

THE EFFECT OF ELECTRICAL INPUTS DURING
BEEF PROCESSING ON RESULTANT MEAT
QUALITY

by

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*Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Agriculture (MScAgric) (Animal Science)*

*at
Stellenbosch University*

The crest of Stellenbosch University, featuring a shield with a blue and white design, topped with a red and white crest, and surrounded by a red and white wreath.

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained is my own, original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

The two main determinants of post-slaughter processing outcomes are rates of pH and temperature decline. Muscle pH and temperature interact continuously during rigor development to affect both the muscle contracture and proteolytic enzyme activity. The pH, however, can be manipulated independently of temperature by electrical inputs applied to the carcass. Electrical inputs that should be considered range from electrical stunning to the various forms of electrical immobilisation (EI) and stimulation (ES) that occur during and after the dressing procedures. EI is used to suppress convulsions that occur after electrical stunning to ensure operator safety to maintain high throughputs speeds while ES is used to induce rapid tenderisation, although having other biochemical and biophysical effects on meat.

The objective of the study was to supply information on the effect of different EI and ES treatments, frequencies and pulse widths on the meat quality of beef. There are very little data on the effect of EI when it is combined with ES on meat quality. This study used two different EI frequencies (high – 800 Hz; HFI and low – 15 Hz; LFI) combined with either high (1040 V; HVS) or medium (300 V; MVS) voltage ES to study the effect of these treatments on meat quality. In the following experiment the EI waveform and ES was standardised using HFI with MVS with the frequency being changed to either 5, 15 or 50 Hz. Then the pulse width of the waveform was changed to 0.1, 0.5, 1 and 10 ms to optimise the ES system.

Meat quality measurements were made from the *Longissimus dorsi* (LD) and *Semimembranosus* (SM) after 1, 5 and 9 days of chilled storage at 0°C. The LD (shear force = 94.3±2.2; cooking loss = 26.85±0.29; retail drip = 0.996±0.037; storage drip = 2.78±0.155; WBC (water binding capacity) = 45.4±0.36) had significantly lower shear force and higher water binding capacity than the SM (shear force = 103.7±2.5; cooking loss = 34.63±0.25; retail drip = 2.12±0.103; storage drip = 3.63±0.245; WBC = 59.3±0.57). Day of assessment (Day 1 = 122.7±2.9; Day 5 = 87.7±2.2; Day 9 = 81.0±2.4) had a significant effect on tenderness of the LD as shear force declined with an increase of day of assessment. The LFI HVS (storage drip = 3.30±0.223; shear force = 102.9±4.5) produced significantly greater drip during storage and shear force values when compared to the HFI followed by either HVS (storage drip = 2.45±0.261; shear force = 85.2±4.0) or MVS (storage drip = 2.60±0.178; shear force = 94.2±4.2) in the LD, probably attributable to different rates of pH decline post mortem. LFI HVS (a^* = 20.79±0.31; chroma = 22.92) and LFI MVS (a^* = 20.24±0.27; chroma = 22.23±0.30) had a redder and more vivid bloomed colour than HFI HVS (a^* = 19.71±0.33; chroma = 21.49±0.37) and HFI MVS (a^* = 20.00±0.27; chroma = 21.98±0.31), while LFI HVS (a^* = 15.27±0.40) and HFI MVS (a^* = 14.64±0.29) had a redder colour compared to HFI HVS (13.85±0.35) at day 9 for the LD. The oxygen consumption rate (MTT assay) correlated inversely linear ($r = -0.63$ and -0.73) with the a^* values 24 hrs post mortem allowing for 3 hrs of bloom.

Stimulation with 15 Hz (0.47±0.040) and 5 Hz (0.41±0.045) had a higher pH decline (Δ pH) during stimulation than 50 Hz (0.29±0.027). Shear force measurements and cooking loss percentage were obtained from the LD after 24 hrs of chilled storage at 0°C. There were no difference between the stimulation treatments for shear force (15 Hz = 121.3±3.3; 5 Hz = 123.8±7.6; 50 Hz = 114.8±7.94), while cooking loss was higher in 15 Hz (28.8±0.47) than 50 Hz (25.9±0.71) which correlated ($r = 0.43$; $p = 0.01$) with Δ pH.

There were no differences between 10 ms (0.46±0.020), 1 ms (0.43±0.020) and 0.5 ms (0.44±0.019) pulse widths on the Δ pH while 0.1 ms (0.33±0.020) had a lower decline. Stimulation with a 1 ms (94.6±5.6) pulse width had the lowest shear force that varied from 10 (111.3±3.8) and 0.1 ms (111.3±5.8). While cooking loss (0.1 = 25.3±0.48; 0.5 = 26.9±0.67; 1 = 25.9±0.63; 10 = 25.5±0.66) and water-holding capacity (0.1 =

36.1±1.60; 0.5 = 37.3±1.42; 1 = 37.5±1.15; 10 = 36.9±1.45) was not affected in the LD after 24 hrs of chilled storage at 0°C. Colour measurements on the SM indicated that a 0.1 (a* = 19.38±0.50; chroma = 22.70±0.51), 0.5 (a* = 20.89±0.49; chroma = 24.34±0.56) and 10 ms (a* = 19.69±0.46; chroma = 22.98±0.58) pulse width had a deeper red and a more vivid colour than 1 ms (a* = 16.66±0.37; chroma = 19.99±0.32) at day nine of retail display.

In conclusion, HFI improves meat quality when combined with either HVS or MVS and that MVS either improves (colour stability) or has no adverse effects on meat quality (tenderness and WBC) in relation to HVS when combined HFI. In addition, it shows that there are alternative electrical parameters to voltage that can be used to change the pH decline and by changing frequency and pulse width, subtle changes can be made to an ES system. Since every abattoir is different due to layout, chiller space and cooling regime these electrical parameters can be modulated to optimise an electrical stimulation system without expensive modification to the whole system.

