with lower weights and from younger bulls contributed to its faster chilling rate.

**Table 3.1 Effects of electrical stimulation, age at slaughter, and breed type on muscle temperature decline in beef carcasses (Mean ± SD)**

| | Electrical stimulation | | Age at slaughter (Years) | | Breed type | |
|---|---|---|---|---|---|---|
| **Time** | **NES** | **ES** | **5 - 5.5** | **4 - 4.5** | **Nyalawi** | **Mesairi** |
| 10 min | 37.83 ± 0.54 | 37.86 ± 0.47 | 37.92 ± 0.45 | 37.77 ± 0.55 | 37.80 ± 0. 47 | 37.90 ± 0.53 |
| 1 h | 31.89 ± 1.61 | 32.43 ± 1.64 | 32.08 ±1.43 | 32.23 ±1.84 | 32.28 ±1.68 | 32.03 ± 1.61 |
| 3 h | 20.81 ± 1.91 | 20.82 ± 1.63 | **21.40$^{a}$ ± 1.75** | **20.23$^{b}$ ± 1.60** | 20.96 ± 1.76 | 20.67 ± 1.78 |
| 6 h | 11.57 ± 1.14 | 11.66 ± 1.21 | 11.80 ± 0.97 | 11.43 ± 1.33 | 11.75 ± 1.35 | 11.48 ± 0.96 |
| 9 h | 7.60 ± 1.00 | 7.65 ± 1.06 | 7.70 ± 0.94 | 7.55 ± 1.11 | 7.65 ± 1.11 | 7.59 ± 0.95 |
| 12 h | 5.49 ± 0.80 | 5.71 ± 0.91 | **5.92$^{a}$ ± 0.84** | **5.28$^{b}$ ± 0.76** | 5.53 ± 0.97 | 5.68 ± 0.74 |
| 24 h | 3.84 ± 0.39 | 3.95 ± 0.39 | **4.06$^{a}$ ± 0.29** | **3.72$^{b}$ ± 0.41** | 3.92 ± 0.44 | 3.87 ± 0.34 |

[a,b] Row means with different superscripts within electrical stimulation, age at slaughter, and breed type differ significantly ($P$ <0.05)

ES = electrical stimulation; NES = non-electrical stimulation

In terms of meat colour, the ES group had a higher ($P$ <0.001) mean value in lightness (L* = 44.61 ± 1.82) and a lower ($P$ <0.001) mean value in redness (a* = 13.74 ± 1.02) compared with the NES group (L* = 41.61 ± 1.46; a* = 14.65 ± 0.88). However, no effect ($P$ <0.05) on meat yellowness (b*) was detected (Figure 3.2). This shows that electrical stimulation improves meat colour by producing a brighter and better red colour. Similarly, the reports of King *et al*. (2004) and Nazli *et al*. (2010) confirmed that ES-treated beef carcasses had a brighter red colour than untreated ones. Carcasses from the ES group had lower ($P$ <0.05) chroma values (chroma = 20.04 ± 1.01) and higher ($P$ <0.001) hue values (hue = 46.70 ± 2.39) compared with the NES group (chroma = 20.59 ± 1.14; hue = 44.61 ± 1.72) (Figure 3.2). Low muscle pH values early *post mortem* combined with high temperature may explain the improvements in meat colour through increased protein denaturation and consequently increased light scattering properties of meat (Kim *et al*., 2014; Hughes *et al*., 2018).

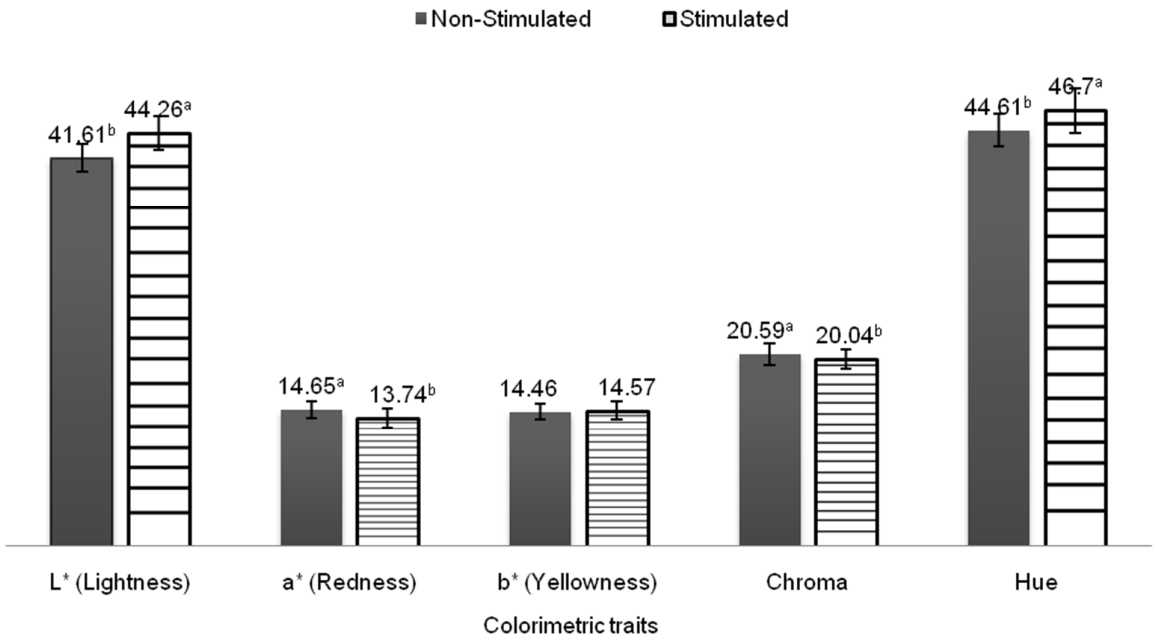


**Figure 3.2 Colorimetric traits of Baggara beef muscle samples between electrical stimulation groups (stimulated vs. non-stimulated)**

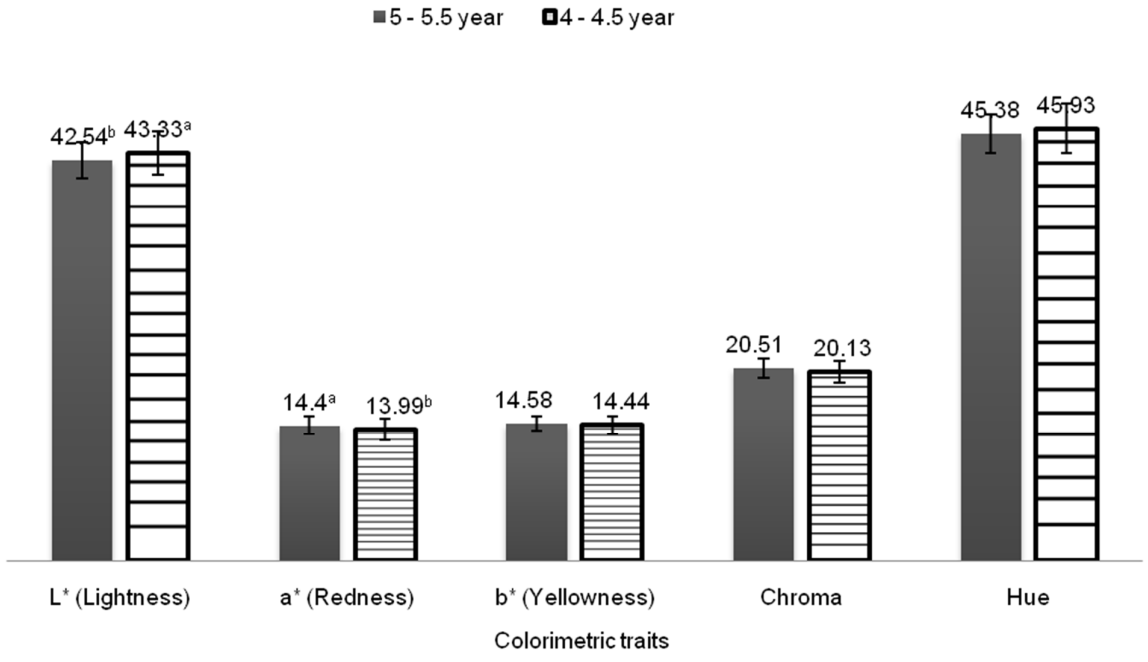


**Figure 3.3 Colorimetric traits of Baggara beef muscle samples between age groups (5 - 5.5 vs. 4 - 4.5)**

The age group had no influence ($P$ >0.05) on chroma and hue values (Figure 3.3). Breed type showed no influence on any of the meat colour traits ($P$ >0.05) (Figure 3.4). However, bulls slaughtered at 4–4.5 years showed the higher ($P$ <0.05) mean value of lightness (L*) and the lower ($P$ <0.05) mean value of redness (a*) compared with animals slaughtered at 5–5.5 years (Figure 3.3). The observed variations in the L* could be due to the differences in myoglobin content, since dark meat is associated with increased myoglobin concentration (Lawrie & Ledward, 2006). In general, the myoglobin concentration increases as the animal ages (Humada *et al*., 2014). With this increase, the meat from older animals tends to be higher in a* value (redness) and lower in L* value (lightness) (Ponnampalam *et al*., 2017). Furthermore, younger animals produce meat with lower a* and chroma values as a result of lower haematin concentration (Vestergaard *et al*., 2000; Gil *et al*., 2001; De Palo *et al*., 2012).

There were only two significant interactions observed for meat colour parameters (Table 3.2). The first interaction ($P$ <0.05) was between breed type and age at slaughter for the meat lightness (L*) parameter. Older Mesairi animals had the lowest L* value (darker) compared with their younger counterparts. The second interaction ($P$ <0.05) was found between breed, age at slaughter and electrical stimulation for hue. Mesairi bulls slaughtered at younger ages and subjected to electrical stimulation tended to have higher hue-angle values (47.95 ± 2.69) than Mesairi bulls slaughtered at older ages and subjected to electrical stimulation (45.19 ± 1.53).

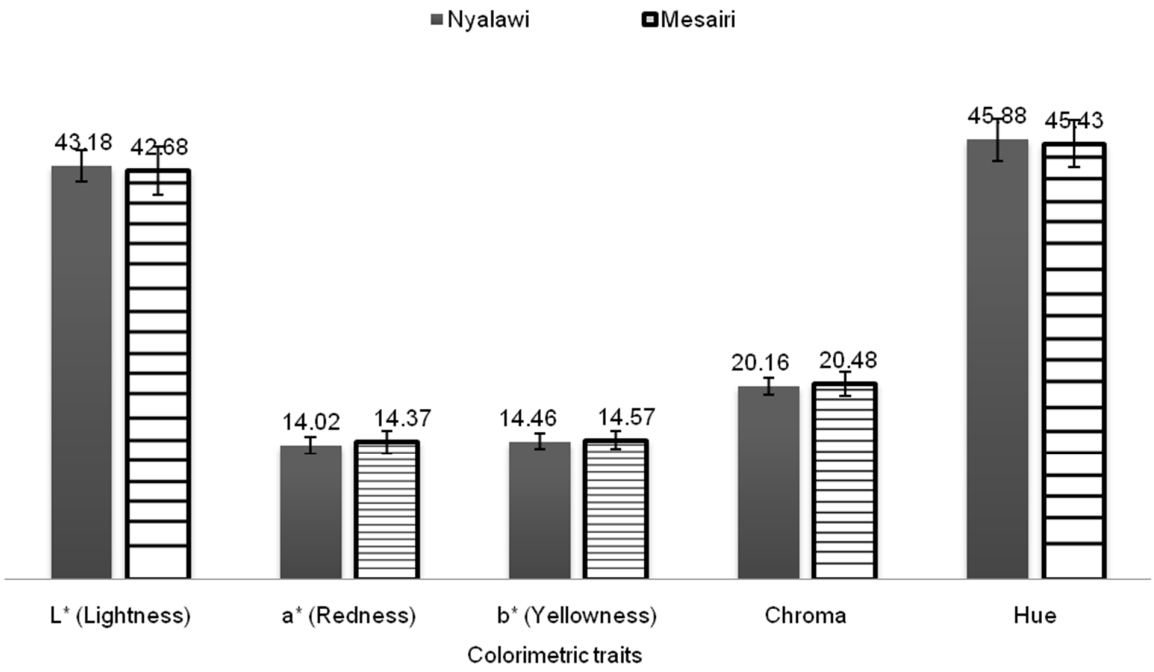


**Figure 3.4 Colorimetric traits of Baggara beef muscle samples between breed type groups (Nyalawi vs. Mesairi breed types)**

Water-holding capacity was affected by ES and age at slaughter (Table 3.3). Electrical stimulation diminished ($P$ <0.01) WHC, but had no effect on cooking losses ($P$ >0.05). A low carcass pH combined with high-temperature causes myosin denaturation, which contributes to lower WHC and higher drip loss (Kim *et al*., 2014). In this study, ES carcasses reached a low pH value (5.77), whereas the carcass temperature was still high (21.40 °C). This observation agrees with the reports of Li *et al*. (2006) and Agbeniga & Webb (2018), who found that ES of beef carcasses resulted in a rapid carcass pH fall with a reduction in the WHC. The water-holding capacity of the muscles from older animals was better ($P$ <0.01) than the muscles of the younger group. These findings might be due to a diminution in muscle water content with increasing animal age.

**Table 3.2 Effects of electrical stimulation, age at slaughter, and breed type on colorimetric traits of Sudanese beef muscle samples (Mean ± SD)**

| Treatments | | | Colorimetric traits | | | | |
|---|---|---|---|---|---|---|---|
| **Breed type** | **Age at slaughter** | **Electrical stimulation** | **L* (Lightness)** | **a* (Redness)** | **b* (Yellowness)** | **Chroma** | **Hue** |
| Nyalawi | 5 - 5.5 years | NES | $41.95^{d} \pm 1.19$ | $14.68^{ab} \pm 0.37$ | $14.58 \pm 0.65$ | $20.69 \pm 0.45$ | $44.78^{bc} \pm 1.63$ |
| | | ES | $44.45^{ab} \pm 0.95$ | $13.80^{ab} \pm 1.01$ | $14.84 \pm 0.67$ | $20.27 \pm 1.03$ | $47.11^{ab} \pm 1.78$ |
| | 4 - 4.5 years | NES | $42.14^{cd} \pm 1.17$ | $14.18^{ab} \pm 0.48$ | $14.24 \pm 1.12$ | $20.10 \pm 1.05$ | $45.06^{bc} \pm 2.05$ |
| | | ES | $44.18^{abc} \pm 1.41$ | $13.44^{b} \pm 0.91$ | $14.18 \pm 0.72$ | $19.56 \pm 0.67$ | $46.57^{abc} \pm 2.74$ |
| Mesairi | 5 - 5.5 years | NES | $40.89^{d} \pm 1.66$ | $14.83^{a} \pm 1.07$ | $14.54 \pm 1.06$ | $20.78 \pm 1.31$ | $44.43^{bc} \pm 2.11$ |
| | | ES | $42.85^{bcd} \pm 1.73$ | $14.29^{ab} \pm 0.97$ | $14.38 \pm 0.84$ | $20.28 \pm 1.16$ | $45.19^{abc} \pm 1.53$ |
| | 4 - 4.5 years | NES | $41.45^{d} \pm 1.64$ | $14.93^{a} \pm 1.23$ | $14.48 \pm 1.00$ | $20.80 \pm 1.49$ | $44.15^{c} \pm 1.49$ |
| | | ES | $45.54^{a} \pm 2.07$ | $13.42^{b} \pm 1.09$ | $14.87 \pm 0.93$ | $20.05 \pm 1.08$ | $47.95^{a} \pm 2.69$ |
| **ANOVA** | | | ***P* value** | ***P* value** | ***P* value** | ***P* value** | ***P* value** |
| Breed type | | | 0.145 | 0.102 | 0.591 | 0.184 | 0.320 |
| Age at slaughter | | | **< 0.05** | **< 0.05** | 0.484 | 0.120 | 0.222 |
| Electrical stimulation | | | **< 0.001** | **< 0.001** | 0.588 | **< 0.05** | **< 0.001** |
| Breed type x Age at slaughter | | | **< 0.05** | 0.914 | 0.079 | 0.259 | 0.133 |
| Breed type x Electrical stimulation | | | 0.279 | 0.615 | 0.971 | 0.772 | 0.691 |
| Age at slaughter x Electrical stimulation | | | 0.223 | 0.319 | 0.772 | 0.694 | 0.220 |
| Breed type x Age at slaughter x Electrical stimulation | | | 0.058 | 0.189 | 0.279 | 0.894 | **< 0.05** |

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

ES = electrical stimulation; NES = non-electrical stimulation

There was an interaction ($P$ <0.05) between age at slaughter and ES. Meat samples from the electrically stimulated carcasses in the older group had a better ($P$ <0.05) WHC (2.70) than meat samples from other groups, followed by ES carcasses in the older group (3.13), NES carcasses in the younger group (3.15) and ES carcasses in the younger group (3.23).

**Table 3.3 Effects of electrical stimulation, age at slaughter and breed type on water-holding capacity, cooking loss, and shear force of Sudanese beef muscle samples (Mean ± SD)**

| Treatments | Parameters | | |
|---|---|---|---|
| | WHC ratio | CL % | SF (kg/1.5 cm$^2$) |
| Electrical stimulation | | | |
| NES | **2.92$^a$ ± 0.43** | 21.72 ± 3.10 | **7.40$^a$ ± 0.81** |
| ES | **3.18$^b$ ± 0.34** | 22.26 ± 2.85 | **5.83$^b$ ± 0.66** |
| Age at slaughter | | | |
| 5 - 5.5 years | **2.91$^a$ ± 0.40** | 21.67 ± 2.93 | **6.99$^a$ ± 1.13** |
| 4 - 4.5 years | **3.19$^b$ ± 0.37** | 22.30 ± 3.09 | **6.24$^b$ ± 0.88** |
| Breed type | | | |
| Nyalawi | 3.01 ± 0.41 | 21.42 ± 2.74 | 6.51 ± 0.91 |
| Mesairi | 3.09 ± 0.41 | 22.56 ± 3.10 | 6.72 ± 1.22 |

[a,b]Column means with different superscripts within electrical stimulation, age at slaughter, and breed type differ significantly ($P$ <0.05)

ES = electrical stimulation; NES = non-electrical stimulation

In terms of cooking loss (CL), the main factors (ES, age at slaughter, and breed type) did not influence the percentage of CL (Table 3.3). However, meat samples from the ES group lost more water as CL (22.26 %) than the NES group (21.72 %), which may be attributed to the variations in WHC and ultimate pH between the two ES groups. Previous studies reported significant losses regarding cooking loss between the ES and NES carcasses (Li *et al*., 2006; Agbeniga & Webb, 2014). Younger bulls also lost more water as CL (22.30 %) than the older animals (21.67%). High moisture content and lower fat content in meat samples from less mature bulls may result in decreasing water binding ability in younger cattle. Likewise, meat samples from the Mesairi-type bulls lost more water as CL (22.56 %) than those from the Nyalawi type (21.42 %).

Concerning shear force (SF), as expected, the meat tenderness was affected by ES and age at slaughter (Table 3.3). The ES carcasses had lower ($P$ <0.001) SF values (5.83 ± 0.66 kg/1.5 $cm^2$) than those from NES carcasses (7.40 ± 0.81 kg/1.5 $cm^2$). As reviewed by Bekhit *et al*. (2014) and Huang *et al*. (2016), ES accelerates carcass pH decline, causing early activation of protease enzymes and protein degradation, which increases the meat tenderization process. The observed effect of ES on SF agrees with previous works (Li *et al*., 2006; McKenna *et al*., 2003; Nazli *et al*., 2010; Mombeni *et al*., 2013; Agbeniga & Webb, 2014). As anticipated, the SF values were affected significantly by age at slaughter (Table 3.3). Meat from younger animals showed lower ($P$ <0.001) SF values (6.24 ± 0.88 kg/1.5 $cm^2$) than those from older animals (6.99 ± 1.13 kg/1.5 $cm^2$). However, breed type had no influence on SF.

A second-order interaction ($P$ <0.05) was observed between age at slaughter and ES on SF. In the NES carcasses, the younger group had lower ($P$ <0.05) SF values (6.88 ± 0.67 kg/1.5 $cm^2$) than the older group (7.91 ± 0.59 kg/1.5 $cm^2$). However, in the ES carcasses, there were no differences ($P$ >0.05) between the younger and older bulls in terms of SF values, but younger bulls still had lower values (5.60 ± 0.54 kg/1.5 $cm^2$) compared with older ones (6.06 ± 0.69 kg/1.5 $cm^2$). Therefore, this study showed that ES reduced the differences in SF values between the younger and older carcasses. There was also a two-way interaction ($P$ <0.05) between breed type and ES for SF values. Regardless of the factors of ES and age at slaughter, the Nyalawi type cattle had a lower ($P$ <0.05) SF value (7.10 ± 0.67 kg/1.5 $cm^2$), compared with the Mesairi type (7.69 ± 0.69 kg/1.5 $cm^2$). However, in the ES group, the Mesairi-type cattle recorded a lower SF value (5.75 ± 0.60 kg/1.5 $cm^2$) than the Nyalawi-type cattle (5.92 ± 0.71 kg/1.5 $cm^2$), but the difference was not significant. Therefore, the Mesairi breed type cattle responded better to ES in terms of tenderness compared with the Nyalawi breed type.

## 3. 5 Conclusion

Breed type did not influence meat quality attributes, but ES and age at slaughter had significant effects. Electrical stimulation accelerated the post mortem pH decline significantly up to 24 hours post mortem. Beef from young animals may help in obtaining high meat quality. This study showed that ES reduced the differences in meat tenderness between younger and older animals. It was also shown that meat from the Mesairi type responded better to ES in terms of tenderness compared with meat from the Nyalawi type. Electrical stimulation of Sudanese Baggara beef carcasses and the use of younger bulls produced more tender meat with better colour. These practices should be adopted in Sudan to improve beef quality.

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## 3.7 Authors' contribution

ECW and ADAB devised the study and performed data analysis. AMM assisted with the experimental animals and abattoir facilities. ADAB performed data collection and wrote the manuscript. ECW provided guidance and reviewed the manuscript.

## 3.8 Conflict of interest declaration

Authors declare that there is no conflict of interest for this work.

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# CHAPTER 4

## Influences of electrical stimulation, slaughter weight, and post-freezing ageing on beef quality and sensory attributes of Sudanese Baggara cattle

Scientific manuscript prepared to be submitted for publication

# Influences of electrical stimulation, slaughter weight, and post-freezing ageing on beef quality and sensory attributes of Sudanese Baggara cattle

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## 4.1 ABSTRACT

The Sudanese beef industry depends on extensive rangeland production systems that yield carcasses that vary markedly in quality compared with those from other developing countries. Therefore, this research aimed to evaluate the effects of electrical stimulation (ES), slaughter weight, and post-freezing ageing on the carcass and meat quality of Sudanese Baggara cattle types (Nyalawi vs Mesairi). Forty bulls from each breed type were allocated to two age groups. ES was randomly applied to 50% of the carcasses and muscle pH and temperature were monitored *post mortem*. Fresh meat colour was measured and *longissimus dorsi* muscle samples were collected and frozen for five months. The frozen samples were thawed and cut into two equal sized steaks. Half of the thawed steaks were immediately processed, while the other half were aged at 4°C for seven days and then processed. The results showed that no cold shortening was detected in the treatment groups. ES and the use of younger animals yielded more tender meat with better colour, but this

was accompanied by a reduction in WHC in Nyalawi cattle. Mesairi cattle responded better to ES in terms of both tenderness and sensory scores than Nyalawi cattle. ES was more effective at improving the meat colour of Nyalawi cattle. In both breed types, the meat aged after freezing/ thawing resulted in higher L* and hue values and lower a* and chroma values in comparison with fresh meat. The aged meat also had better WHC, lower CL, and lower shear force (SF) values than meat that was just frozen/thawed.

**Keywords** Nyalawi, Mesairi, electrical stimulation, post-freezing ageing, *longissimus dorsi* muscle, Meat quality

## 4.2 INTRODUCTION

Sudanese Baggara beef cattle are the dominant and important breed for local consumption and export. The Nyalawi and Mesairi are the main Baggara cattle types, and are managed according to the nomadic production system (Alsiddig *et al*., 2010; Omer *et al*., 2018). The animals in this system are frequently exposed to stresses due to high environmental temperatures, continuous movement, and low quality and quantity of feed (Rahman, 2007). These animals are usually slaughtered at relatively mature ages (≥ four years old), which affects beef quality negatively, especially the meat tenderness. Beef tenderness, colour, flavour, and juiciness are critical factors that influence the beef quality and its acceptability to consumers (Shackelford *et al*., 2001). Of these factors, tenderness is recognised as one of the most important characteristics (Lusk *et al*., 1999), while colour is regarded as the main characteristic that consumers note when purchasing meat (Mancini and Hunt, 2005). There are several factors that influence meat quality, such as animal species, breed, gender, age at slaughter, body weight, nutrition, and pre- and post-slaughter handling (Guerrero *et al*., 2013).

At post-slaughter, electrical stimulation (ES) of the carcass has been adopted extensively in the meat industry to develop tenderness and other quality attributes (Adeyemi and Sazili, 2014). ES causes extensive muscle contraction, thus hastening *post-mortem* (pm) glycolysis and

accelerating the onset of rigor mortis before the carcass' temperature falls within the range of cold shortening risk (Hwang *et al*., 2003). It was also found that ES could contribute to the tenderisation process via structural changes to muscle fibres, either through physical disruption or by accelerating the proteolysis rate as a result of increased $Ca^{2+}$ releases (Hwang *et al*., 2003; Kadim *et al*., 2009b; Yang *et al*., 2019). However, there are inconsistencies among researchers on the effects of ES on the quality of meat. For example, some researchers found positive influences (Hwang and Thompson, 2001; McKenna *et al*., 2003; Nazli *et al*., 2010; Mombeni *et al*., 2013; Agbeniga and Webb, 2014), while others found no influences (Botha *et al*., 2009; Van den Berg, 2009; Kim *et al*., 2013) or negative influences (Simmons *et al*., 2008). The contradicting in the reports could be due to differences in breed, animal age and species, and their responses to ES (Adeyemi and Sazili, 2014). Samples from Nyalawi cattle generally had superior phenotypic beef characteristics (Eltahir *et al*., 2018) and meat quality traits (Biraima *et al*., 2014) over the Mesairi cattle. However, a recent study by Biraima *et al*. (2019) showed that breed type (Nyalawi vs Mesairi) did not influence the quality of beef. The authors speculated that Nyalawi and Mesairi cattles might have different responses to ES, which may have reduced the differences in quality of meat between the two breed types. Although research about the commercial application of ES was considered seriously in 1973 (Lawrie and Ledward, 2006), it is not yet practiced in the Sudanese meat industry. Due to the lower weights of the carcasses of Sudanese Baggara Zebu cattle, they can chill faster and be more susceptible to cold shortening. Therefore, the adoption of ES technique would help to avoid the risk of cold shortening.

Besides the ES technique, meat tenderness could also be improved by allowing more muscle proteolysis via ageing. Meat freezing has been widely practised for long-term *post mortem* storage (Kim and Kim, 2017). It is thought that the calpains proteolytic system plays a pivotal role in *post mortem* muscle breakdown and in determining the ultimate tenderness of

meat (Frylinck *et al*., 2015; Koohmaraie and Geesink, 2006). Kristensen *et al*. (2006) reported that calpain activity is stable for several months when meat is stored at -20°C. Furthermore, some authors have indicated that calpain could be more active when meat is subjected to post-freezing ageing; and this might result in more tender meat compared with normal ageing prior to freezing (Grayson *et al*., 2014; Kim and Kim, 2017; Whipple and Koohmaraie, 1992). Therefore, the post-freezing ageing intervention may be useful in improving meat tenderness, and may be another option in managing the quality of beef in Sudan. The effect of slaughter weight on Sudanese Baggara beef quality was not assessed previously.

The purposes of this study were to 1) assess the influence of ES on the *post mortem* pH/ temperature relationship and beef quality attributes of the Nyalawi and Mesairi cattle breed types; 2) evaluate the effects of slaughter weight and post-freezing ageing on beef quality attributes of the Nyalawi and Mesairi cattle breed types; and 3) estimate the association between SF values and sensory tenderness scores.

## 4.3 MATERIALS AND METHODS

### *4.3.1 Experimental design and treatments*

Experimental procedures were approved by the Animal Ethical Committee of the University of Pretoria, South Africa (approval number: EC076-17). The trial was carried out at the Animal Production Research Center (KUKU) in Sudan. Two main types of Baggara cattle – Nyalawi (n = 40) and Mesairi (n = 40) – were obtained from the KUKU feedlot. Each breed type was selected according to body weight, and subdivided into group 1 (240-260 kg, ranging in age from 4 to 4.5 year old) and group 2 (280-310 kg, ranging in age from 5 to 5.5 year old). They were kept without feed and given only *ad libitum* access to water for 12 hours before slaughter. The cattle were humanely killed and dressed according to the standard Halal method. This method of slaughtering lawful animals has several conditions to be fulfilled. A very sharp knife should be used to cut the throat and major blood vessels in the neck but leaving

the spinal cord intact. During slaughtering itself, the specific phrase in the Arabic language "Bismillah, Allahu Akbar", which means "In the name of Allah, Allah is the greatest", should be pronounced by the slaughter-man, and the animal should be alive (Abdullah *et al*., 2011).

Electrical stimulation (ES) was randomly applied to 50% of the carcasses at 20 minutes pm within each breed type and body weight group. ES was applied for 30 seconds by using an electrical stimulator (voltage, 110 V; JARVIS, Model ECS-1, South Africa). The ambient temperature of the abattoir was 25 to 30°C. Carcasses were moved to the chilling room (2-4°C) at approximately 45 min pm and chilled for 24 h before removing the muscle samples. Muscle pH and temperature were measured using a portable pH/temperature meter (Hanna Instruments, code HI99163) specially designed for the meat processing industry. Measurements were recorded at about 10 min, three h, 6 h, 9 h, 12 h, and 24 h pm. They were taken from the same depth at the $10^{th}$ and $11^{th}$ rib of the left side *longissimus dorsi* muscle. At 24 h pm, the left *longissimus dorsi* muscles were taken between the $9^{th}$ and $12^{th}$ rib, and the meat colour was measured, after which each sample was kept in a labelled plastic bag and frozen at -20°C for five months. The frozen samples were thawed at 4°C for 36 h, and then each muscle was cut into two equal steaks. Half of the steaks of each treatment were used immediately for meat quality analysis (meat colour, WHC, CL, SF, and sensory evaluation), while the others half were aged at 4°C for seven days until processed (meat colour, WHC, CL, and SF) (Figure 4.1).

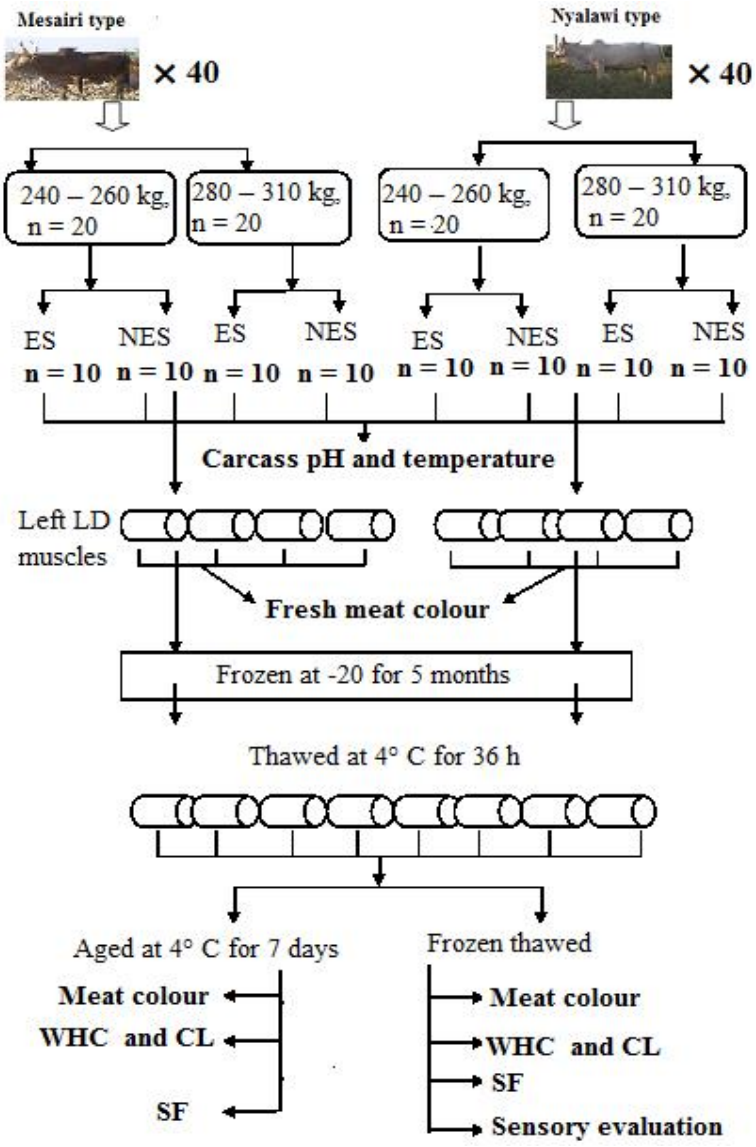


**Figure 4.1 Schematic diagram of the experimental design**

### *4.3.2 Instrumental colour measurements*

The beef samples were left to bloom at 20°C ± 1°C (room temperature) for 20 min. All readings were taken using a Hunter Lab ColorFlex EZ (Model 45/0 LAV, Hunter Laboratory Associates, Inc., Reston, Virginia, USA) using illuminant D65 at 10° standard observer to determine the L* (lightness), a* (redness) and b* (yellowness) values. Chroma (red colour intensity/saturation index) and hue-angle (meat discoloration) were computed using the equations "chroma = $(a^{*2}+b^{*2})^{1/2}$ and hue-angle = $\tan^{-1}$ (b*/a*)" (Hunt *et al*., 1991). The ColorFlex EZ was standardised before readings against black and white tiles following the manufacturer's instructions. At each sampling day, readings were obtained from three different random locations on each beef sample, and the mean was used for statistical analysis.