OPSOMMING

Die tempo van pH en temperatuur daling is die twee hoof bepalings van na-slag prosesserings uitkomst. Spier pH en temperatuur het 'n gedurige interaksie tydens *rigor* ontwikkeling en beïnvloed die spier sametrekking en proteolitiese ensiem aktiwiteit. Die spier pH kan onafhanklik van temperatuur gemanipuleer word, deur elektriese golfvorms deur die karkas te stuur. Die elektriese golfvorms wat in ag geneem moet word varieer van elektriese impulse tydens bedwelming tot die verskeie golfvorms van elektriese immobilisasie (EI) en stimulasie (ES) wat gebruik kan word gedurende en na die slagproses. EI word gebruik om konvulsies te beheer wat ontstaan na elektriese bedwelming om werker veiligheid en hoë deurvloei tempos te verseker, terwyl ES die verouderings proses versnel, alhoewel dit ander biochemiese en biofisiese uitwerkings het op vleis.

Die studie het verneem om inligting te verskaf oor die effek van verskillende EI en ES kombinasies, frekwensie en puls wydtes op die kwaliteit van beesvleis. Daar is baie min inligting van EI in kombinasie met ES se effek op vleis kwaliteit. Die studie het gebruik gemaak van twee verskillende (EI) frekwensies (hoog – 800 Hz; HFI and laag – 15 Hz; LFI) wat gekombineer is met of hoë (1040 V; HVS) of medium (300 V; MVS) spanning ES se effek op vleis kwaliteit. In die volgende eksperiment was die EI golfvorm en die ES gestandaardiseer en HFI met MVS was gebruik met die frekwensie wat verander is tussen 5, 15 en 50 Hz. Daarna was die pulse wydte van die golfvorm verander tussen 0.1, 0.5, 1 en 10 ms om die ES sisteem te optimaliseer.

Vleis kwaliteit van die *Longissimus dorsi* (LD) en *Semimembranosus* (SM) spiere was bepaal na 1, 5 en 9 dae van verkoelde storing teen 0°C. Die LD (skeurkrag = 94.3 ± 2.2 ; kookverlies = 26.85 ± 0.29 ; kleinhandel drup verlies = 0.996 ± 0.037 ; storing drup verlies = 2.78 ± 0.155 ; WBV (water bindings vermoë) = 45.4 ± 0.36) het 'n betekenisvolle laer skeurkrag waardes en hoër water bindings vermoë gehad in vergelyking met die SM (skeurkrag = 103.7 ± 2.5 ; kookverlies = 34.63 ± 0.25 ; kleinhandel drupverlies = 2.12 ± 0.103 ; bergings drupverlies = 3.63 ± 0.245 ; WBV = 59.3 ± 0.57). Die dag van assesering (Dag 1 = 122.7 ± 2.9 ; Dag 5 = 87.7 ± 2.2 ; Dag 9 = 81.0 ± 2.4) het 'n betekenisvolle effek gehad op die skeur krag waardes en het afgeneem met 'n toename in die dag van assesering. LFI HVS (storing drupverlies = 3.30 ± 0.223 ; skeurkrag = 102.9 ± 4.5) het betekenisvolle hoër vog verliese gehad tydens verkoelde storing en skeur krag wanneer dit vergelyk word met HFI gevolg deur of HVS (storing drupverlies = 2.45 ± 0.261 ; skeurkrag = 85.2 ± 4.0) of MVS (storing drupverlies = 2.60 ± 0.178 ; skeurkrag = 94.2 ± 4.2). LFI HVS ($a^* = 20.79 \pm 0.31$; chroma = 22.92) en LFI MVS ($a^* = 20.24 \pm 0.27$; chroma = 22.23 ± 0.30) het 'n helder en dieper rooi kleur gehad in vergelyking met HFI HVS ($a^* = 19.71 \pm 0.33$; chroma = 21.49 ± 0.37) en HFI MVS ($a^* = 20.00 \pm 0.27$; chroma = 21.98 ± 0.31), terwyl LFI HVS ($a^* = 15.27 \pm 0.40$) en HFI MVS ($a^* = 14.64 \pm 0.29$) 'n rooier en helderer kleur as HFI HVS (13.85 ± 0.35) gehad het in die LD. Die suurstof verbruik tempo (MTT analise) korreleer omgekeerd ($r = -0.63$ en -0.73) met die a^* waardes 24 hr *post mortem* na 3 hr van blootstelling van lug.

Stimulasie met 15 (0.47 ± 0.040) en 5 Hz (0.41 ± 0.045) het 'n hoër pH daling (ΔpH) tydens stimulasie as 50 Hz (0.29 ± 0.027). Skeurkrag waardes en kookverliese is verkry vanaf die LD na 1 dag van verkoelde storing teen 0°C. Daar was geen verskil tussen stimulasie frekwensie se effek of skeurkrag (15 Hz = 121.3 ± 3.3 ; 5 Hz = 123.8 ± 7.6 ; 50 Hz = 114.8 ± 7.94) nie, terwyl die kookverliese hoër was in die 15 Hz (28.8 ± 0.47) as 50 Hz (25.9 ± 0.71) behandeling wat gekorreleer ($r = 0.43$; $p = 0.01$) het met ΔpH .

Daar was geen verskill tussen 10 (0.46±0.020), 1 (0.43±0.020) en 0.5 ms (0.44±0.019) puls wydtes se effek op Δ pH nie, terwyl 0.1 (0.33±0.020) ms 'n kleiner afname tot gevolg gehad het. Stimulasie met 'n 1 ms (94.6±5.6) puls wydte het die laagste skeurkrag gehad wat verskil het van die 10 (111.3±3.8) and 0.1 ms (111.3±5.8) puls wydtes, terwyl kookverliese (0.1 = 25.3±0.48; 0.5 = 26.9±0.67; 1 = 25.9±0.63; 10 = 25.5±0.66) en waterbindingsvermoë (0.1 = 36.1±1.60; 0.5 = 37.3±1.42; 1 = 37.5±1.15; 10 = 36.9±1.45) nie beïnvloed was nie. Kleur metings van die SM het getoon dat 'n 0.1 (a^* = 19.38±0.50; chroma = 22.70±0.51), 0.5 (a^* = 20.89±0.49; chroma = 24.34±0.56) en 10 ms (a^* = 19.69±0.46; chroma = 22.98±0.58) puls wydtes die helder en dieper rooi kleur gehad het as 1 ms (a^* = 16.66±0.37; chroma = 19.99±0.32) teen dag 9 van kleinhandel vertoning.