### *4.3.3 Water-holding capacity and cooking loss*

Water-holding capacity (WHC) was performed on each sampling day, as described by Babiker and Lawrie (1983). A 0.5 g minced meat sample was placed on humidified Whatman filter paper (No.4), stored in a desiccator over-saturated potassium chloride solution, and pressed between two plexiglass plates at 25 kilograms (kg) weight for two minutes. The area of the meat was defined with a ballpoint pen and the paper was dried. Meat and loose water areas (in $cm^2$) were determined with a planimeter to calculate the WHC ratio using the equation "WHC ratio = (loose water area – meat film area)/meat film area". A large WHC ratio indicates the high watery condition of the meat or a reduction in the WHC of the muscle. Concerning cooking losses, samples were weighed and boiled in a water bath at 80ºC to an internal temperature of 70ºC. The cooked samples were then stored overnight in a refrigerated condition at 4ºC. The samples were later blotted dry and weighed. Cooking loss percentages were calculated using the equation "the dividing of the weight loss (g) after cooking by the initial weight before cooking and then multiplying by 100" (Honikel, 1998).

### *4.3.4 Shear force measurements*

The instrumental tenderness was measured on the same samples used for the CL evaluations. The meat SF measurements were determined by using a Warner-Bratzler device (G-R Elec. Mfg. Co., Manhattan, Kansas 606502). Rectangular samples with a cross-sectional area of 1.5 cm² (1 x 1.5 cm) and 10 cm long were obtained from the cooked meat. Single peak force values in kg required to cut the meat samples by shearing perpendicular to the muscle fibres were taken. An average of two single peak force values per sample on each sampling day were taken for statistical analysis.

### *4.3.5 Sensory evaluation*

Sensory evaluation was performed by nine semi-trained panelists who were final-year undergraduate students at the Department of Meat Production, University of Khartoum, Sudan. The cooked samples were judged for aroma, colour, juiciness, flavour, tenderness, and overall acceptability using a sensory unstructured scaling method (Munoz and Civille, 1998). The panelists were instructed to record their evaluations of each sample by making a vertical mark on a horizontal line (10 cm long) at the place that best reflected their perception of each trait. The left end (0 cm) of the line was labelled as ‘not light’, ‘intense’, ‘juicy’, ‘tasty’, ‘tender’, and ‘acceptable’, while the right end (10 cm) was labelled as ‘extremely light’, ‘intense’, ‘juicy’, ‘tasty’, ‘tender’, and ‘acceptable’. Each sample was wrapped in aluminum foil and cooked in an electric oven at 163˚C to an internal temperature of 75˚C (Griffin *et al*., 1985). Slices of about one inch square were obtained from the cooked meat and then labelled with a random code number. The slices were randomly served warm on plates to be evaluated. Water was supplied to rinse the mouth before testing each sample. The panelists were asked to abstain from food, smoking, and drinking fluids (except for water) for one hour before each session.

### *4.3.6 Statistical analysis*

The data were analysed using the general linear models (GLM) procedure of Statistix 8.0 for Windows (2003, Analytical Software, Tallahassee, FL, USA). The model used in the analysis within each breed type as follows:

$$Y_{ijk} = \mu + A_i + B_j + C_k + A_iB_j + A_iC_k + B_jC_k + A_i B_jC_k + E_{ijk}$$

Where $Y_{ijk}$ = dependent variables (pH, temperature, and meat quality parameters), $\mu$ = overall mean, $A_i$= effect of ES, $B_j$= effect of slaughter weight, $C_k$= effect of post-freezing ageing, $A_iB_j$ = interactive effect of ES and slaughter weight, $A_iC_k$ = interactive effect of ES and post-freezing ageing, $B_jC_k$ = interactive effect of slaughter weight and post-freezing ageing, $A_iB_jC_k$ = interactive effect of ES, slaughter weight and post-freezing ageing and $E_{ijk}$ = random error. Interactions were insignificant in the majority of cases; consequently, means of the main effect were presented throughout. Significant interactions were provided in the text where appropriate. The significant interaction means were separated by Tukey's test at a 5% significance level. Correlations between the SF values and the sensory tenderness scores were estimated using the Pearson correlation coefficient of SPSS 11.5 for Windows (2003, SPSS version 11.5, SPSS Inc., Chicago, IL, USA). Data were expressed as means ± standard deviation (SD).

## 4.4 RESULTS AND DISCUSSION

### *4.4.1 Carcass pH/temperature relationship*

The effects of ES on the *post mortem* pH/temperature decline in Nyalawi and Mesairi carcasses are illustrated by the graph in Figure 4.2. The ES groups of Nyalawi and Mesairi breed types had a rapid decline in carcass pH up to 24 h pm, compared with the non-electrical stimulation (NES) groups ($P < 0.001$; Figure 4.2). Similar observations were reported in cattle (Nazli *et al*., 2010; Mombeni *et al*., 2013) and other species (Kadim *et al*., 2009a; Kadim *et al.*, 2009b; Kadim *et al.,* 2010; Cetin *et al*., 2012). However, in the current study, the pH decline

rate of the control groups (the NES carcasses) was relatively fast. This may indicate a high concentration of muscle glycogen, since the fast rate of pH decline indicates a high level of muscle glycogen (Gardner *et al*., 2006).

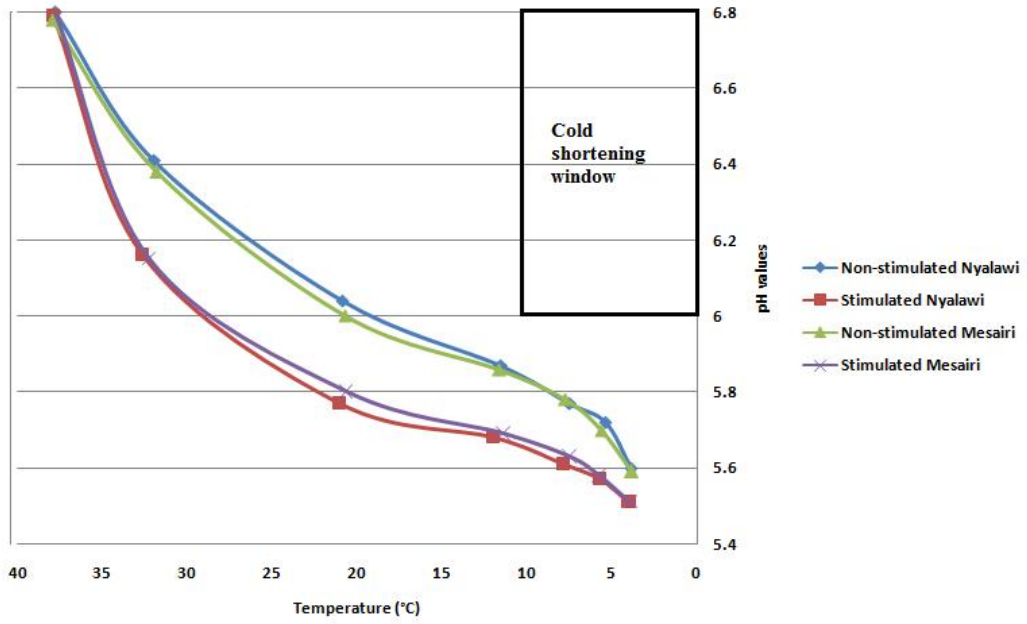


**Figure 4.2 Temperature/pH relationship of electrically stimulated and non-stimulated carcasses of Nyalawi and Mesairi breed types**

As reviewed by Thompson (2002), cold shortening happens if the carcass temperature falls below 10°C and the pH is above 6.0 when the ATP level is still high. The cold shortening risk was anticipated in the NES groups. However, in this study, no cold shortening was detected (Figure 4.2). The results of O'Neill *et al*. (2018) demonstrated that sarcomere shortening could be avoided by reducing the hours of fasting before slaughter to less than the conventional period of 24 hours, which results in a rapid pH decline due to a high level of muscle glycogen. In the current study, animals were offered feed up to 12 hours before slaughter, which might have raised the concentration of muscle glycogen and contributed to preventing the risk of cold shortening.

### *4.4.2 Instrumental colour measurements*

The influences of ES, slaughter weight, and ageing post-freezing/thawing on meat colour are summarised in Table 4.1. ES treatment of the carcasses resulted in higher L* values, lower a* values (not significant in the Mesairi type) and higher hue values (not significant in the Mesairi type) when compared with the controls. The ES treatment did not act in the same way on the chroma values of the two breed types. The chroma values of the Mesairi meat were not influenced by ES, while the electrically stimulated carcasses from the Nyalawi cattle had a lower ($P < 0.01$) chroma value than the NES group. ES showed to be more effective in improving a*, chroma and hue values of Nyalawi cattle than Mesairi cattle. The increase in muscle lightness was probably due to the fast pH decline caused by the ES treatment, which could result in increasing the light scattering properties of meat by increasing the protein denaturation at high temperatures (Hughes *et al*., 2018). The observed effect of ES on redness agrees with Li *et al*. (2011), who reported that ES reduced the redness of the *longissimus dorsi* muscle from bovines at 24 h pm. Furthermore, in the current study, the muscle pH of the ES samples was significantly lower than in the NES samples, which could explain the observed effect of ES on the a* value, since muscle redness decreases with a decreasing pH level (Lawrie and Ledward, 2006). The results of Zullo *et al*. (2006) indicated that ES causes better bleeding and reduces the content of blood in haemoglobin, which leads to lower a* and chroma values and a higher hue value. Electrical stimulation (ES) did not affect muscle yellowness (b* values) in the two breed types. Similar findings were observed by Kadim *et al*. (2009a, 2009b, and 2010) in cattle, camel, and goat meat.

The effect of slaughter weight on meat colour is shown in Table 4.1. Nyalawi cattle demonstrated a significant variation in meat colour parameters between lighter and heavier carcasses. By contrast, meat colour of Mesairi cattle was not influenced by slaughter weight except for muscle lightness. Meat from the lighter bulls (240 – 260 kg) had lower a* and

chroma values (not significant in the Mesairi type), and higher L* values (not significant in the Nyalawi type) than meat from the heavier (280 – 310 kg) bulls. Similar results for the same breed were observed by Biraima and Webb (2018). Generally, meat from older animals tends to be a darker (lower L* values) and redder (higher a*) colour than meat from younger, lighter animals (Ponnampalam *et al*., 2017). The darker redder meat might be the result of a higher myoglobin content since meat colour depends on the myoglobin content. Indeed, the concentration of muscle myoglobin increases as the animal ages (Lawrie and Ledward, 2006).

**Table 4.1 Effects of electrical stimulation, slaughter weight, and ageing after freezing/thawing on meat colour of Nyalawi and Mesairi cattle types (mean ± SD)**

| | **Nyalawi breed type parameters** | | | | |
|---|---|---|---|---|---|
| **Treatments** | **L*** | **a*** | **b*** | **Chroma** | **Hue angle** |
| ES treatment | | | | | |
| NES | **43.52$^{b}$± 2.33** | **12.27$^{a}$ ± 2.32** | 15.15 ± 1.20 | **19.63$^{a}$ ± 1.23** | **51.23$^{b}$ ± 6.80** |
| ES | **45.67$^{a}$ ± 2.31** | **11.09$^{b}$ ± 2.74** | 15.17 ± 1.10 | **18.96$^{b}$ ± 1.39** | **54.24$^{a}$ ± 7.91** |
| **Slaughter weight** | | | | | |
| 280 - 310 kg | 44.56 ± 2.58 | **11.94$^{a}$ ± 2.59** | **14.94$^{b}$ ± 1.19** | **19.63$^{a}$ ± 1.29** | 52.53 ± 7.32 |
| 240 - 260 kg | 44.63 ± 2.55 | **11.42$^{b}$ ± 2.61** | **15.39$^{a}$ ± 1.06** | **18.97$^{b}$ ± 1.34** | 52.94 ± 7.73 |
| **Ageing** | | | | | |
| Fresh meat | **43.18$^{b}$ ± 1.63** | **14.02$^{a}$ ± 0.85** | **14.46$^{b}$ ± 0.83** | **20.16$^{a}$ ± 0.90** | **45.88$^{b}$ ± 2.17** |
| Frozen/thaw-ed/aged | **46.01$^{a}$ ± 2.53** | **9.33$^{b}$ ± 1.30** | **15.86$^{a}$ ± 0.97** | **18.44$^{b}$ ±1.16** | **59.59$^{a}$ ± 3.51** |
| | **Mesairi breed type parameters** | | | | |
| **Treatments** | L* | a* | b* | Chroma | Hue angle |
| **ES treatment** | | | | | |
| NES | **43.39$^{a}$ ± 3.26** | 12.08 ± 2.75 | 14.93 ± 1.17 | 19.42 ± 1.62 | 51.27 ± 7.72 |
| ES | **46.18$^{b}$ ± 3.21** | 11.63 ± 2.62 | 15.23 ± 1.27 | 19.32 ±1.56 | 52.98 ± 7.33 |
| Slaughter weight | | | | | |
| 280 - 310 kg | **43.91$^{a}$ ± 3.21** | 12.06 ± 2.81 | 15.12 ± 1.39 | 19.51 ± 1.76 | 51.82 ± 7.67 |
| 240 - 260 kg | **45.66$^{b}$ ± 3.61** | 11.65 ± 2.56 | 15.04 ±1.05 | 19.23 ± 1.39 | 52.42 ± 7.47 |
| **Ageing** | | | | | |
| Fresh meat | **42.68$^{a}$± 2.50** | **14.22$^{b}$ ± 1.07** | **14.49$^{a}$ ± 0.89** | **20.37$^{b}$ ± 1.14** | **45.43$^{a}$ ± 2.47** |
| Frozen/thaw-ed/aged | **46.89$^{b}$ ± 3.10** | **9.49$^{a}$ ± 1.39** | **15.67$^{b}$ ± 1.24** | **18.37$^{a}$ ± 1.30** | **58.82$^{b}$ ± 4.09** |

$^{a,b}$ Column means within ES treatment, slaughter weight, and ageing, with different superscripts differ (P <0.05).

NES = not electrically stimulated; ES = electrically stimulated.

L* = lightness; a* = redness; b* = yellowness.

Ageing after freezing/thawing improved ($P < 0.001$) the muscle lightness (L* value) and yellowness (b* value) compared to the fresh meat in both breed types (Table 4.1). In general, ageing causes an increase in the light-scattering properties of meat, either through the loss of water as purge or through the degradation of myofibrillar proteins (Kim *et al*., 2011; Mortensen *et al*., 2006; Li *et al*., 2014). This may enhance the L* and b* values. However, the thaw drip loss and purge loss were not measured during this study, and so should be considered in future research. The aged meat of the Nyalawi breed type was lower ($P < 0.001$) in a* value (a* = 9.33 ± 1.30) than the initial meat redness (a* = 14.02 ± 0.85). Also, the aged meat of the Mesairi breed type had a lower ($P < 0.001$) a* value (a* = 9.49 ± 1.39) compared with the fresh meat redness (a* = 14.22 ± 1.07). In both breed types, the post-freezing ageing showed a significant reduction in chroma values and an increase in hue values (Table 4.1). The observed effect of post-freezing ageing on the redness and chroma values is similar to the findings with ovine *longissimus* muscle (Van der Stok, 2015). These findings may be a result of changing myoglobin to metmyoglobin due to ageing, which reduces the amount of oxymyoglobin (red colour) in the muscle. Demos and Mandigo (1996) reported that the storage of ground beef at 4°C for seven days changed the myoglobin to metmyoglobin by approximately 65%. The decrease in redness and increase in yellowness could explain the rising hue value. Among the Mesairi breed type a significant interaction was detected between ES and slaughter weight for the L* value; the colour of the meat from smaller ES carcasses was brighter ($P < 0.05$) than its counterparts.

### *4.4.3 Water-holding capacity (WHC), cooking loss (CL), and shear force (SF) measurements*

The effects of ES, slaughter weight, and ageing after freezing/thawing on WHC, CL, and SF are shown in Table 4.2. ES did not affect the WHC of meat samples from the Nyalawi and Mesairi cattle breeds in the same way. ES reduced the WHC in the Nyalawi cattle ($P < 0.001$; Table 4.2), while meat from the Mesairi cattle was not affected by ES. There are contradictions

in authors' reports on the effect of ES on WHC in beef. Li *et al*. (2006) and Agbeniga and Webb (2018) found that ES resulted in a reduction of WHC. In contrast, McKenna *et al*. (2003) found that ES did not influence WHC. These variations in the reports, as reviewed by Adeyemi and Sazili (2014), might be due to differences in breed, animal age and species and their responses to electrical stimulation. ES did not influence ($P > 0.05$) the CL in both breed types, this agrees with Li *et al*. (2011) and Yang *et al*. (2019), who noted that the CL percentage of the ES beef carcasses was similar to the NES ones. Similar results were also observed in pigs (Channon *et al*., 2003), who reported that ES enhanced the percentage of purge loss without a significant effect on the CL of the pork *longissimus et lumborum* muscle. In both breed types, the SF values were significantly lower after ES treatment than for the NES carcasses (Table 4.2); however, the ES appears to be most effective in enhancing tenderness of the Mesairi cattle compared with the Nyalawi cattle. Similar phenomena were reported in beef carcasses (Hwang and Thompson, 2001; McKenna *et al*., 2003; Li *et al*., 2006; Nazli *et al*., 2010; Mombeni *et al*., 2013; Agbeniga and Webb, 2014; Agbeniga and Webb, 2018; Yang *et al*., 2019), lamb carcasses (Polidori *et al*., 1999; Kahraman and Ergun, 2009; Cetin *et al*., 2012), goat carcasses (Biswas *et al*., 2007; Cetin *et al*., 2012; Kadim *et al*., 2010) and camel carcasses (Kadim *et al*., 2009a and 2009b). As mentioned above, no cold shortening was detected in the NES groups. Therefore, the detected effects of ES on SF values support the theory that ES can improve meat tenderness through structural changes to muscle fibres either through physical disruption or by accelerating the rate of proteolysis as a result of increased $Ca^{2+}$ release (Kadim *et al*., 2009b; Yang *et al*., 2019).

Meat from group 2 (280 – 310 kg) of both breed types had a better WHC than that in group 1 (240 – 260 kg), but was statistically significant only in the Nyalawi breed type ($P < 0.001$; Table 4.2). Mohammed (2004) reported better ($P < 0.001$) WHC for heavier bulls from the same Baggara cattle than in lighter bulls. No significant differences between animal weights

were detected for CL in the breed types. In contrast, Mohammed (2004) reported that Sudanese Baggara bulls with a lower weight had a significantly lower CL percentage than the heavier ones. These variations in reports could be due to the methods used to determine the cooking losses. In the current study, the meat samples were cooked at 80ºC to achieve an internal temperature of 70ºC, while Mohammed (2004) cooked the samples at 80ºC for 90 minutes. Regarding the SF values in both breed types, the lighter animals (240 – 260 kg) had lower SF values ($P < 0.001$) than the heavier ones (280 – 310 kg; Table 4.2). Similar results for the same breed were reported by Mohammed (2004).

This study indicated that the post-freezing ageing treatment had a better WHC and a lower CL than normal freezing in both breed types (Table 4.2). Meat aged after freezing/thawing from the Nyalawi breed type resulted in a better ($P < 0.001$) WHC (WHC ratio = 2.61 ± 0.38) and a lower ($P < 0.01$) CL (CL% = 19.33 ± 2.79) compared with just freezing and thawing (WHC ratio = 3.01 ± 0.41; CL% = 21.42 ± 2.74). The Mesairi breed type also showed a better WHC and a lower CL (WHC ratio = 2.74 ± 0.38; CL% = 19.73 ± 2.73; $P < 0.001$) than just freezing and thawing (WHC ratio = 3.09 ± 0.41; CL% = 22.56 ± 3.10). Similarly, the finding of Balan *et al.* (2019) reported that post-freezing ageing improved the WHC of the *longissimus lumborum* muscle from lamb. The observed effect of post-freezing ageing on CL agrees with the finding of Grayson *et al.* (2014), who reported that beef aged post-freezing had a significantly lower CL than meat just frozen/thawed.

**Table 4.2 Effects of electrical stimulation, slaughter weight, and ageing after freezing/thawing on water-holding capacity, cooking loss, and shear force of Nyalawi and Mesairi cattle types (mean ± SD)**

| Treatments | Nyalawi breed type parameters | | |
|---|---|---|---|
| | WHC ratio | CL % | SF (kg/1.5 cm$^2$) |
| **ES treatment** | | | |
| NES | **2.65$^a$± 0.40** | 19.83 ± 3.19 | **6. 50$^b$ ± 1.17** |
| ES | **2.96$^b$ ± 0.43** | 20.92 ± 2.59 | **5.40$^a$ ± 0.85** |
| **Slaughter weight** | | | |
| 280 – 310 kg | **2.69$^a$ ± 0.44** | 19.91 ± 3.23 | **6.54$^b$ ± 1.00** |
| 240 – 260 kg | **2.93$^b$ ± 0.41** | 20.84± 2.58 | **5.35$^a$ ± 0 .99** |
| **Ageing** | | | |
| Fresh meat | **3.01$^b$ ± 0.41** | **21.42$^b$ ± 2.74** | **6.51$^b$ ± 0.91** |
| Frozen/thawed/aged | **2.61$^a$ ± 0.38** | **19.33$^a$ ± 2.79** | **5.39$^a$ ± 1.11** |
| **Treatments** | **Mesairi breed type parameters** | | |
| | **WHC ratio** | **CL %** | **SF (kg/1.5 cm$^2$)** |
| **ES treatment** | | | |
| NES | 2.85 ± 0.45 | 21.04 ± 3.35 | **7.10$^b$ ± 1.01** |
| ES | 2.99 ± 0.41 | 21.25 ± 3.15 | **5.37$^a$ ± 0.84** |
| **Slaughter weight** | | | |
| 280 – 310 kg | 2.86 ± 0.47 | 20.78 ± 3.47 | **6.69$^b$ ± 1.28** |
| 240 – 260 kg | 2.97 ± 0.39 | 21.51 ± 2.98 | **5.78$^a$ ± 1.09** |
| **Ageing** | | | |
| Frozen/thawed | **3.09$^b$ ± 0.41** | **22.56$^b$ ± 3.10** | **6.72$^b$ ± 1.22** |
| Frozen/thawed/aged | **2.74$^a$ ± 0.38** | **19.73$^a$ ± 2.73** | **5.75$^a$ ± 1.13** |

$^{a,b}$ Column means within ES-treatment, slaughter weight, and ageing with different superscripts differ ($P < 0.05$).

NES = not electrically stimulated; ES = electrically stimulated.

WHC = water-holding capacity; CL = cooking loss; SF = shear force.

For both breed types, the SF values of meat aged after freezing/thawing were lower ($P < 0.001$) than for meat just frozen and thawed (Table 4.2). This result is similar to reports from several beef studies (Aroeira *et al*., 2016; Grayson *et al*., 2014; Kim and Kim, 2017) and a lamb study (Balan *et al*., 2019). The observed effects of post-freezing ageing on tenderness confirmed the findings of Kristensen *et al*. (2006), who found that the calpain activity is stable for several months when meat is stored at -20°C; and Aroeira *et al*. (2016) indicated that beef samples aged after being frozen (for 40 days at -20°C) showed greater proteolysis than frozen/thawed only and fresh-aged samples.

Within the Nyalawi breed type, significant interactions between slaughter weight and ES were found in the meat's WHC and SF. The heavier NES carcasses were better ($P < 0.01$) for WHC than other carcasses. The SF value did not differ ($P > 0.05$) between lighter NES carcasses (5.70 ± 1.00) and the older ES group (5.80 ± 0.73); however, the most tender meat was observed in the lighter ES group (5.01 ± 0.80; $P < 0.01$), while the toughest meat was in the heavier NES animals (7.29 ± 0.61; $P < 0.01$). An interaction was also observed ($P < 0.01$) between slaughter weight and post-freezing ageing for the SF values. The aged meat from group 1 (240 – 260 kg) was the most tender (4.58 ± 0.46), while the frozen/thawed only meat from group 2 (280 – 310 kg) was the toughest (6.89 ± 0.93). Among the Mesairi breed type, there was interaction ($P < 0.01$) between slaughter weight and ES for the SF value. The lower value was seen in the lighter ES group (5.07 ± 0.80), followed by the heavier ES group (5.66 ± 0.78) and then by the lighter NES group (6.48 ± 0.87) and the heavier NES group (7.72 ± 0.71).

### *4.4.4 Sensory evaluations*

Table 4.3 presents the results of the effects of ES and slaughter weight on sensory attributes. No interaction was observed between ES and slaughter weight for sensory evaluations. The ES group of the Mesairi cattle had scored higher in tenderness ($P < 0.01$) and overall acceptability ($P < 0.05$) scores than the NES group. In contrast, the ES carcasses of the Nyalawi cattle showed a tendency towards a higher ($P = 0.06$) tenderness score (5.98 ± 0.69) than the NES group (5.45 ± 0.83). However, the overall acceptability among the Nyalawi group was not influenced by the ES treatment. The ES group of the Mesairi cattle also had higher scores for sensory colour, aroma and flavour than the NES group, but these were not statistically significant. Therefore, Mesairi cattle showed more positive responses to ES in terms of sensory attributes compared with Nyalawi cattle. The sensory colour, tenderness, aroma and flavour of meat have been reported to be improved by ES (Nazli *et al*., 2010; Mota-

Rojas *et al*., 2012). The use of ES in beef carcasses may enhance the flavor and aroma by accumulating peptides and glutamic acid, which are believed to produce better flavour (Warriss, 2010). In both breed types, the ES groups showed very small, insignificant increases in the juiciness scores compared with the NES groups. There are contradictions in other authors' reports on the influence of ES on meat juiciness. Some authors have shown higher juiciness scores (Hwang and Thompson, 2001), while some found no influence (Davel *et al*., 2003) and yet others showed a reduction in juiciness (Ferguson *et al*., 2000). These variations in the reports, may be due to differences in the effects of ES on the ultimate pH, the physical disruption of meat structure, and proteolytic activity acceleration (Adeyemi and Sazili, 2014). It is well-known that high overall acceptability scores are positively associated with increased tenderness, juiciness, or flavour scores (O'Quinn *et al*., 2018).

**Table 4.3 Effects of electrical stimulation and slaughter weight on meat sensory evaluation of Nyalawi and Mesairi breed types (Mean ± SD)**

| **Nyalawi type** | | | | |
|---|---|---|---|---|
| | **ES treatment** | | **Slaughter weight** | |
| Item | NES | ES | 280 - 310 kg | 240 - 260 kg |
| Colour | 4.98 ± 1.44 | 4.86 ± 1.26 | 4.67 ± 1.21 | 5.17 ±1.45 |
| Aroma | 5.31 ± 1.12 | 5.03 ± 0.98 | 5.37 ± 1.05 | 4.97 ± 1.04 |
| Juiciness | 4.85 ± 0.81 | 4.87 ± 1.02 | 4.80 ± 0.79 | 4.92 ± 1.04 |
| Flavour | 5.08 ± 0.85 | 5.12 ± 0.88 | 5.19 ± 0.73 | 5.01 ± 0.97 |
| Tenderness | 5.45 ± 0.83 | 5.98 ± 0.69 | 5.54 ± 0.68 | 5.88 ± 0.89 |
| Overall acceptability | 5.30 ± 0.95 | 5.69 ± 0.81 | 5. 43 ± 0.83 | 5.52 ± 0.97 |
| **Mesairi type** | | | | |
| | **ES treatment** | | **Slaughter weight** | |
| Item | NES | ES | 280 - 310 kg | 240 - 260 kg |
| Colour | 4.40 ± 0.87 | 4.99 ± 1.69 | 4.50 ± 1.76 | 4.95 ± 1.02 |
| Aroma | 4.83 ± 0.56 | 5.10 ± 0.86 | 5.05 ± 0.84 | 4.92 ± 0.68 |
| Juiciness | 5.05 ± 0.97 | 5.21 ± 0.63 | 5.12 ± 0.86 | 5.16 ± 0.73 |
| Flavour | 4.71 ± 0.66 | 5.10 ± 1.32 | 5.15 ± 1.21 | 4.73 ± 0.98 |
| Tenderness | **4.82$^{b}$ ± 0.82** | **5.79$^{a}$± 1.14** | 5.61 ± 1.38 | 5.17 ± 0.81 |
| Overall acceptability | **5.05$^{b}$ ± 0.83** | **5.69$^{a}$ ± 0.76** | 5. 89 ± 0.73 | 5.35 ± 0.95 |

[a,b] Row means within ES treatment and slaughter weight with different superscripts differ ($P < 0.05$).

NES = not electrically stimulated; ES = electrically stimulated.

The slaughter weight did not influence ($P > 0.05$) the sensory evaluations (Table 4.3). However, Mohammed (2004) reported that Baggara bulls slaughtered at 250 kg were more tender ($P < 0.01$) than bulls slaughtered at either 300 or 350 kg. That author further reported that sensory colour increased as slaughter weight increased, while juiciness decreased without any significant effect on flavour. In the present study, bulls from the Nyalawi breed type slaughtered at 240 – 260 kg had an insignificant increase in tenderness and overall acceptability scores compared with bulls slaughtered at 280 – 310 kg. The lighter bulls (240 – 260 kg) from both breed types had a slightly insignificant increase in colour and juiciness scores compared with heavy bulls (280 – 310 kg).

The Pearson correlation coefficients between the SF values and the sensory tenderness scores were -0.39 and -0.47 for the Nyalawi breed type and the Mesairi breed type respectively. Generally, the correlation coefficients were within the range reported for cattle. Lorenzen *et al*. (2003) and Dikeman *et al*. (2005) reported that there is substantial variability in the correlations between SF values and sensory tenderness scores between laboratories, even when evaluated by trained panelists, and vary from -0.11 to -0.72.

## 4.5 CONCLUSIONS

This study showed that ES treatment increased *post mortem* pH decline significantly compared with cattle in the NES control group. In both breed types, no risk of cold shortening was observed. ES and the use of lighter bulls improved tenderness and colour of meat, but with some detrimental effects on WHC in meat samples from Nyalawi cattle. The effectiveness of ES in improving meat tenderness and sensory scores differed between the two breed types and Mesairi cattle responded better to ES than Nyalawi cattle. However, the use of ES showed to be more effective at improving the beef colour of carcasses from Nyalawi cattle. In both breed types, the meat from bulls slaughtered at 240 – 260 kg had lower SF values than the meat from animals slaughtered at 280 – 310 kg. However, the panelists were unable to detect any

differences in sensory attributes between the slaughter weight groups. Although post-freezing ageing had a negative effect on the colour stability of the meat, it had a beneficial influence on beef tenderness. This study also showed a good negative correlation between the instrumental tenderness measurements (SF) and the sensory tenderness scores. Therefore, the ES techniques should be introduced into the Sudanese beef industry to improve beef quality. The production of lighter carcasses yields more desirable beef quality; so Baggara keepers should be encouraged to slaughter their animals at a lighter weight. Post-freezing ageing may be a practical option to improve tenderness if it is not improved through normal ageing.

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## 4.7 CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest in this work.

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# CHAPTER 5

## Effects of age and protease extract of *Solanum dubium* seed on beef eating-quality of longissimus muscle from Sudanese Baggara cattle

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# Effects of age and protease extract of *Solanum dubium* seed on beef eating-quality of *longissimus* muscle from Sudanese Baggara cattle

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## 5.1 Abstract

The ability of protease extract from *Solanum dubium* seeds to improve beef tenderness of two age groups of Sudanese Baggara cattle was investigated. Samples of *longissimus dorsi* muscle were obtained from 4 – 4.5 and 5 – 5.5 year old bulls. Ultimate pH and meat colour were determined at 24 hours *post mortem* (pm). Muscle samples were sliced into two steaks; one (10 per cent muscle weight) was injected with the protease extract, while the other was left as a control. Samples were incubated at 4°C for 24 hours. Muscle pH, colour, cooking loss (CL), Warner-Bratzler shear force (WBSF), and sensory attributes were determined at 48 h pm ($pH_{48}$). Meat from younger bulls had a brighter and better red colour, a higher cooking loss, and was more tender than that of older bulls. *Solanum dubium* seed extract did not affect ($P > 0.05$) meat pH, lightness (L*), redness (a*), chroma, or sensory colour, aroma, or flavour. However, protease extract increased b* values, %CL, juiciness and overall acceptability scores compared to non-injected samples. Protease extract improved beef tenderness significantly in term of both instrumental and sensory measures. The current study highlights the potential benefits of extract from *S. dubium* seeds as a meat tenderiser for the Sudanese meat industry.

**Keywords:** *Longissimus dorsi* muscle, meat tenderness, protease extract, sensory evaluation, *Solanum dubium*.

## 5.2 Introduction

Sudanese Baggara beef cattle are kept by the nomadic Baggara tribes in extensive rangeland production systems. These cattle provide the source of beef for local and exportation markets (Alsiddig, Babiker, Galal, & Mohammed, 2010). Baggara cattle are always exposed to stressors – for instance, herding, high temperatures, and shortage of water and feed, particularly during summer (Rahman, 2007). These stressors may depress the growth of cattle. However, the nomadic Baggara tribes rear the cattle mainly as a store of wealth (Fahey & Leonard, 2008). The cattle are thus sold and slaughtered at relatively mature ages (≥ four years old). All these factors may influence meat quality negatively and, in particular, its tenderness. Generally, *post mortem* proteolysis decreases as the animal's age increases; beef from old cattle thus has fewer endogenous protease enzymes for the *post mortem* tenderisation process (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2004; Whipple & Koohmaraie, 1992). Beef consumers consider meat tenderness, colour, juiciness, and flavour as very important quality traits (Mancini & Hunt, 2005; Shackelford *et al*., 2001). Among these traits, tenderness is preferred over the others by beef consumers, and they are willing to buy tender steaks at higher prices (Lusk, Fox, Schroeder, Mintert, & Koohmaraie, 1999).