Ter opsomming, lei HFI tot beter vleis kwaliteit wanneer dit gekombineer word met of HVS of MVS. Verder lei MVS tot of 'n verbetering (kleur stabiliteit) of geen nadelige effek op vleis kwaliteit (sagtheid en WBV) in vergelyking met HVS wanneer dit gekombineer word met HFI. Die studie bewys ook dat daar ander elektriese parameters bestaan as spanning, wat verander kan word om die pH daling te beïnvloed. Deur die frekwensie en pulswydte te verander, kan klein veranderinge aangebring word aan 'n ES sisteem. Elke abattoir is verskillend as gevolg van uitleg, koelkamer spasie en verkoelings tempo en hierdie elektriese parameters kan verander word om 'n ES sisteem te optimiseer sonder enige duur veranderinge aan die hele sisteem.

ACKNOWLEDGEMENTS

On the completion of this thesis, I would like to express my sincerest appreciation and gratitude to the following people and institutions:

- Prof. L.C. Hoffman of the Department of Animal Sciences, Stellenbosch University, my supervisor, for his knowledge, guidance and for his ability to challenge my thinking pattern throughout my study. He made it possible to conduct my research in New Zealand, and for this I am extremely grateful;
- Dr. N.J. Simmons of Carne Technologies, Cambridge, New Zealand, my co-supervisor, for her friendship, opportunity to work at Carne Technologies, guidance, support and advice throughout my study;
- NRF (National Research Foundation) for the two year scholarship that partly funded this study;
- MWNZ (Meat and Wool New Zealand) for financial assistance;
- MLA (Meat and Livestock Australia) for financial assistance;
- Dr. C.C. Daly of Carne Technologies, Cambridge, New Zealand, for his incredible knowledge, support and advice throughout my study;
- T.L. Cummings, N.V. Johnson, J.M. McGurk, S.K. Morgan and T. Lomas, the personnel of Carne Technologies, Cambridge, New Zealand, for their friendship and technical assistance during this study;
- Stellenbosch University for providing me with a structure where I can learn and grow;
- My parents, Anthonie and Alwynna, for their endless love, encouragement and support;
- To my fellow students from Animal Science, for their endless emotional support, patience, advice, encouragement, good humour and friendship;
- All my friends and family for their encouragement, humour, friendship and emotional support.
- To my housemates in New Zealand, Anthony, Becki and Dandan for taking a stranger into their home and becoming life long friends;
- To my flatmates, Lizelle and Liezl, for the great times, friendship and dealing with my mood swings;

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
EI	Electrical immobilisation
ES	Electrical stimulation
HFI	High frequency immobilisation
LFI	Low frequency immobilisation
HVS	High voltage stimulation
MVS	Medium voltage stimulation
V	Voltage
mA	milliamps
mins	minutes
Hz	Hertz
s	seconds
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
P _i	Inorganic phosphate
DHP	Dihydropyridine
SHD	Succinic dehydrogenase
Mb	Myoglobin
MbO	Oxymyoglobin
MMb	Metmyoglobin
LD	<i>Longissimus dorsi</i>
SM	<i>Semimembranosus</i>
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
pH _u	Ultimate pH
ΔpH	pH decline during stimulation
PSE	Pale, soft and exudative
DFD	Dark, firm and dry
WBC	Water binding capacity
L*	Lightness
a*	Red-green colour range
b*	Blue-yellow colour range

NOTES

The language and style used in this thesis are in accordance with the requirements of the scientific journal, South African Journal of Animal Science. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between the chapters has, therefore, been unavoidable.

Results of this study have been represented at the following symposium:

1. Lombard, A.C., Hoffman, L.C., Johnson, N.V., McGurk, J.M., Cummings, T.L., Daly, C.C. & Simmons, N.J., 2008. The effect of different electrical immobilisation and stimulation procedures on meat quality of beef. In: Proceedings of the 54th International Congress of Meat Science and Technology, 10-16 August, 2008, Cape Town, South Africa.

LIST OF CONTENTS

Chapter 1	1
Introduction	1
1.1 References	3
Chapter 2	5
Literature review	5
2.1 Introduction	5
2.2 Skeletal muscle	5
2.2.1 Morphology	6
2.2.2 Ultrastructure	6
2.2.3 Proteins involved in contraction	7
2.2.4 Muscle contraction	7
2.3 Meat quality characteristics	8
2.3.1 Colour	8
2.3.2 Water-binding capacity	11
2.3.3 Tenderness	13
2.4 Processing options related to pH and temperature interaction	13
2.5 Cold and rigor shortening	14
2.6 The effects of temperature at the time of rigor on the calpains	15
2.7 Diverse market requirements	16
2.8 Electrical stimulation	17
2.8.1 Historical overview	17
2.8.2 Mechanism and effect of electrical stimulation on pH decline	17
2.9 Effects of electrical stimulation on quality characteristics	19
2.9.1 Colour	19
2.9.2 Water-binding capacity	20
2.9.3 Tenderness	20
2.9.3.1 Possible reasons for increased tenderness	20
2.9.3.2 Shear-force values	22
2.9.3.3 Sarcomere length	23
2.9.3.4 Proteolysis	23
2.9.3.5 Calpain and calpastatin activity	25
2.9.3.6 Calcium levels	26
2.9.3.7 Ageing	26
2.9 Electrical Immobilisation	27
2.10 Classification of Electrical inputs	29
2.10.1 Pulse frequency	32
2.10.2 Pulse width and amplitude	33
2.10.3 Pulse polarity	34
2.11 Physiological response to stimulation	34
2.12 Conclusion and Objectives	36
2.13 References	37
Chapter 3	45
Electrical immobilisation and stimulation of beef carcasses and its effect on meat quality	45
3.1 Introduction	45
3.2 Materials and methods	47
3.2.1 Animals and treatments	47
3.2.2 pH and temperature measurements	48
3.2.3 Shear force and cooking loss	48
3.2.3 Drip loss (retail and storage)	48