Endogenous calpain protease enzymes play an essential role in the meat tenderisation process by hydrolysing the muscle proteins (Koohmaraie & Geesink, 2006). However, *post mortem* proteolysis is relatively limited because the calpains undergo autolysis (Goll, Thompson, Li, Wei, & Cong, 2003). Due to this self-autolysis, calpains do not over-tenderise meat. Therefore, the use of exogenous enzymes can contribute greatly to improving meat tenderness. Exogenous protease enzymes extracted from plant, bacterial, and fungal sources are widely used to improve meat tenderness by hydrolysing the meat proteins. Many studies

report that plant protease enzymes, such as papain, bromelain, ficin, zingibain, and actinidin, together with the microbial protease enzymes *Aspergillus oryzae* and *Bacillus subtili*s, have been widely used in the meat industry as meat tenderisers (Ashie, Sorensen, & Nielsen, 2002; Bekhit, Hopkins, Geesink, Bekhit, & Franks, 2014; Doneva *et al*., 2015; Han, Morton, Bekhit, & Sedcole, 2009; Ionescu, Aprodu, & Pascaru, 2008; Istrati, Vizireanu, Dima, & Dinica, 2012; Liu, Xiong, & Rentfrow, 2011; Rawdkuen & Benjakul, 2012; Sullivan & Calkins, 2010). Dubiumin is a serine protease enzyme found in the seeds of the *Solanum dubium* plant, and it has massive proteolytic activity (Ahmed, Morishima, Babiker, & Mori, 2009b). This proteolytic activity could make it another promising meat tenderiser.

*Solanum dubium,* a recognised wild plant in Sudan known as Gubbein, grows during the rainy season. Its seeds have long been used by dairy farmers as a traditional plant protease for manufacturing cheese. The protease enzyme from this plant has a wide range of stability at temperatures between 20 and 90°C and a pH between 3.0 and 12 (Ahmed, Morishima, Babiker, & Mori 2009a; Ahmed *et al*., 2009b). However, previous studies of this enzyme have focused mainly on its application in dairy industries (Abdalla, Ali, & Mohamed, 2010; Ahmed *et al*., 2009a; Ahmed *et al*., 2009b; El-Owni, Kheir, & Abdalla, 2011; Kheir, El Owni, & Abdalla, 2011; Talib, Abubakar, Jideani, & Hassan, 2009; Talib, Abubakar, & Jideani, 2011; Yousif, McMahon, & Shammet, 1996). Uphof (1968, as cited by Ahmed *et al*., 2009b) reported that seeds from this plant have been used as a traditional method of removing hair from animal hides. It has been reported that the seeds from the *Solanum dubium* (Gubbein) plant are non-toxic for humans. Recently, Mohamed, Hassan, Kabbashi, Ayoub, and Mohamed (2016) studied the acute toxicity of the aqueous extract from the *Solanum dubium* seeds in rats. The authors reported that no sign of toxicity or behavioural changes were detected in the rats throughout the study period, even at the highest doses. The authors further stated that no changes in the haematological and biochemical parameters were found.

The quest for a meat tenderiser for the meat industry is a continuous challenge (Maróstica & Pastore, 2010). The literature thus supports the notion of both safe and excessive proteolytic activity in *Solanum dubium* seeds, in addition to the availability of raw materials. As the first experimental approach, the protease extract from the seeds of the *Solanum dubium* plant was investigated as a meat tenderiser in the *longissimus dorsi* muscle from Sudanese Baggara cattle.

The objective of this study was to assess the effects of *S. dubium* seed protease extract and age at slaughter on *longissimus* muscle pH, colour, cooking loss (CL), Warner-Bratzler shear force (WBSF), and eating quality traits of Sudanese Baggara cattle.

## 5.3 Materials and methods

### *5.3.1 Experimental design and sampling*

Animal ethics approval was granted by the Animal Ethical Committee of the University of Pretoria, South Africa (approval number: EC076-17). A total of 30 Sudanese Baggara bulls (Nyalawi breed type) from the Animal Production Research Centre (KUKU) in Sudan were used in this study. Bulls were randomly selected according to their age groups: group1 (4 – 4.5 years old, weighing 240 – 260kg) and group2 (5 – 5.5 years old, weighing 301.67 ± 16.22kg). Bulls were fed the same diet and humanely slaughtered at the slaughterhouse of the Animal Production Research Centre. The carcasses were placed in the chilling room (2°C – 4°C) at about 40 minutes post-slaughter, and chilled for 24 hours before sampling. The samples of *longissimus dorsi* muscle were removed between the 9$^{th}$ and 12$^{th}$ rib of the left side of each carcass at 24 hours post-slaughter. The ultimate pH ($pH_{24}$) and instrumental colour were determined at 24 hours pm. Samples were then transported to the Meat Science Laboratory (Department of Meat Production, Faculty of Animal Production, University of Khartoum, Sudan) in plastic bags in an insulated icebox and stored at 4°C ± 1°C for about 30 minutes before the injection treatment was performed (Figure 5.1).

### *5.3.2 Sample preparation and injection treatment*

*Longissimus* muscle samples were collected and trimmed of excess visible fat and connective tissue. Each muscle sample was then cut into two equal steaks; one was used for the injection treatment, and the other was left as control (no injection). The yellow fruits of *Solanum dubium* were collected from North Kordofan State, Sudan. The yellow coats were carefully cleaned manually, and the seeds were powdered using an electric grinder. A preliminary investigation was done to identify the concentration of the extract that could be injected into the muscles. Two different amounts of the powdered seed of the *Solanum dubium* plant (5g and 2.5g) were mixed with 30 mL of distilled water and stirred for 30 minutes at room temperature using a magnetic stirrer, and then filtered using a nylon mesh strainer. The aqueous extracts were centrifuged at 6000 rpm for 10 minutes. The action of the proteolytic enzyme of the final aqueous extracts was examined by adding 5 mL of the extract into heated milk at about 60°C, and coagulation was observed, as shown in Figure 5.2. The supernatant was then used for meat injection. Based on maximum tenderisation occurring without a negative effect on eating quality, a concentration of 5g/30mL was selected for the present study. An injector with a single needle was used for muscle injection. The entire quantity of aqueous extract was pumped manually into whole muscle mass. The volume of injection solution was 10 per cent of muscle weight (Han *et al*., 2009; Ilian *et al*., 2004; Liu *et al.*, 2011). As reported by Han *et al*. (2009) and Liu *et al*. (2011), the injection of meat with water did not influence tenderness. Consequently, in the current study, the water injection treatment was avoided, and we focused only on injecting with the aqueous protease extract. Injected and non-injected muscle samples were packed in plastic bags and incubated overnight at 4°C ± 1°C to allow proteolytic degradation. The $pH_{48}$, instrumental colour, CL, WBSF, and sensory attributes were then evaluated (Figure 5.1).

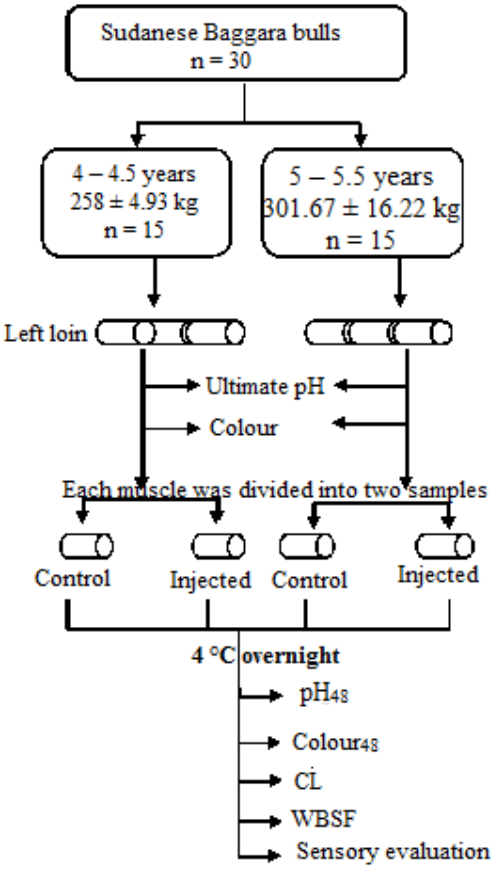


**Figure 5.1 Diagram of the experimental design**

**Figure 5.2 Coagulation of milk due to the proteolytic activity of the *Solanum* extract**

### *5.3.3 Measurement of pH*

The muscle pH was recorded with a portable pH meter (Hanna Instruments, code-HI99163) specially designed for the meat processing industry.

### *5.3.4 Instrumental colour*

For instrumental colour evaluations, samples were left to bloom at room temperature at 20°C ± 1°C for 20 minutes. All instrumental colour readings were taken using a Hunter Lab ColorFlex EZ (Model 45/0 LAV, Hunter Laboratory Associates, Inc., Reston, Virginia, USA) using illuminant D65 at 10° standard observer to determine L* values (lightness, 0 = black and 100 = white), a* (redness, positive values = red and negative values = green), and b* (yellowness, positive values = yellow and negative values = blue). Chroma (saturation) and hue-angle (meat discoloration) were calculated using the equations "chroma = $(a^{*2}+b^{*2})^{1/2}$, and hue-angle = $\tan^{-1}$ (b*/a*)" (Hunt *et al*., 1991). The mean of three random readings was used for statistical analysis. The ColorFlex EZ was standardised immediately before readings against the black and white tiles according to the guidelines of the manufacturer.

### *5.3.5 Cooking loss and Warner-Bratzler shear force measurements*

For cooking loss (CL), each muscle was weighed and placed in a plastic bag and cooked in a water bath at 80ºC until the internal temperature reached 70ºC. The cooked muscles were chilled overnight at 4°C. The samples were later blotted dry and weighed. The CL percentage was determined using the equation "cooking loss % = ((initial sample weight - sample weight after cooking)/ initial sample weight) x 100" (Honikel, 1998).

Warner-Bratzler shear force (WBSF) values were determined on the same samples used for the CL evaluations. A single peak force value in kilograms (kg) using a Warner-Bratzler instrument (G-R Elec. Mfg. Co. Manhattan, KANSAS 606502) was required to cut the cooked samples by shearing perpendicular to the muscle fibres. Rectangular slices with a cross section

of 1.5 $cm^2$ (1 x 1.5 cm) and 10 cm long were removed from the cooked samples (Szczesniak, 1963). A mean of two single peak force values per sample was recorded for statistical analysis.

### *5.3.6 Sensory analysis*

For sensory analysis, each meat sample was wrapped in aluminium foil and cooked in an electric oven at 163˚C to an internal temperature of 75˚C (Griffin, Smith, Rhee, & Johnson, 1985). Cooked samples were divided into small slices (2.54$cm^2$) and randomly labelled with a code number. They were then offered warm, on dishes, to be assessed. Cooked meat samples were assessed in terms of colour, aroma, juiciness, flavour, tenderness, and overall acceptability using a sensory unstructured scaling method (Munoz and Civille, 1998). The panellists were requested to judge each sample by making a vertical mark on a 10 cm long horizontal line at the point that best reflected their perception of the magnitude of each attribute. The left end (0 cm) of the line was marked as not light, intense, juicy, tasty, tender, and acceptable. The right end (10 cm) was marked as extremely light, intense, juicy, tasty, tender, and acceptable. The eight semi-trained panellists who contributed to this study were final-year undergraduate students at the Department of Meat Production, University of Khartoum, Sudan. Water was provided to remove traces of the previous sample. The panellists were requested to refrain from smoking, eating, or drinking fluids other than water for one hour prior to each session.

### *5.3.7 Statistical analyses*

The data collected at 24 hours pm (ultimate pH and instrumental colour) of the two age groups were analysed using an independent samples t-test. The data collected after the injection treatment ($pH_{48}$, instrumental colour at 48 hours pm, CL, WBSF, and sensory analysis) were analysed using a general linear model (GLM) procedure. The model used in the analysis as follows:

$$Y_{ij} = \mu + A_i + B_j + A_iB_j + E_{ij}$$

Where $Y_{ij}$ = dependent variables, $\mu$ = overall mean, $A_i$= effect of injection, $B_j$= effect of age at slaughter, $A_iB_j$ = interactive effect of injection and age at slaughter and $E_{ij}$ = random error. Data processing was conducted using Statistix 8.0 for Windows (2003, Analytical Software, Tallahassee, FL, USA). Data were expressed as mean values ± standard deviation (SD).

## 5.4 Results and discussion

### *5.4.1 Ultimate pH and instrumental colour at 24 hours pm*

Table 5.1 shows the effect of age at slaughter on the ultimate pH, and on instrumental colour at 24 hours pm. In general, the pH values at 24 hours for the current study were within the normal range compared with other studies of beef cattle (Du Plessis & Hoffman, 2007; Marti, Realini, Bach, Pérez-Juan, & Devant, 2013; Coleman *et al*., 2016; Van Ba *et al*., 2018). The pH at 24 hours pm was not influenced by age at slaughter (Table 5.1).

**Table 5.1 Ultimate pH and instrumental colour (mean ± standard deviation) as affected by age at slaughter at 24 hours *post mortem***

| | **Age at slaughter (years)** | | **P-value** |
|---|---|---|---|
| Item | **4 – 4.5** | **5 – 5.5** | |
| Ultimate pH | 5.60 ± 0.08 | 5.62 ± 0.05 | 0.229 |
| Lightness (L*) | **43.20 ± 2.65** | **41.01 ± 2.19** | **< 0.05** |
| Redness (a*) | **13.63 ± 1.69** | **15.40 ± 1.16** | **< 0.01** |
| Yellowness (b*) | 14.10 ± 1.09 | 14.93 ± 1.46 | 0.088 |
| Chroma | **19.63 ± 1.80** | **21.46 ± 1.66** | **< 0.05** |
| Hue-angle | **46.09 ± 2.60** | **44.06 ± 2.24** | **< 0.05** |

Similar findings were reported by Czyżak-Runowska *et al*. (2017) and Kadim *et al*. (2006). As expected, meat from older bulls (5 – 5.5 years of age) had significantly lower L* (darker) and higher a* (redder) values than meat from younger animals (4 – 4.5 years of age; Table 5.1). Yellowness (b*) was not affected ($P > 0.05$) by age at slaughter (Table 5.1). However, the age at slaughter influenced the chroma and hue-angle ($P < 0.05$; Table 5.1). These observations are consistent with those of other studies of bovine meat (Marenčić, Ivanković, Kozačinski, Popović, & Cvrtila, 2018; Marti *et al*., 2013; Du Plessis & Hoffman, 2007), lamb

meat (Polidori, Pucciarelli, Cammertoni, Polzonetti, & Vincenzetti, 2017), camel meat (Kadim *et al*., 2006), and horse meat (De Palo, Maggiolino, Centoducati, & Tateo, 2012). In general, the colour of meat depends on the myoglobin content (Lawrie & Ledward, 2006). Older animals have high myoglobin, which results in a darker and redder meat than in younger animals (Ponnampalam, Hopkins, Bruce, Baldi, & Bekhit, 2017).

### *5.4.2 Instrumental colour at 48 hours pm*

The instrumental colour at 48 hours pm (after the incubation period) for all treatment groups is shown in Table 5.2. No interaction ($P > 0.05$) was detected between age at slaughter and injection for colour values. The aqueous extract injection did not influence ($P > 0.05$) muscle lightness (L*), redness (a*), or chroma values (Table 5.2). However, injected meat had higher ($P < 0.05$) b* values (15.56 ± 1.67), and higher ($P < 0.05$) hue values (46.74 ± 3.24) than those from non-injected muscles (b* = 14.72 ± 1.30; hue = 44.83 ± 2.72) (Table 5.2). It is well-known that exogenous proteolytic enzymes cause the protein degradation that tenderises meat (Bekhit *et al*., 2014; Doneva *et al*., 2015; Liu *et al*., 2011; Han *et al*., 2009), enhancing the light-scattering properties of muscles and resulting in higher instrumental colour values (Hughes, Clarke, Purslow, & Warner, 2018). However, in this study, the injected protease extract of *S. dubium* improved tenderness considerably with no significant influence on muscle lightness, redness, or chroma values. These contradictions can be attributed to the colour of the aqueous extract, which is relatively dark yellow. The colour of the aqueous extract may also explain the observed increase in muscle yellowness (b*) due to injection treatment. On the other hand, the observed insignificant effect of the protease extract on the L*, a*, and chroma values may be due to muscle pH, since pH is very important in meat colour (Abril *et al*., 2001). In this study, the $pH_{48}$ was almost the same between the injected and non-injected groups.

The age at slaughter also influenced the instrumental colour at 48 hours pm (Table 5.2). The colour values were almost the same as those observed at 24 hours pm. Jayasooriya, Torley,

D'arcy, and Bhandari (2007) reported no significant differences in the colour values of bovine *semitendinosus* and *longissimus* muscles between day 0 and day 3 of ageing. However, the authors observed a significant increase in the colour values after seven days of ageing.