3.2.4	Water-binding capacity	49
3.2.5	Colour and colour stability	49
3.2.6	MTT (Tetrazolium Salt) Assay	50
3.2.7	Myofibrillar density assay.....	50
3.2.8	Statistical analysis	50
3.3	Results.....	51
3.3.1	pH decline	51
3.3.2	Temperature	52
3.3.3	Shear force.....	52
3.3.4	Cooking loss.....	54
3.3.5	Drip loss	55
3.3.6	Water-binding capacity	57
3.3.7	Colour.....	58
3.3.8	MTT assay.....	68
3.3.9	Myofibrillar density.....	69
3.4	Discussion	70
3.4.1	pH.....	70
3.4.2	Shear force.....	71
3.4.3	WBC.....	72
3.4.4	Colour stability.....	73
3.4.5	MTT and Myofibrillar density.....	75
3.5	Conclusion	75
3.6	References	76
The effect of different electrical stimulation frequencies on muscle pH decline and beef tenderness		80
4.1	Introduction	81
4.2	Materials and methods.....	83
4.2.1	Treatments and pH measurement	83
4.2.2	Shear force and Cooking loss.....	83
4.2.3	Statistical analysis	83
4.3	Results.....	84
4.3.1	pH.....	84
4.3.2	Shear force and cooking loss	84
4.3	Discussion	85
4.3.1	pH.....	85
4.3.2	Cooking loss.....	86
4.3.3	Shear force.....	86
4.4	Conclusion.....	87
4.4	References	88
Chapter 5.....		90
The effect of small pulse widths during electrical stimulation on muscle pH decline and denaturation of beef proteins		90
5.1	Introduction	90
5.2	Materials and methods.....	92
5.2.1	Animals and treatments	92
5.2.2	pH measurement.....	92
5.2.3	Shear force and cooking loss	92
5.2.4	Water-binding capacity	93
5.2.5	Colour stability.....	93
5.2.6	Statistical analysis	93
5.3	Results.....	94
5.3.1	pH.....	94
5.3.2	Cooking loss.....	95
5.3.3	Shear force.....	96

5.3.4 WBC.....	96
5.3.5 Retail Colour Display.....	97
5.4 Discussion	101
5.4.1 pH.....	101
5.4.2 Shear-force values	101
5.4.3 WBC.....	102
5.4.4 Colour stability.....	103
5.5 Conclusion.....	104
5.6 References	105
Chapter 6	108
Conclusion	108
6.1 References	110

Chapter 1

INTRODUCTION

The inconsistency in the eating-quality characteristics of meat, predominantly tenderness, is probably the most critical problem faced by the meat industry worldwide. Consumers consider tenderness to be the most important component of meat quality. A number of procedures to ensure meat tenderness have been developed (such as suspension via the pelvic bone, mechanical restraint of muscles, conditioning, cooler ageing, high temperature conditioning, delayed chilling, blade or needle tenderisation, use of tropical plant or fungal enzymes, etc.) (Lawrie, 1998). All of these procedures cause changes in meat tenderness by affecting the contracting machinery (muscle fibres), the collecting, harnessing and reinforcing structures (connective tissues) or both. The factors affecting muscle tenderness the last few decades have been extensively researched. First, the connective-tissue component received the greatest attention but more recently the state of muscle contraction following *rigor mortis* has been most intensively studied (Cross, 1979).

The discovery by Locker & Hagyard (1963) that muscle shortening occur during *rigor mortis* and it causes meat to toughen has led to the realisation that post-mortem treatments sometimes outweigh live-animal factors such as breed, age, stress and pre-slaughter handling in determining meat quality (Cross, 1979). The two main determinants of post-mortem processing outcomes are rates of pH and temperature decline (Simmons *et al.*, 2006). Muscle pH and temperature continuously interact during *rigor* development to affect both muscle contracture (Tornberg, 1996) and proteolytic enzyme activity (Dransfield *et al.*, 1992). One of the most important factors in enhancing tenderisation (or reducing toughening) during high temperature conditioning of muscle is reducing pH while the muscle temperature is still relatively high. This not only reduces the amount of shortening but also increases tenderness in the absence of differences in shortening (Dutson & Pearson, 1985). The pH can, however, be manipulated independently of temperature through electrical stimulation (ES), and this presents the opportunity to manipulate meat-quality outcomes.

The concept of an electrical shock to improve tenderness was first described by Benjamin Franklin in 1749, who determined that ES of turkeys improved tenderness (Savell *et al.*, 1977). Franklin reported that “killing turkeys electrically, with the pleasant side effect that it made them uncommonly tender” was the first practical application that had been found for electricity within the meat-processing industry. Rediscovery of the use of ES for meat animals (Carse, 1973) occurred in New Zealand, largely as a consequence of the realisation that frozen lamb had the tendency of being tough, and this set the scene for many studies in New Zealand on the effects of the ES of carcasses on the eating quality of lamb.

In commercial abattoirs, pre-rigor rapid chilling of carcasses has been used to limit evaporative losses, time in chiller and microbial growth of the by lowering the surface temperature to around 2 °C at a rapid rate (James & Bailey, 1989). However, the application of rapid chilling creates the risk of producing tough meat caused by the cold-induced shortening. Lowering the muscle temperature below 10°C before *rigor* has been attained causes cold shortening. Therefore, it is critical to take measures to avoid this with rapid chilling. Electrical stimulation has proved to be one of the effective methods to prevent cold-induced shortening

(Locker & Hagyard, 1963). Electrical stimulation of muscles soon after slaughter hastens the onset of *rigor mortis* and provides the basis for the process of rapidly lowering muscle pH in lamb and beef, thus avoiding the toughening effects of cold shortening and thaw shortening (Crystall & Devine, 1978).

Electrical stimulation tends to decrease shear force values compared to non-stimulated meat (Strydom *et al.*, 2005), ensuring that the tenderness level is consumer acceptable and can be moved into the market earlier. As ageing progresses, the differences between ES and non-stimulated samples decrease (Strydom *et al.*, 2005) and can disappear completely if enough time is allowed (Chrystall & Daly, 1996) and cold-induced contractions are avoided. If carcasses receive excessive amounts of stimulation, shear force values after ageing are higher in the stimulated carcasses compared to non-stimulated treatment, especially when chilling is slow (Geesink *et al.*, 2001; Koh *et al.*, 1987). In addition, excess stimulation can reduce water-holding capacity (Eikelenboom *et al.*, 1981) and colour stability of the muscles (Unruh *et al.*, 1986). The effects of excess stimulation can be attributed to the coincidence of high temperatures and low muscle pH, conditions known to denature muscle structural proteins (Offer, 1991) and accelerate the autolysis of calpains (Simmons *et al.*, 1996). Severe *rigor* contractions also occur if *rigor* is attained at high carcass temperatures (Hertzman *et al.*, 1993) and this can be expected to affect the capacity of the meat to tenderise. Shortening during *rigor* is temperature dependent (Locker & Hagyard, 1963) and, therefore, the temperature of the muscle as it enters *rigor* is an important factor in predicting the tenderness of meat. Heat- or rigor shortening normally occurs above 20°C (up to 30%) and cold shortening between 0°C and 14°C (up to 50%). Cold shortening and rigor shortening can be classified as extremes in processing. In between lie a field of different processing options for diverse markets. The optimum balance needs to be obtained between the sometimes conflicting requirements of the products for these diverse markets. By controlling the post-mortem pH and temperature decline, *rigor* development can be managed, to provide precise meat-quality outcomes (Simmons *et al.*, 2008).

The application of ES in sheep and beef carcasses in the processing industry has been erratic around the world; this may be a reflection of a general lack of knowledge on how to optimise the technology. In South Africa, only about 30% of abattoirs use ES, and it is generally used for the wrong reasons. A low-voltage system is used after exsanguination and is incorrectly applied to increase blood drainage from the carcass. Although ES does increase blood drainage, its use should be for the processor to increase the rate of tenderisation. If ES is applied correctly, the meat has a higher degree of tenderness at an early stage post mortem, thus allowing the processor to have a market-ready product earlier. The product can be moved faster to the customer, decreasing the time of capital turnover. Electrical stimulation could also be used on the carcasses of older animals to reduce variations in tenderness that may have resulted because of age, nutrition, animal history and stress during slaughter. ES is an important application in modern abattoirs, and with the use of rapid chilling, it is beneficial to the supplier and consumer. When ES is used within a processing plant, the system and the electrical characteristics applied will be determined by the market the plant serves. It is well known that stimulation increases the rate of post-mortem glycolysis; however, other biochemical and biophysical effects have also been indicated with the use of ES (Hwang *et al.*, 2003).

In many abattoirs electrical stunning and immobilisation is combined with electrical stimulation where Halal slaughter is practised. Head-only electrical stunning meets the requirement for instantaneous and sustained loss of consciousness that allows exsanguination to be the primary cause of death. However, the epilepsy

triggered by a head-only stun cause severe convulsions that can cause injury to operators and slow down throughput rates. The standard procedure used in New Zealand plants for both sheep- and beef slaughter has been to follow the electrical stun with a period of electrical immobilisation (EI) during the bleeding procedure to suppress convulsive activity and thus allow the workers to safely undertake further work on the carcass (Simmons *et al.*, 2006). This is a relatively new technology and decreases the processing time needed on each carcass, while ensuring operator safety. However, the interaction between EI and ES and its effect on meat quality have not been intensively studied.

The objective of this study is to supply data on the effect of different EI and stimulation treatments, frequencies and pulse widths on the meat quality of beef. Abattoirs are combining electrical stunning with electrical immobilisation as a procedure to maintain high throughput speeds. There is very little data on the effect of electrical immobilisation combined with ES on meat quality. This study will make use of two different immobilisation frequencies (high – 800 Hz and low – 15 Hz) combined with either high (1040 V) or medium (300 V) voltage ES to determine the effect on meat quality. In a second experiment, the electrical immobilisation waveform is standardised as is the ES in relation to high- or medium voltage, with the frequency being modulated. In the following experiment the pulse width of the waveform is changed to optimise the ES system. The following chapters will provide background information on general aspects of ES and illustrate the procedure for optimising an ES system in accordance to the specific market requirement.

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Chapter 2

Literature review

2.1 Introduction

Electrical stimulation (ES) is used in this thesis article to apply to the situation in which an electrical current is passed through a carcass under carcass chilling regimes with the aim of ensuring that the meat is tender (Devine *et al.*, 2004). Tenderness is the most important meat-quality characteristic for the consumer (Wood *et al.*, 1998) and can be measured either subjectively by consumer panels or by means of objective measurements such as shear force (the force required to shear a piece of cooked meat) (Strydom *et al.*, 2005). It has been shown that ES improves tenderness for cattle, sheep, goats, deer and some poultry species. Its application has introduced other problems, but the overall improvement in tenderness outweighs these other problems.