**Table 5.2 Instrumental colour at 48 hours *post mortem* (mean ± standard deviation) as affected by *S. dubium* seed injection and age at slaughter.**

| | Injection | | Age at slaughter (years) | | P-value | | |
|---|---|---|---|---|---|---|---|
| Item | Control | Injected | 4 – 4.5 | 5 – 5.5 | Injection | Age | Injection x Age |
| L* | 42.00 ± 3.05 | 42.79 ± 2.21 | **43.31 ± 3.29** | **41.48 ± 2.21** | 0.292 | **< 0.05** | 0.984 |
| a* | 14.82 ± 1.36 | 14.65 ± 1.68 | **14.07 ± 1.60** | **15.39 ± 1.10** | 0.638 | **< 0.0001** | 0.287 |
| b* | **14.72 ± 1.30** | **15.56 ± 1.67** | 14.88 ± 1.44 | 15.40 ± 1.62 | **< 0.05** | 0.190 | 0.893 |
| Chroma | 20.91 ± 1.60 | 21.40 ± 2.04 | **20.52 ± 1.75** | **21.79 ± 1.70** | 0.279 | **< 0.01** | 0.532 |
| Hue-angle | **44.83 ± 2.72** | **46.74 ± 3.24** | **46.65 ± 3.42** | **44.91 ± 2.54** | **< 0.05** | **< 0.05** | 0.415 |

### *5.4.3 The pH48, cooking loss, and Warner-Bratzler shear force*

The effects of injection and age at slaughter on pH, CL, and WBSF are shown in Table 5.3. No interaction ($P > 0.05$) was observed between age at slaughter and injection for $pH_{48}$, CL, and WBSF values (Table 5.3). The pH is very important in meat processing, as it directly affects meat colour, shelf life, and quality (Simela, 2005). Generally, the degradation of muscle proteins by exogenous enzymes leads to the presence of free amino acids, which may result in a low muscle pH (Ketnawa & Rawdkuen, 2011). However, in the current study, the aqueous extract injection did not affect ($P > 0.05$) meat pH (Table 5.3). This observation was probably due to the pH of the aqueous extract, which was 5.74, and which may have contributed to reducing the variations in pH values between the injected and non–injected samples. Age at slaughter also did not influence ($P > 0.05$) muscle $pH_{48}$ (Table 5.3). Nogalski *et al*. (2018) reported that the pH of beef *longissimus* muscle at 48 hours pm ranged between 5.52 and 5.57, and was not influenced by age at slaughter. The injected muscles with the aqueous extract had a higher ($P < 0.001$) CL percentage (27.59 ± 2.70) than the control muscles (18.16 ± 3.12)

(Table 5.3). Obviously, the injected fluids (aqueous protease extract) were not well bound in the meat, which may have increased the CL percentage in the injected muscles. The injected exogenous protease enzyme may also increase the breakdown of muscle fibres and enlarge the spaces between fibres for water movement, which may result in a higher CL percentage. In agreement with the current work, Liu *et al*. (2011) reported that injected muscles with 10 per cent water or 10 per cent protease aqueous extract of kiwifruit significantly increased the CL percentage compared with control samples (non-injected). Meat from younger animals had a greater ($P < 0.05$) CL percentage than older animals (Table 5.3). This finding may be attributable to the decrease in meat moisture content as animal age increases (Schönfeldt, Naudé, & Boshoff, 2010).

**Table 5.3 $pH_{48}$, cooking loss, and shear force (mean ± standard deviation) as affected by *S. dubium* protease injection and age at slaughter.**

| | Injection | | Age at slaughter (years) | | P-value | | |
|---|---|---|---|---|---|---|---|
| Item | **Control** | **Injected** | **4 – 4.5** | **5 – 5.5** | **Injection** | **Age** | **Injection x Age** |
| $pH_{48}$ | 5.54 ± 0.10 | 5.49 ± 0.13 | 5.50 ± 0.11 | 5.52 ± 0.13 | 0.067 | 0.680 | 0.991 |
| CL% | **18.16 ± 3.12** | **27.59 ± 2.70** | **23.78 ± 5.00** | **21.98 ± 6.03** | **< 0.001** | **< 0.05** | 0.672 |
| WBSF (kg/1.5 $cm^2$) | **6.00 ± 1.20** | **2.12 ± 0.76** | **3.41 ± 1.93** | **4.71 ± 2.29** | **< 0.0001** | **< 0.05** | 0.218 |

For WBSF measurements, the injected protease aqueous extract reduced the WBSF values by almost 65 per cent. Therefore, muscles injected with the protease extract had a lower ($P < 0.0001$) WBSF value (2.12 ± 0.76 kg) than the non-injected ones (6 ± 1.20 kg) (Table 5.3). The present study indicates that a protease extract of *S. dubium* seeds is a powerful source for reducing meat toughness. The highly proteolytic ability of the protease enzyme from the *S. dubium* seeds may explain the observed effect of the protease extract injection on the WBSF values. Previous studies have reported massive proteolytic activity of the *S. dubium* seeds'

aqueous extract on milk clotting and cheese-making (Abdalla *et al*., 2010; Ahmed *et al*., 2009a; Ahmed *et al*., 2009b; El Owni *et al*., 2011; Kheir *et al*., 2011; Talib *et al*., 2009; Talib *et al*., 2011; Yousif *et al*., 1996). As anticipated, the WBSF value was lower ($P < 0.05$) for younger animals (3.41 ± 1.93 kg) than that for older animals (4.71± 2.29 kg) (Table 5.3). Similar reports have demonstrated that WBSF values increase with increasing animal age (Kadim *et al*., 2006; Du Plessis & Hoffman, 2007; Purchas, Hartley, Xun, & Grant, 1997; Polidori *et al*., 2017; Li *et al*., 2018).

### *5.4.4 Sensory analysis*

There was no significant interaction between age at slaughter and injection for sensory attributes. The sensory evaluation scores of the injected and non-injected samples are illustrated in Figure 5.3. Panelists did not detect any significant difference between the injected and non-injected samples regarding colour, aroma, or flavour, while the meat from injected samples had higher ($P < 0.001$) sensory scores for juiciness (6.30 ± 1.21), tenderness (7.90 ± 1.22), and overall acceptability (7.39 ± 1.26) than the control samples (5.00 ± 1.21, 5.04 ± 1.37, and 5.70 ± 1.38 respectively). Due to the bitterness of *S. dubium* seeds, the muscles treated with the protease extract were anticipated to have a bitter flavour. However, no negative effect on aroma or flavour was detected in the current study. This effect of the extract from the *S. dubium* seeds on sensory evaluation has been reported in dairy technology. The study of Kheir *et al*. (2011) did not show any negative effect on sensory colour, flavour, texture, or saltiness in the cheese made with the protease extract of *S. dubium* seeds. The good eating quality observed may be due to the loss of bitter compounds during cooking since, in our own research, the water lost as CL percentage was about 9.4 per cent greater in the injected samples than in the control ones. This study thus indicates that the protease extract of *S. dubium* seeds could be appropriate for dry heat cooking methods, such as roasting and broiling. The higher tenderness scores in the injected muscles can be explained by the protease enzyme of *S. dubium* seed extract. The

improved overall acceptability from the injection of protease extract may be a result of improved tenderness and juiciness, since there is a strong positive correlation between the overall acceptability and the tenderness and juiciness (O'Quinn, Legako, Brooks, & Miller, 2018).

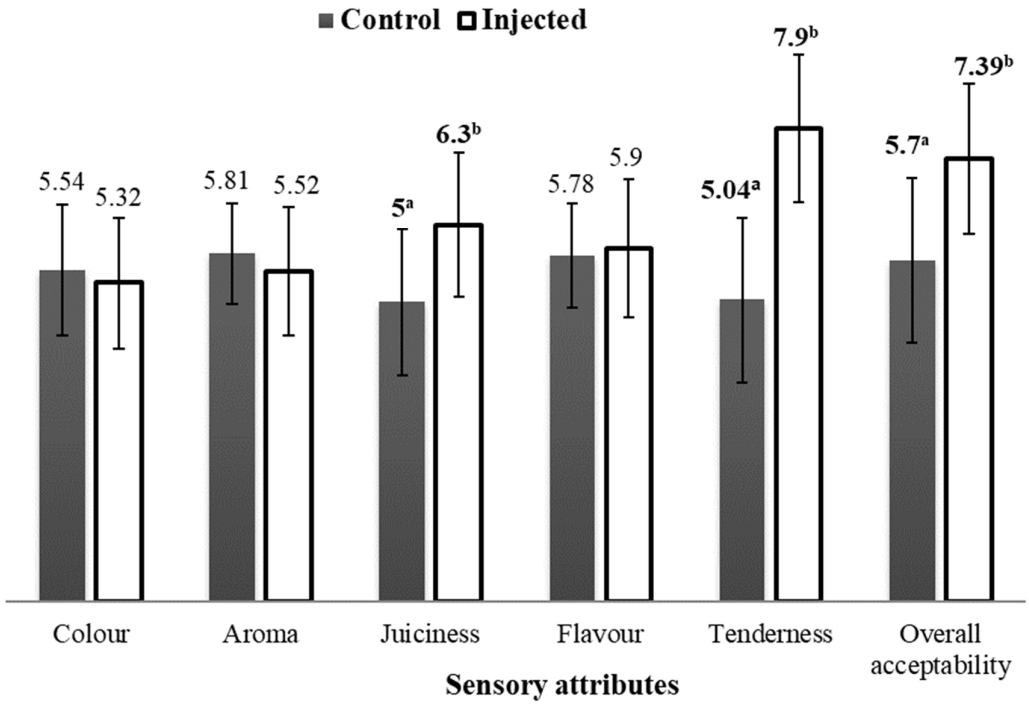


**Figure 5.3 Sensory analysis of beef *longissimus* muscle (mean ± standard deviation) between injection treatments (injected vs non-injected)**

The sensory evaluation scores of the age groups (4 – 4.5 vs 5 – 5.5 years) are shown in Figure 5.4. Regardless of the injection effect, meat from younger bulls had slightly higher scores for sensory analysis than older bulls, but the differences were not significant. Improvement in tenderness by the injection extract treatment may further reduce the differences in sensory evaluation between the age groups.

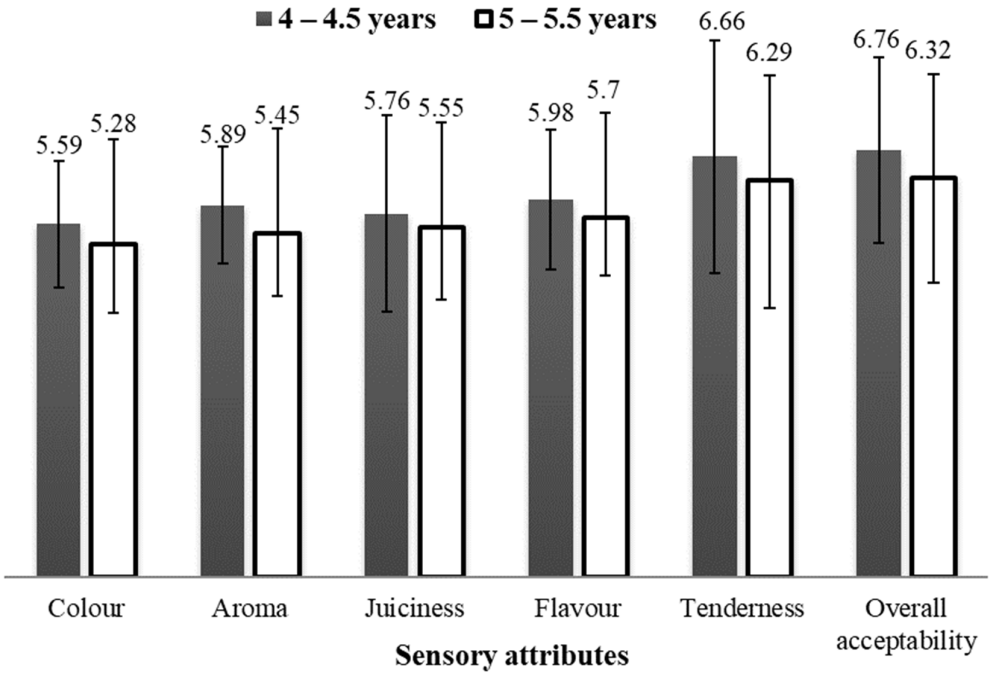


**Figure 5.4 Sensory analysis of beef *longissimus* muscles (mean ± standard deviation) between age groups (4 – 4.5 vs 5 – 5.5 years)**

## 5.5 Conclusions

The current study showed the successful use of protease extract from *S. dubium* seeds as a promising and novel source of an exogenous enzyme for meat tenderisation. The protease extract of *S. dubium* greatly improved the tenderness without negative impacts on the instrumental colour or sensory attributes. This study also showed that meat from younger bulls had a better colour, a higher CL percentage, and was more tender than that from older bulls. It was suggested that the dry heat cooking method used in this study removed the bitter compounds during cooking. Thus, the application of *S. dubium* protease extract using dry heat cooking methods may help to achieve more tender meat with good eating qualities. If the bitter compounds of *S. dubium* seeds can be removed, the application of a purified enzyme in the meat industry seems more promising than using only dry heat cooking methods. A study of a purified enzyme is therefore needed. A protease extract of *S. dubium* should be exploited by

the Sudanese meat industry as a commercial meat tenderiser to improve meat quality. Further studies are required to assess the effects of the *S. dubium* protease enzyme on the histochemistry and degradation of myofibrillar and collagen proteins.

## 5.6 Authors' contributions

Ahmed Dayain Abdalla Biraima conceived the idea, performed the experiments and the laboratory analyses, and wrote the original paper. The authors performed the data analysis. Edward Cottington Webb provided guidance and reviewed the paper.

## 5.7 Conflicts of interest

The authors declare that there is no conflict of interest for this work.

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# CHAPTER 6

## Meat quality and the microstructural and biochemical properties of beef samples injected with protease extract from Gubbain (*Solanum dubium*) seed

Scientific paper prepared to be submitted for publication

# Meat quality and the microstructural and biochemical properties of beef samples injected with protease extract from Gubbain (*Solanum dubium*) seed

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## 6.1 ABSTRACT

The tenderising effects of *S. dubium* protease extract in beef *m. longissimus thoracis et lumborum* (LTL) were investigated. Twelve LTL samples were obtained from both sides of several carcasses at 24 h *post mortem*. Each sample was cut into two equal samples and then randomised for treatments (injection with *S. dubium* protease extract vs no injection). After 24 h of incubation, the muscle was analysed for colour, sarcomere length (SL), myofibril fragment length (MFL), Warner-Bratzler shear force (WBSF), collagen solubility, muscle fibre types, quantification meat degradation, and SDS-PAGE. The injected muscles yielded lower ($P < 0.0001$) WBSF values with a better L* ($P < 0.05$) compared with samples of the control muscles. The results of MFL and the quantification of meat degradation reflected the great ability of the protease extract to degrade myofibrillar proteins. Collagen was hydrolysed significantly by the protease extract. SDS-PAGE showed the presence of several new lower molecular weight bands after treating muscle with the protease extract. The protease enzyme from *S. dubium* seeds may be a novel and promising option as a meat tenderiser.

**Keywords:** Tenderization, *Solanum dubium* protease, microstructural, collagen, *m. longissimus thoracis et lumborum*.

## 6.2 INTRODUCTION

Tenderness is deemed to be one of the most important attributes of meat quality, and a consumer preference (Shackelford *et al*., 2001). *Post mortem* proteolytic degradation of myofibrillar and connective tissue (collagen) proteins plays a major role in meat tenderisation. The endogenous calpain protease enzyme contributes considerably to tenderisation during ageing. However, the endogenous proteolytic enzymes do not break down the muscle fibre structure or degrade the connective tissue collagen protein sufficiently (Bekhit, 2017; Koohmaraie & Geesink, 2006). The injection of meat with exogenous protease enzymes extracted from plant, bacterial, and fungal sources is a popular method to improve meat tenderness (Arshad *et al*., 2016).

Several plant protease enzymes have been used for the tenderisation of meat, such as papain, bromelain, ficin, actinidin, and zingibain. Protease tenderisers derived from microbes such as Aspergillus oryzae and Bacillus subtilis are also used (Ashie, Sorensen, & Nielsen, 2002; Bekhit, Hopkins, Geesink, Bekhit, & Franks, 2014; Doneva *et al.*, 2015; Han, Morton, Bekhit, & Sedcole, 2009; Ionescu, Aprodu, & Pascaru, 2008; Istrati, Vizireanu, Dima, & Dinica, 2012; Liu, Xiong, & Rentfrow, 2011; Rawdkuen & Benjakul, 2012; Sullivan & Calkins, 2010). Gubbain (*Solanum dubium*) seeds contain Dubiumin serine protease, which is a good source of the exogenous proteolytic enzyme for food industries (Ahmed, Morishima, Babiker, & Mori, 2009b). The initial study by Biraima and Webb (2018) showed that protease extract from the seeds of *Solanum dubium* improved tenderness considerably, without any negative impact on meat colour or sensory properties in the *longissimus* muscle of Sudanese Baggara cattle.

Gubbein (*Solanum dubium*) is a recognised wild plant in Sudan that grows during the rainy season and is known as ‘Gubbein’. The seeds are usually used by dairy farmers as a traditional protease for clotting milk and manufacturing white soft cheese. Recently, several

studies have been cited on the application of proteolytic enzyme from the seeds of Gubbein (*Solanum dubium*) in dairy technology (Abdalla, Ali, & Mohamed, 2010; Ahmed, Morishima, Babiker, & Mori 2009a; Ahmed *et al*., 2009b; El-Owni, Kheir, & Abdalla, 2011; Kheir, El Owni, & Abdalla, 2011; Talib, Abubakar, & Jideani, 2011; Talib, Abubakar, Jideani, & Hassan, 2009; Yousif, McMahon, & Shammet, 1996). It was also reported by Uphof (1968, as cited by Ahmed *et al*., 2009b) that the seeds from this plant have been used as a traditional method for removing hair from animal hides. It has been reported that the seeds of the *Solanum dubium* plant are non-toxic for humans (Mohamed, Hassan, Kabbashi, Ayoub, & Mohamed, 2016). This study aimed to evaluate the effects of protease extract from the seeds of the *S. dubium* plant on meat quality characteristics, collagen solubility, muscle fibre types, quantification of meat degradation, and SDS-PAGE pattern of beef *m. longissimus thoracis et lumborum* (LTL). This study also examined the association between muscle fibre types and meat colour.