ES of muscle hastens the process of rigor mortis in slaughtered animals by causing the muscle to work by means of glycolysis, resulting in an immediate pH drop (ΔpH) followed by a change in the rate of the pH decline (dpH/dt) (Chrystall & Devine, 1980). These two effects ensure that the muscle enters rigor at a high muscle temperature, and cold induced shortening is avoided; it also allows ageing to start at a higher temperature, and consequently the aging process is more rapid (Simmons *et al.*, 2008). However, there are other mechanisms also involved in meat tenderisation, such as structural disruptions (Takahashi *et al.*, 1987) and enzyme modification (Simmons *et al.*, 1996).

ES can be applied early on post-mortem (normally within 10 minutes) using low voltages ($< 100\text{ V}$) that operate via the nervous system. As the time elapsed post-mortem before stimulation is administered increases, a higher voltage is needed to directly stimulate the muscle (Lawrie, 1998). The electrical parameters generally used must be based on the consideration of the appropriate waveform, pulse frequency, pulse width, duration, pre-stimulation delay and the chilling rate (Devine *et al.*, 2004).

ES has been shown to enhance certain quality-characteristics of meat, such as lean colour and tenderness (Strydom *et al.*, 2005). The packager, retailer and consumer could therefore benefit if ES is used as an integral part of the process of converting muscle into meat. ES seems to be an important application in modern abattoirs, and with the use of rapid chilling, it is beneficial to both the supplier and consumer (Li *et al.*, 2006).

2.2 Skeletal muscle

It is important to understand the morphology, structure and proteins involved and the mechanism of muscle contraction, as ES causes contraction of the muscle fibres which results in anaerobic glycolysis and a subsequent drop in pH (Chrystall & Devine, 1980). In the process of converting muscle to meat, these mechanisms also influence the meat quality as pertaining to tenderness, water-binding capacity and colour. A good understanding of muscle physiology is important when ES is applied, since the contraction response is governed by the electrical parameters, muscle fibre type, initial glycogen stores within the muscle, the temperature of muscle and the time elapsed after exsanguination before the stimulation is applied (Chrystall

& Devine, 1985). It is therefore important to know the mechanisms involved with muscle contraction as influenced by the formerly mentioned parameters. The following section gives a brief introduction to muscle physiology.

2.2.1 Morphology

A skeletal muscle is composed of extended fibres running in parallel. Fibres are multinucleate cells formed from the combination of single nucleated myoblasts. In large limb muscles, fibres may reach a length of 30 cm with a diameter of 100 µm. The cell membrane (sarcolemma) surrounds the cytoplasm (sarcoplasm) (Rehfeldth *et al.*, 2004). The sarcoplasm contains from several hundreds to thousands of contractile elements, the myofibrils, each of which is 1–2 µm in diameter. A myofibril is compartmentalised into as many as 10 000 repeating units, called sarcomeres, joined together by dense material at Z-lines (Lawrie, 1998). These repeating units in myofibrils provide the muscle fibre with repeated cross-striations, which leads to the alternative term 'striated muscle' for skeletal muscle (Rehfeldth *et al.*, 2004). The sarcolemma is invaginated at each sarcomere to form blind-ending transverse tubes (T-tubules) that run into the centre of the fibre and play an important role in the activation of contraction. Running longitudinally between the repeating T-tubules are blind-ending membrane tubes or sacs, called sarcoplasmic reticulum. The ends of the tubes of the sarcoplasmic reticulum, terminal cisternae, lie alongside closely to the membranes of the T-tubules, forming triads. A triad is part of a T-tubule and the terminal cisternae on either side and is the site of excitation–contraction coupling (Lawrie, 1998).

Fibres are categorised by their speed of contraction, from slow to fast. Mainly, speed of contraction depends on the activity of myosin ATPase. Slow fibres receive a rich blood supply and have oxidative metabolisms, whereas fast fibres can operate anaerobically and do not have such a rich blood supply. All striated muscles are composed of a mix of slow and fast fibres with the ratio depending on their function and the amount and type of exercise (Lawrie, 1998).

2.2.2 Ultrastructure

Fibres are made up of, amongst others, contractile proteins. These contractile proteins are known as myosin and actin. Inside a sarcomere, the filaments of actin are attached to the Z-line by actinin (Huf-Lonergan & Lonergan, 2005). Lying between the thin actin filaments are thick myosin filaments that are not firmly attached to anything (Lawrie, 1998). They are kept in place by elastic protein molecules of titin which stretch from one Z-line to the next. The action of titin and nebulin, an inelastic protein lying next to actin filaments and attached to one Z-line of each sarcomere, ensures that actin and myosin exist in a very ordered way. Associated with actin filaments are two inhibitory proteins, troponin and tropomyosin, which prevent any uncontrolled reaction with myosin (Wareham, 2005c). The partial overlapping of thin filaments (actin) and thick filaments (myosin) results in distinct banding across each sarcomere of light areas (I band) where there is no overlap and dark areas (A band), where there is overlap. Thin filaments (the filaments comprise more than just actin) do not extend across the sarcomere, therefore the central region of the A band is lighter (H zone) (Rehfeldth *et al.*, 2004). The reaction between actin and myosin is responsible for the production of force and movement as the two types of filament are able to slide along each other, thus drawing the Z-bands together and shortening each sarcomere and, consequently, the whole muscle fibre (Lawrie, 1998).

2.2.3 Proteins involved in contraction

Myosin has at least 10 isoforms. Each myosin molecule consists of two heavy (2000 amino acids) alpha-helical protein chains, wound together to form a rod-like tail and two tadpole-like heads, each attached to a flexible neck. Myosin contains an active site that reacts with actin, and a portion that allows movement of the head (Lawrie, 1998). One molecule of myosin is 150 nm long. About 250 molecules make up a thick filament in a sarcomere. The molecules are wound together in such a way that the heads are clustered at each end of the thick filament, resulting in the central portion being just a bundle of myosin tails (Wareham, 2005c).

The actin molecule is a globular protein (G-actin), whilst the actin of the thin filament in a sarcomere is a polymerised form called F-actin. The thin filament is thus composed of two F-actin filaments wound together like two strands of beads. Each 'bead' of G-actin in the filament has a binding site for a myosin head (Wareham, 2005c).

Tropomyosin (70 kDa) is an elongated protein polymer that is wrapped around the actin filament and partly obscures the binding sites. In such a position, myosin heads bind only weakly and cannot create a power stroke. Troponin is a complex of three proteins associated with tropomyosin. Troponin I is inhibitory, troponin T binds to tropomyosin and troponin C binds reversibly to Ca^{2+} . The Ca^{2+} binding pulls tropomyosin away from the myosin-binding sites. In such a position, myosin heads can bind and carry out their power stroke (Wareham, 2005c).

2.2.4 Muscle contraction

In order for an action potential in the motor axon to activate the muscle fibre by exciting the electrically excitable sarcolemma, several complex steps have to be completed. These include the release of the chemical transmitter acetylcholine (ACh) from the axon terminal by exocytosis, the activation by transmitter of nicotinic ACh receptors at the end-plate, the transient depolarisation and repolarisation leading to an end-plate potential and, finally, the production of the sarcolemmal action potential. The process of chemical transmission at the neuromuscular junction induces a delay of about 0.5 ms between the time of arrival of an action potential pre-synaptically and the appearance of a muscle action potential (Wareham, 2005a).

The end-plate potential is a large depolarisation of about 70–80 mV. It is also slower than an action potential, lasting 10–15 ms, mainly owing to its slow repolarisation. In the case of the end-plate potential, repolarisation depends on the chemical breakdown of ACh by acetylcholinesterase to prevent further reactions with ACh receptors and to allow the channels to close. Thus, the properties of an end-plate potential differ in many respects from those of a normal action potential. Nevertheless, because the fast depolarising phase of the end-plate potential is associated with a relatively intense current, it is always strong enough to activate voltage-gated Na^+ channels on the sarcolemma. The end-plate potential activates Na^+ channels in the sub-synaptic folds and the combination of the end-plate potential and the depolarisation resulting from activated Na^+ sub-synaptic channels, is sufficient to overcome the considerable membrane capacitance of the end-plate region and to excite the population of Na^+ channels outside the end-plate. Once these Na^+ channels open, an all-or-none action potential is generated, and a muscle action potential propagates in all directions from the end-plate across the sarcolemma. This action potential is responsible for activating muscle contraction via the process known as excitation–contraction coupling (Wareham, 2005b).

The sarcoplasmic reticulum contains a high concentration of calcium as a result of the activity of an inwardly-directed calcium pump. The release of this calcium is the link between a muscle action potential and contraction. The sarcolemmal action potential travels into the centre of a muscle fibre along T-tubules. At each triad, the depolarisation activates voltage-sensitive dihydropyridine (DHP)-binding calcium channels. These activate ryanodine-binding calcium channels on the sarcoplasmic reticulum membrane. This activation, caused by either a physical link between the two types of calcium channels or calcium entering via the activated DHP channel, opens the ryanodine calcium channels and releases calcium from the sarcoplasmic reticulum. The calcium binds to troponin C, which moves tropomyosin on actin, exposes the actin binding sites and starts the contraction cycle. Once the myosin heads have bonded to the actin fibers, a cross-bridge has formed, making it possible for contraction to occur. When the cross-bridges form, two chemicals, adenosine diphosphate (ADP) and inorganic phosphate (P_i), are released into the sarcoplasm. The release of these chemicals results in the myosin heads bending, causing the actin fibers to be pulled past the myosin fibers. The sliding of the actin filament past the myosin filament results in a shrinking of the sarcomere. The shrinking of the sarcomeres is what causes force to be generated in the muscle (Wareham, 2005c).

When the myosin head has cocked back to its furthest position, a new chemical, adenosine triphosphate (ATP) binds to the myosin head, causing the myosin head to release the actin-binding site. This allows the myosin head to swing back to its original position and prepare for another contraction. Once bound to the myosin, the ATP is hydrolysed into ADP and P_i by the myosin (myosin is an ATPase) and the ADP and P_i causes the myosin head to cock back and prepare to once again bind with the actin fiber. Provided the sarcoplasmic calcium concentration is kept high, by the repeated arrival of action potentials in the T-tubules, the contraction cycle continues. However, as soon as the action potentials cease, calcium is rapidly sequestered back into the sarcoplasmic reticulum system, tropomyosin returns to cover the actin binding sites and the muscle relaxes (Wareham, 2005c).

2.3 Meat quality characteristics

In considering, how meat-animals' muscles develop and works, a distinction needs to be made between muscle and meat. Meat, as defined by Lawrie (1998), largely reflects the chemical and structural nature of muscle which is in the post-mortem state and differs from muscle due to a series of biochemical and biophysical changes that is initiated in muscle at the death of the animal. These biochemical and biophysical changes influence the quality of meat (Hwang *et al.*, 2003). There are many attributes that contribute to the quality of meat, and these can be defined by the following: tenderness, texture, fat levels, drip or purge, colour and the microbiology of the meat (Lawrie, 1998). These elements of meat quality are based on the visual quality, eating quality or a combination of the two. In other words, meat quality can be considered in terms of the appearance, which will then influence the purchase decision, and quality attributes that become obvious upon eating. Basic knowledge of meat quality is important in understanding and explaining subsequent changes in meat quality attributes following ES.

2.3.1 Colour

Colour is the single most important factor of meat products that influences consumer buying decisions and affects their perception of the freshness of the product (Bickerstaffe, 1996; Sanders *et al.*, 1997). However,

meat colour can be controlled if the many factors that influence it are understood. Colour occurs when electromagnetic radiation in the visible range is emitted or reflected by atoms or molecules. Colour is ultimately related to the electron structure of the pigment molecule because some incoming energy can be absorbed by these electrons. Light directed at meat contains varying amounts of energy at wavelengths in the visible range. When light strikes an opaque surface, some wavelengths are absorbed by the pigments and other are reflected. The light energy that is reflected back to the eye is missing the colour associated with the wavelengths that have been absorbed. It is this reflected light, which is now missing some wavelengths, that shows colour (McDougall, 1983).