## 6.3 MATERIALS AND METHODS

### *6.3.1 Sampling*

Animal ethics approval was granted by the Animal Ethical Committee of the University of Pretoria, South Africa (approval number: EC076-17). Six Afrikaner x Bonsmara crossbred steers of 12 months old and an average weight of 386 ± 25 kg were used in this study. The steers were slaughtered according to standard procedures at the abattoir of the Agricultural Research Council – Animal Production Institute (ARC-API), Irene, Gauteng, South Africa. No electrical stimulation was used and, after dressing, the carcasses were placed directly in a chiller at 4°C for 24 h before sampling. A total of 12 steaks of the *m. longissimus thoracis et lumborum* (LTL) were obtained from the 10$^{th}$ rib to the last lumbar vertebrae of both carcass sides at 24 h *post mortem*.

### *6.3.2 Sample preparation and injection treatment*

The visible fat and connective tissue were trimmed from the samples. Each LTL steak was cut perpendicular to its longitudinal axis into two equal samples and then randomised for treatments (injection with *S. dubium* protease extract vs no injection). Dry yellow fruits of *Solanum dubium* were collected in January 2018 from the rangeland of North Kordofan State, Sudan. The yellow coats of the Gubbein (*Solanum dubium*) fruits were removed by hand to obtain the seeds, which were powdered using an electric grinder. The powdered seeds were packed in plastic bags and placed in a container, then transported to ARC-API, Irene, Gauteng, South Africa for analysis. The powdered seeds of the *Solanum dubium* were checked, and the Department of Agriculture, Forestry and Fisheries (DAFF) in South Africa issued a letter to confirm that a permit in terms of the Agricultural Pests Act, 1983 (Act No. 36 of 1983) was not required for the importation of plant products in the form of powder. No phytosanitary certificate was required.

The dry powdered seeds of the *S. dubium* (83.33 g) were mixed with 500 mL of distilled water and stirred for 30 minutes using a magnetic stirrer (5g/30mL). The extract was filtered using a nylon mesh strainer and centrifuged at 6000 rpm for 10 minutes. Then the supernatant was used for the injections. The protease enzyme of the final aqueous extract was confirmed by adding 5 mL of the extract to 50 mL of heated milk (60°C); milk clotting was then seen after about one minute. About two litres of fresh aqueous protease extract was prepared before injection. The muscles were manually injected with 10% aqueous protease extract (muscle weight basis) perpendicular to their muscle fibre orientations using a syringe with a single needle (Biraima & Webb, 2018; Han *et al*., 2009; Ilian *et al*., 2004; Liu *et al*., 2011). The entire volume of the aqueous extract was uniformly injected in different portions of the whole muscle mass. The samples (both injected and control) were then vacuum packed, labelled, and stored for 24 h at 4°C to allow the proteases to degrade the muscle proteins. After the storage period,

muscles of about 50 g each were cut from the steaks and used to evaluate the meat colour and sarcomere lengths. Samples for WBSF and collagen solubility were vacuum packed, labelled, and kept frozen at −20°C until processing, while samples for MFL, muscle fibre types, quantification of meat degradation, and the SDS-PAGE pattern were frozen in liquid nitrogen and stored at −80°C until they were processed.

### *6.3.3 Meat colour*

Beef samples (15 mm thickness) were bloomed at 18°C (room temperature) for one hour before measuring the meat colour. The colour readings (CIE L*, a*, b*, chroma, and hue-angle) were taken with a Konica Minolta CM-600d/CM-700d spectrophotometer using illuminant D65 at 10° observer angle, measurement aperture 8 mm. The Konica Minolta was calibrated before the readings, following the manufacturer's instructions. Three random recordings were taken on each muscle sample (Pophiwa, Webb, & Frylinck, 2016).

### *6.3.4 Sarcomere length (SL) measurements*

The sarcomere lengths (SL) of the injected and the control muscles were measured at 48 h *post mortem* (after 24 h of incubation). Muscle tissue (2 g) was homogenised in distilled water (Dreyer *et al*., 1979; Hegarty & Naudé, 1970). A small drop of each homogenate was placed on a microscope slide and covered with a cover glass, and then examined under an Olympus B340 microscope at 31,000 magnification, connected to a video camera and image analyser (Olympus, Tokyo, Japan). A mean of 50 sarcomere lengths per sample was recorded for statistical analysis.

### *6.3.5 Warner-Bratzler shear force (WBSF) determinations*

The frozen beef samples were thawed at 4°C for 24 h, and then about 200 g was removed from each sample steak and broiled (American Meat Science Association [AMSA], 1995) in a broiling oven (Mielé model H217; Mielé & Cie, Gütersloh, Germany) at 260°C (pre-set) to an internal temperature of 70°C and cooled down to room temperature (18°C). Six cores

from each cooked sample were removed parallel to the fibre orientation, using a hollow metal probe with an 8 cm length and 1.27 cm diameter. A Warner-Bratzler shear device attached to the Universal Instron apparatus (Model 4301; Instron Ltd, Buckinghamshire, UK; crosshead speed 200 mm/min) was used to shear the cores perpendicular to the muscle fibre orientation. An average of six single peak force values (kg) per sample was taken for statistical analysis.

### *6.3.6 Myofibril fragment length (MFL) measurements*

The lengths of the myofibril fragments of the injected and non-injected steaks were measured using an Olympus BX41 system microscope and video image analysis (VIA; Soft Imaging System, Olympus, Japan). The myofibrils were extracted using the method of Culler, Parrish, Smith, and Cross (1978) as modified by Heinze and Bruggemann (1994). The MFL measurements were determined at a magnification of 400×. A mean of 100 MFL (µm) per sample was recorded for statistical analysis.

### *6.3.7 Collagen solubility*

The collagen solubility of the injected and non-injected samples was determined following the procedure of Bergman and Loxley (1963), Hill (1966), and Weber (1973).

### *6.3.8 Muscle fibre types*

Muscle fibre typing was done on 100 g of the samples that were frozen in liquid nitrogen and stored at −80°C. The nitro-blue tetrazolium procedure of Malaty and Bourne (1953) was used for the histochemical demonstration of succinate dehydrogenase in mitochondria. The fibres were categorised under 100X magnification by VIA into red, intermediate, and white according to the intensity of the staining reaction. The VIA was also used to measure the fibre cross-sectional areas.

### *6.3.9 Quantification of meat degradation*

To determine the fibre detachment, fibre breaks, and percentage fibre separation score, blocks of approximately 7mm× 4mm were cut from the frozen muscles and fixed on a Cryotome disk. A Shandon Cryotome E (Thermo Fisher Scientific, Pittsburgh, USA) was used

to obtain sections of 15 µm thickness by cutting parallel to the orientation of the muscle fibres, and they were then mounted on a microscope slide. Two sections from each muscle sample were stained with Amaranth (Sigma A 1016-100G), after which the stained sections were observed under a microscope (Olympus BX41 system) at a magnification of 100× (Olympus, Tokyo, Japan). The entire muscle fibre areas and the fibre detachments (% white to red area) in a field of 0.57 $mm^2$ were measured using the AnalySIS Life Science software package (Soft Imaging Systems Gmbh, Münster, Germany). The fibre breaks were scored by the analyst on a scale of 1-5 (Taylor & Frylinck, 2003).

### *6.3.10 SDS-PAGE*

The changes in the myofibrillar proteins in the *m. longissimus thoracis et lumborum* (LTL) were also measured by means of SDS–PAGE. Protein was extracted from 200 mg of the LTL samples that were frozen in liquid nitrogen and stored at -80°C. Each sample was homogenised in 1 mL TES buffer and extracted as described by Jia, Hollung, Therkildsen, Hildrum, and Bendixen (2006). Protein concentrations were determined with the RC-DC protein assay kit (Bio-Rad, USA) at 750 nm in a Universal Micro Plate Rader (Bio-tek Elx 800) with bovine serum albumin (BSA), as standard. Twelve per cent gel of SDS-PAGE was used to separate the protein bands using the Ettan DALTsix large format vertical system (GE Healthcare Bio-Sciences), after which the Coomassie brilliant blue G250 stain was used to stain the protein bands in PAGE gels. A Chemi-Doc™ MP imaging system (Bio-Rad Hercules, CA, USA) was used to image and process the gels.

### *6.3.11 Statistical analyses*

The data were analysed using the independent samples t-test of SPSS 11.5 for Windows (2003, SPSS version 11.5, SPSS Inc., Chicago, IL, USA). The fibre breaks scores were transformed to ranks before running the analysis. Correlations were estimated using Pearson

correlation coefficients (SPSS, 2003). The data were expressed as mean values ± standard deviation (SD).

## 6.4 RESULTS AND DISCUSSION

### *6.4.1 Meat quality characteristics and collagen solubility*

Injection with the protease extract of Gubbain (*S. dubium*) seeds did not influence ($P > 0.05$) the meat colour parameters except for lightness (L*) values (Table 1). The meat injected with the protease extract showed higher L* values than the control samples. These findings were somewhat similar to the observations in the preliminary study conducted on *longissimus* muscles from Sudanese beef cattle (Biraima & Webb, 2018). There were no differences ($P > 0.05$) between the injected and the non-injected muscles for SL, while the meat samples treated with the protease extract showed significantly shorter MFL and lower WBSF values (Table 1). The length of the myofibril fragments can give a good indication of the amount of myofibrillar protein degradation. The observed effect of the protease extract injection on MFL was probably because the fragment length became shorter due to proteolysis by the exogenous proteolytic enzyme of the *S. dubium* seeds (Figure 6.1). In this study, the injection with the protease extract lowered the WBSF values by nearly 62% relative to the control samples. In support of this, Biraima and Webb (2018) reported that beef steaks injected with the protease extract of *S. dubium* seeds had significantly lower WBSF values (2.12 kg) than non-injected steaks (6.00 kg), and produced more tender meat by almost 65% relative to the non-injected meat. The shorter MFL, with an increase in the myofibrillar fragmentation of treated LTL with the protease extract (Figure 6.1B), may explain the observed improvement in tenderness, since the shorter length of the myofibril fragments contributes to more tender meat (Frylinck *et al*., 2009).

**Table 6.1 The effect of *S. dubium* protease extract injections on the meat quality characteristics of beef *m. longissimus thoracis et lumborum* (LTL) samples.**

| | Injection | | |
|---|---|---|---|
| Item | **Control** | **Injected** | **P-value** |
| Lightness (L*) | **32.36 ± 0.54** | **33.36 ± 1.55** | **< 0.05** |
| Redness (a*) | 11.34 ± 0.85 | 11.38 ± 1.13 | 0.931 |
| Yellowness (b*) | 11.95 ± 0.86 | 12.45 ± 1.18 | 0.253 |
| Chroma | 16.48 ± 1.13 | 16.88 ± 1.51 | 0.471 |
| Hue-angle | 46.50 ± 1.45 | 47.54 ± 2.12 | 0.176 |
| Sarcomere length (µm) | 1.90 ± 0.05 | 1.92 ± 0.06 | 0.421 |
| Myofibril fragment length (µm) | **33.09 ± 2.33** | **23.65 ± 3.22** | **< 0.0001** |
| Warner-Bratzler shear force (kg) | **5.13 ± 1.01** | **1.95 ± 0.70** | **< 0.0001** |

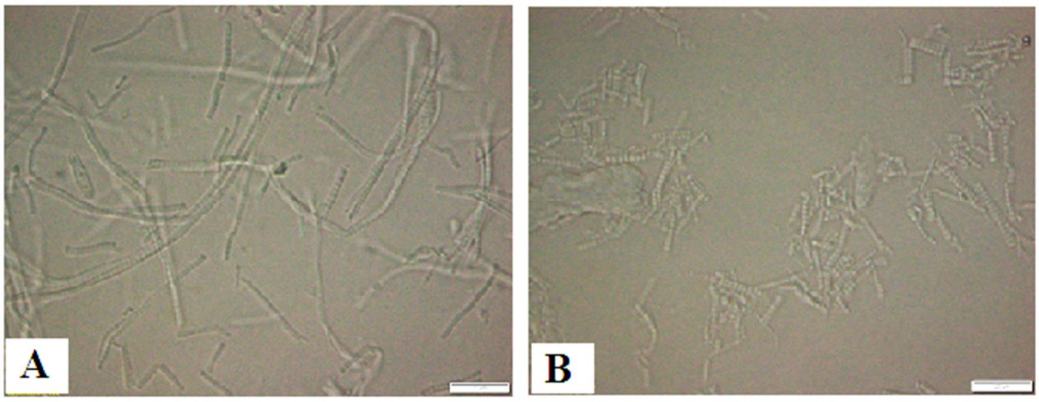


**Figure 6.1 Myofibril fragment lengths (MFL-µm) of beef *m. longissimus thoracis et lumborum* (LTL) samples subjected to different injection treatments (injected vs control). A = control sample (non-injected), while B = injected sample with protease extract of *S. dubium* seeds.**

Collagen is one of the major protein components of animal connective tissues, and is the main factor in determining the tenderness and texture of meat (Torrescano, Sanchez-Escalante, Gimenez, Roncales, & Beltrán 2003; Weston, Rogers, & Althen, 2002). The muscles injected with the aqueous protease extract of *S. dubium* seeds showed a higher ($P < 0.0001$) percentage of collagen solubility than the non-injected samples (Figure 6.2). This great hydrolysis of collagen protein was probably due to the tenderising effects of the exogenous

protease enzyme, which may explain the lower WBSF values observed in the injected muscle samples.

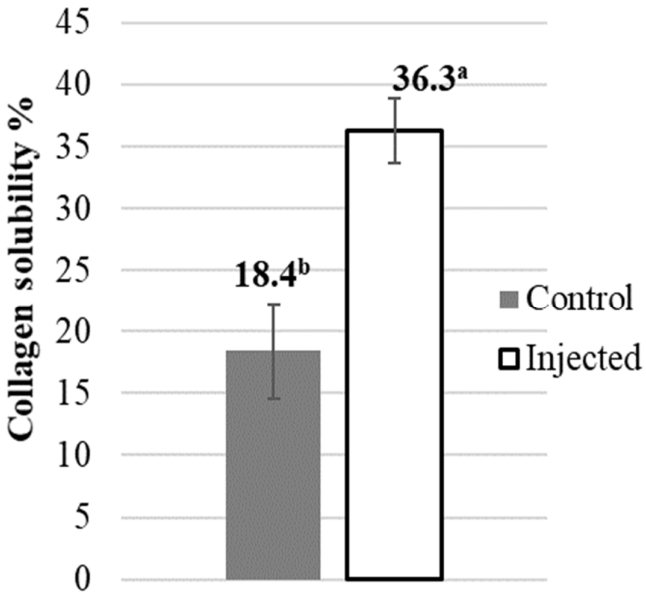


**Figure 6.2 Effects of *S. dubium* protease extract injections on the collagen solubility (%) of beef *m. longissimus thoracis et lumborum* (LTL) samples.**

### *6.4.2 Muscle fibre types and quantification meat degradation*

No differences ($P > 0.05$) were observed between the injected treatments (injected vs control) for muscle fibre types (% fibre type and fibre areas) (Table 2). Figure 6.3 presents examples of histological images of the beef LTL samples that were subject to different injection treatments (injected vs control). The muscle fibres from the muscles injected with the protease extract of *S. dubium* seeds were observed to have more fractures and breaks (Figure 6.3C and Figure 6.3D) than those from the control samples (Figure 6.3A and Figure 6.3B). These observed fractures and breaks were possibly due to the action of the exogenous protease enzyme of *S. dubium* seeds.

**Table 6.2 Effects of *S. dubium* protease extract injections on the muscle fibre types of beef *m. longissimus thoracis et lumborum* (LTL) samples.**

| | Injection | | |
|---|---|---|---|
| Item | **Control** | **Injected** | **P-value** |
| Fibre type % | | | |
| Red fibre Type 1 | 35.62 ± 3.83 | 35.66 ± 3.45 | 0.977 |
| Intermediate fibre Type IIA | 26.35 ± 4.72 | 26.95 ± 2.19 | 0.694 |
| White fibre Type IIB | 38.03 ± 2.80 | 37.39 ± 2.92 | 0.591 |
| Fibre areas ($\mu m^2$) | | | |
| Red fibre Type 1 | 2582.67 ± 614.93 | 2311.42 ± 228.04 | 0.166 |
| Intermediate fibre Type IIA | 3355.75 ± 697.53 | 3150.42 ± 307.37 | 0.361 |
| White fibre Type IIB | 5480.92 ± 998.06 | 5189.75 ± 601.79 | 0.396 |

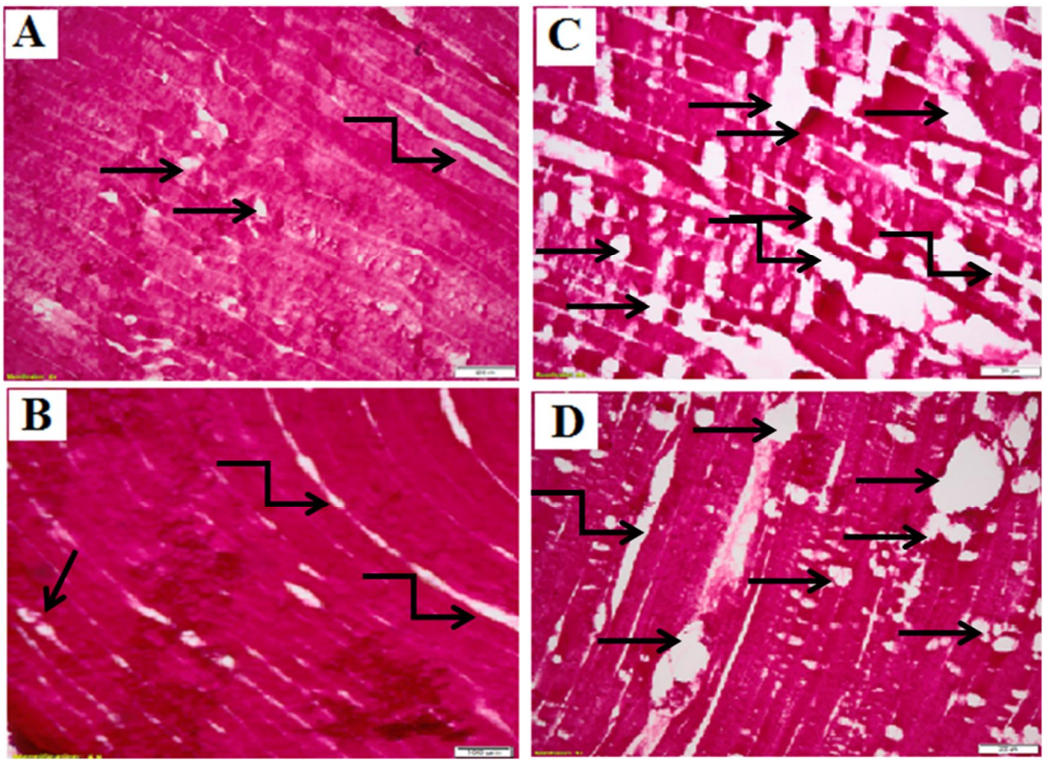


**Figure 6.3 Longitudinal sections of beef *m. longissimus thoracis et lumborum* (LTL) of control samples (non-injected) (A, B) and samples injected with protease extract of *S. dubium* seeds (C, D). Fractured muscle fibres are indicated by straight arrows, whereas breaks are indicated by elbow arrows.**

Fibre breaks and detachment (% white to red area) were quantified and found to be higher ($P < 0.0001$) for injected muscles than for non-injected ones (Table 6.3). These findings may also explain the lower WBSF values of the injected muscles, since muscle fibre fractures, breaks, and detachment are indications of the proteolytic action of enzymes that relate to meat tenderness (Taylor & Frylinck, 2003; Veiseth-Kent, Hollung, Ofstad, Aass, & Hildrum, 2010).

**Table 6.3 Rank means (mean) for the fibre breaks score and means (± SD) for fibre detachment of beef *m. longissimus thoracis et lumborum* (LTL) samples affected by injection treatment (injection with *S. dubium* protease extract vs control).**

| | Injection | | |
|---|---|---|---|
| Item | **Control** | **Injected** | **P-value** |
| Fibre breaks score (1-5) | **7.17 (1.58)** | **17.83 (4.5)** | **< 0.0001** |
| Fibre detachment: % white area | **16.92 ± 5.33** | **25.92 ± 4.06** | **< 0.0001** |

### *6.4.3 SDS-PAGE*

The results of the SDS–PAGE pattern indicated pronounced proteolytic changes between the injected and non-injected muscles (Figure 6.4A). The myofibrillar proteins extracted from the meat injected with the protease extract of *S. dubium* seeds had a lower number of protein bands of high molecular weights (50 – 230 kDa) (Figure 6.4A and Figure 6.4B) than the control samples (Figure 6.4A and Figure 6.4C). However, the number of protein bands of low molecular weights (22.2 kDa and below) increased in the injected meat (Figure 6.4A and Figure 6.4B) compared with the control samples (Figure 6.4A and Figure 6.4C). These fragments of low molecular weight proteins were probably result of degradation. Therefore, these observations indicated that the exogenous protease extract of *S. dubium* seeds degraded the myofibril proteins of high molecular weights into lower molecular weights, which resulted in more tender meat. Liu *et al*. (2011) reported that meat injected with the protease enzyme of kiwifruit juice showed a significant loss of higher molecular weight fractions with

the presence of many new lower molecular weight bands underneath, because of the breakdown of the myosin-heavy chain, thus improving the meat's tenderness.

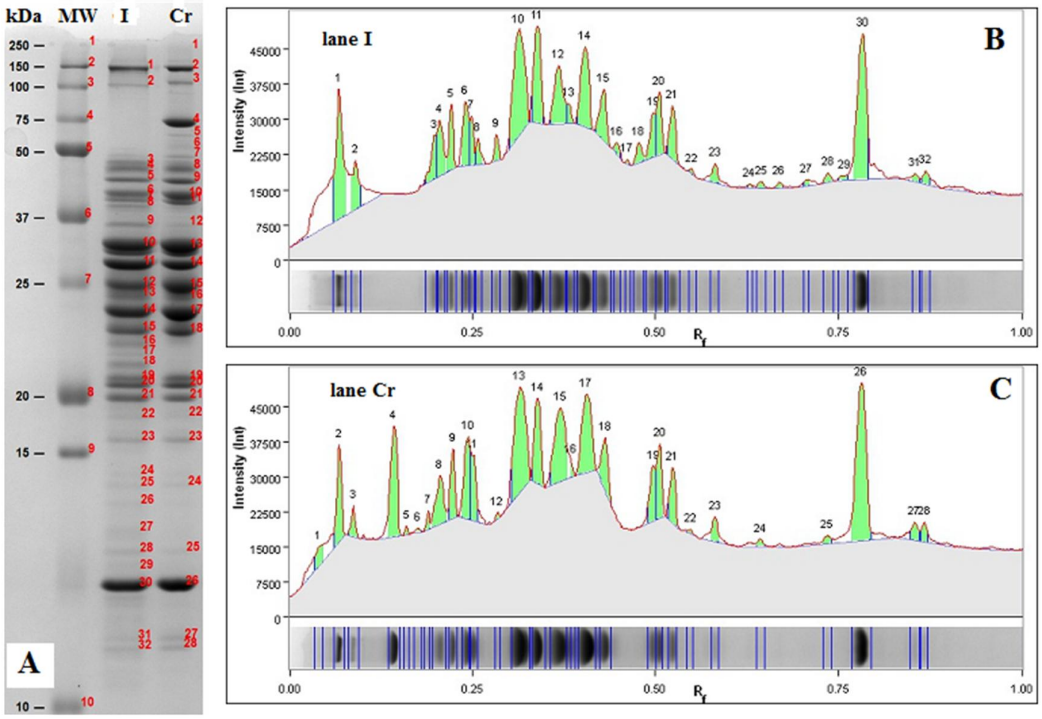


**Figure 6.4A. SDS-PAGE pattern of myofibrillar proteins isolated from beef *m. longissimus thoracis et lumborum* (LTL) subjected to injection treatment (injected vs control). Lane MW molecular weight marker; lane I, injected muscle sample with protease extract of *S. dubium* seeds; lane Cr, control muscle sample (non-injected).** Figure 6.4B. **The expression of the bands from the injected muscle sample (lane I).** Figure 6.4C. **The expression of the bands from the control muscle sample (lane Cr).**

### *6.4.4 Correlation coefficients*

The correlation coefficients between the muscle fibre types (% fibre type and fibre areas) and meat colour (L*, a*, b*, chroma, and hue-angle) are presented in Table 6.4. There was no significant correlation between the muscle fibre areas and the meat colour, while the red fibre Type 1 % and intermediate fibre Type IIA % showed a good and significant correlation with some colour parameters. Red fibre Type 1 % showed a good positive

correlation with redness (r = 0.528, P < 0.01) and chroma (r = 0.451, P < 0.05). Kim *et al.* (2010) reported that the myoglobin content increases as the proportion of red muscle fibres increases, leading to high redness and low lightness. With this, the chroma increases and the hue-angle decreases. In the present study, the Red Type 1 proportion had negative correlations with lightness (r = - 0.330) and hue-angle (- 0.262), but they were not significant. Intermediate fibre Type IIA % showed a good negative correlation with redness (r = - 0.518, P < 0.01) and a good positive correlation with hue-angle (r = 0.441, P < 0.05). This was contrary to previous reports that found positive relationships between fibre Type IIA composition and redness (Kim *et al.*, 2010; Kim, Yang, & Jeong, 2016). The negative correlation is difficult to explain; however, the intermediate fibre Type IIA is characterised by a lower myoglobin content than the red fibre Type I, and colour is more related to the myoglobin content (Lawrie & Ledward, 2006).

**Table 6.4 Correlation coefficients between muscle fibre types and meat colour of beef *m. longissimus thoracis et lumborum* (LTL).**

| | Lightness (L*) | Redness (a*) | Yellowness (b*) | Chroma | Hue-angle |
|---|---|---|---|---|---|
| **Fibre type %** | | | | | |
| Red fibre Type 1 | - 0.330 | **0.528**** | 0.327 | **0.451*** | - 0.262 |
| Intermediate fibre Type IIA | 0.239 | **- 0.518**** | - 0.180 | - 0.362 | **0.441*** |
| White fibre Type IIB | 0.112 | - 0.005 | - 0.183 | - 0.106 | - 0.234 |
| **Fibre areas (µm$^2$)** | | | | | |
| Red fibre Type 1 | - 0.114 | - 0.135 | - 0.207 | - 0.189 | - 0.088 |
| Intermediate fibre Type IIA | - 0.169 | - 0.086 | - 0.096 | - 0.100 | - 0.004 |
| White fibre Type IIB | - 0.168 | - 0.256 | - 0.366 | - 0.340 | - 0.140 |

** P < 0.01

* P < 0.05

**Table 6.5 Correlation coefficients between Warner-Bratzler shear force and histological characteristics and collagen solubility of beef *m. longissimus thoracis et lumborum* (LTL).**

| | Sarcomere length (μm) | Myofibril fragment length (μm) | Fibre breaks | Fibre detachment | Collagen solubility |
|---|---|---|---|---|---|
| Warner-Bratzler shear force (kg) | - 0.207 | **0.797*** ** | **- 0.681**** | **- 0.566**** | **- 0.934*** ** |

*** $P < 0.001$

** $P < 0.01$

The Warner-Bratzler shear force (WBSF) showed high negative correlations with fibre breaks ($r = - 0.681$, $P < 0.01$), fibre detachment ($r = - 0.566$, $P < 0.01$), and collagen solubility ($r = - 0.934$, $P < 0.001$), and strong positive correlations with MFL ($r = 0.797$, $P < 0.001$) (Table 5). Low and insignificant negative correlations were detected between the WBSF and SL ($r = - 0.207$, $P > 0.05$) (Table 5). Similarly, Taylor and Frylinck (2003) assessed the quantification of sarcomere length and myofibrillar structure degradation in different beef breeds, and stated that meat tenderness is related to muscle fibre fractures, breaks, and detachment, but not to the lengths of a sarcomere. However, Stolowski *et al*. (2006) reported that tenderness increases as sarcomere lengths increase. The strong relationship between collagen solubility and WBSF was expected since, in this study, the exogenous protease enzyme from the seeds of *S. dubium* hydrolysed the collagen greatly, resulting in more tender meat. Good relationships between myofibrillar fragmentation and tenderness have been reported (Muchenje, Dzama, Chimonyo, Raats, & Strydom, 2008; Strydom, Naude, Smith, Scholtz, & Van Wyk, 2000). In general, shorter MFLs or a higher myofibril fragmentation index (MFI) are positively associated with proteolysis and a decreased shear force (Dosler, Polak, Zlender, & Gasperlin, 2007; Frylinck *et al*., 2009). Strydom, Frylinck and Smith (2005) reported that MFL was a good predictor of improved tenderness during prolonged ageing. The current study indicated that MFL was a good predictor of the differences in tenderness.

## 6.5 CONCLUSIONS

The present study confirmed that the protease present in the seeds of *S. dubium* was a powerful meat tenderiser, and resulted in a lower shear force without a negative influence on meat colour. The results for MFL, quantification of myofibrillar structure degradation, and SDS-PAGE reflected the strong proteolytic activity of the protease on myofibrillar proteins. The protease extract of *S. dubium* seeds showed a great hydrolysis of myofibrillar and connective tissue (collagen) proteins. This study showed that the proportions of red and intermediate fibre types are a good indicator of meat redness. The results further revealed that meat tenderness is strongly correlated with collagen solubility, fibre breaks, fibre detachments, and MFLs. The protease enzyme from the seeds of *S. dubium* is a promising source of an exogenous proteolytic enzyme that could have a wide application in the meat industry. Further research is required to identify the specific proteins affected by the protease extract of *S. dubium* seeds and the tenderising effect of purified dubiumin enzyme. The current results could pave the way for a promising new meat tenderiser to improve the tenderness of meat.

## 6.6 Conflicts of interest

The authors declare that there is no conflict of interest in this work.

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# CHAPTER 7

## Conclusions, Recommendations, and Areas for Further Research

### 7.1 Conclusions

The Sudanese beef industry is in a unique situation when compared with other developing countries. Its production of beef depends mainly on nomadic systems in its extensive rangelands. The indigenous Baggara cattle types produce carcasses that vary markedly in quality. Cattle are sold and slaughtered at mature ages (≥ four years old). Nor is any intervention (electrical stimulation [ES], enzymatic) used to manage beef quality. Therefore, this study aimed to evaluate the effects of pre-slaughter factors such as breed, age, and weight at slaughter on the carcasses and meat quality of Sudanese Baggara cattle. This study also assessed the effects of *post mortem* interventions such as ES, post-freezing ageing, and enzymatic proteases (protease extract from seeds of the Gubbain plant) on beef quality of Sudanese Baggara cattle. Additionally, the study assessed the microstructural and biochemical properties of beef muscles treated with the Gubbain protease. ES hastened the rate of pH decline up to 24 h *post mortem*, compared with non-electrical stimulation. Based on the results, the ES of Sudanese Baggara beef carcasses and the use of younger animals yielded more tender beef with better colour. However, this was accompanied by a reduction in water-holding capacity (WHC). There were no breed differences in the characteristics of the meat quality; however, carcasses of the Mesairi breed type responded better to ES in terms of tenderness than those of the Nyalawi breed type. It was observed that there was no risk of cold shortening. Although post-freezing ageing had an adverse effect on the colour stability of the meat, it had a beneficial influence on its tenderness. Panelists did not find differences in their meat sensory evaluation due to age and weight at slaughter. In contrast, the variations in sensory tenderness

and overall acceptability due to ES and treatments with the protease extract of *Solanum dubium* seed were recognised.

Aqueous extract from the seeds of the Gubbain plant (*Solanum dubium*) was able to improve both the instrumental and the sensory measures of tenderness without an adverse impact on its colour and eating qualities. Based on the results of myofibril fragment length (MFL), SDS-PAGE, and the quantification of myofibrillar structure degradation, the protease extract from the Gubbain plant showed a strong proteolytic activity on myofibrillar proteins. The protease extract also significantly hydrolysed the proteins of connective tissue (collagen). The current results could pave the way for a promising new meat tenderiser to improve meat tenderness. The invention of using protease from the seeds of the Gubbain plant (*Solanum dubium*) as a meat tenderiser has been patented in Sudan by the General Intellectual Property Registrar (national patent No. 4114).

This study has shown that there is the potential to produce good quality beef from Sudanese Baggara cattle, if certain interventions (ES, slaughtering younger and lighter bulls, post-freezing ageing, and injection with protease from *Solanum dubium* seeds) are practised.

## 7.2 Recommendations

Based on the results of the current study, the recommendations include the following:

1. Electrical stimulation (ES) technique should be implemented in Sudan to improve beef quality.
2. Baggara breeders should be encouraged to sell cattle at a younger age for further feedlot fattening before slaughter.
3. Ageing after freezing may be a useful option to enhance beef tenderness.
4. A protease extract from the seeds of the Gubbain plant (*Solanum dubium*) should be developed and used by the Sudanese meat industry as a commercial meat tenderiser.

## 7.3 Areas for further research

Future studies could cover the following suggested topics:

1. The use of high voltage electrical stimulation of Sudanese beef carcasses to yield better meat quality.
2. Using a purified protease enzyme of *Solanum dubium* seeds in the meat industry.
3. The technology of the recombinant production of enzymes as a help in producing the protease enzyme of the Gubbain plant (*Solanum dubium*) for further research and commercial purposes.
4. Identifying the specific proteins that are influenced by the proteolytic enzyme of *Solanum dubium* plant.
5. Study the tenderising effects of other naturally growing plants and herbs in Sudan.
6. Identify the best practical route of administering *Solanum dubium* enzyme into the live animal, carcass or wholesale cuts.