Meat colour is the result of the concentration of pigments, myoglobin (Mb), its chemical state and the light-scattering properties of meat (Lawrie, 1998). Myoglobin is a water soluble metalloprotein that stores oxygen for aerobic metabolism in the muscle. It consists of a protein and a non-protein porphyrin ring with a central iron atom. The iron is a key factor in meat colour, but the crucial factors are the oxidation state of the iron and which compounds are attached to the iron fraction of the molecule (Brewer, 2004).

In fresh meat the Mb can exist in three forms: the purple, reduced myoglobin (Mb); the cherry-red, oxygenated oxymyoglobin (MbO) and the greyish-brown, oxidised metmyoglobin (MMb). These three forms are continuously being inter-converted (Van Laack & Smulders, 1990). After cutting the fresh meat, the colour of the meat is quite dark (purplish-red) and as the oxygen makes contact with the meat surface, it is absorbed and binds to the iron ion. As the Mb is oxygenated, it blooms and this pigment is called MbO (Lawrie, 1998). This is the colour that customers relate with freshness. The depth and the rate of penetration are dependent on the rate of oxygen diffusion – the diffusion being deeper as the meat is exposed to oxygen for longer. Mb and MbO have the capability to be oxidised, which turns the pigment to a brown colour and yields MMb. The brownish layer forms under the MbO and slowly starts to extend to the outside surface. The meat will thus discolour and become unacceptable to consumers (Van Laack & Smulders, 1990). The accumulation of MMb is dependent on oxygen diffusion (Brooks, 1938), oxygen consumption rate (Atkinson & Follet, 1973), rate of oxidation (Lawrie, 1998) and the enzymatic reduction of MMb (Ledward, 1985). The pigments Mb, MbO and MMb can be changed from one to the other, depending on the conditions at which the meat is stored (Figure. 1). After cooking, a brown pigment called denatured MMb is formed, which normally cannot be altered to form another pigment (Lawrie, 1998).

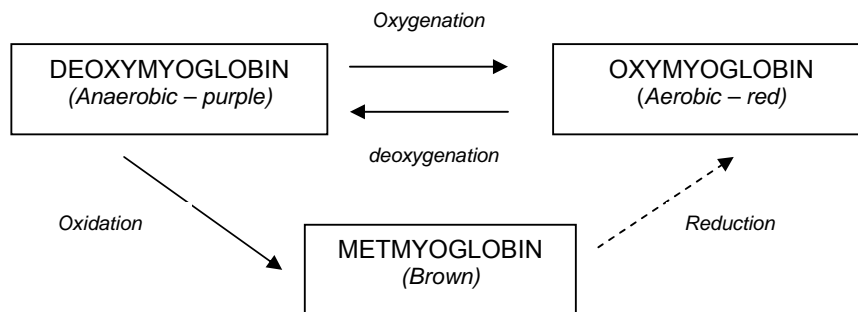


Figure 1 Principles of meat colour (Adapted from Lawrie, 1998)

Apart from the chemical state of Mb, physical and chemical factors such as pH, water-holding capacity and muscle structure affect the perceived colour (Lawrie, 1998). The rate at and extent to which muscle pH declines post-mortem are both variable and have a great impact on the colour of meat and meat products. The normal pH decline in muscles is from approximately 7.0 - 7.2 down to near pH 5.5 - 5.7 over about 24 hours. If the pH declines to pH of 5.5 - 5.7 within 45 minutes or less, the muscle will become very pale and soft in appearance (PSE). A very low ultimate pH (< 5.4) will also cause the meat to have a paler colour. If the pH does not drop noticeably post-mortem, the meat will be dark with a dull, dry surface (DFD). As the ultimate pH increases, the meat gradually becomes darker. This darkening of colour becomes noticeable when the muscle pH exceeds 5.7. The colour changes observed with PSE and DFD meat are mostly due to structural changes in muscle (Faustman & Cassens, 1990). The changes in pH affect the charge on the proteins that constitute the thick and thin filaments. Electrostatic repulsion keeps defined spacing between the fibres in muscle, and the charge is reduced by a drop in pH or by denaturation of myosin. In both cases, the spacing between filaments decreases, increasing the extent to which light is reflected (because the spacing is closer to the wavelength of light). A greater reflection of light means the colour is paler and less red, compared with the greater translucency and a darker colour when light penetrates deeper into the meat (Seideman *et al.*, 1984, Conforth, 1994).

High ultimate pH can affect the colour stability of fresh meat because it affects cytochrome enzyme activity and the rate of oxygenation. Reducing enzymes are necessary to convert MMb back to MbO. MMb-reducing activity increases with increasing pH (Ledward *et al.*, 1986) and thereby the meat surface turns brown quicker, thus reducing retail display of the meat. The high ultimate pH restricts lattice shrinkage, which means light penetrates more deeply into the surface, producing a translucent colour and, because less light is reflected back to the observer, a darker colour. The higher pH favours oxygen consumption, so the oxygenation of Mb is reduced and the colour remains dominated by deoxymyoglobin (Lawrie, 1998).

The rate at which MMb forms depends on two main factors: firstly, the level of antioxidants in the meat – by increasing antioxidant levels, the rate of Mb oxidation is reduced (Xiong *et al.*, 1993). Hence, supplementation with vitamin E, for example, can improve retail-colour stability. The second contributing factor is the extent of the oxygenation of the surface layers, which determines the proportion of Mb in the MbO form. When this proportion is high, the development of the brown MMb is inhibited, and colour stability is increased (Van Laack & Smulders, 1990).

The third aspect of MMb formation is the survival of the reductase enzyme responsible for converting the brown MMb back to myoglobin (Lawrie, 1998). However, the activity of this enzyme is gradually lost as time elapses post-mortem. Therefore, increased storage times, beneficial for tenderness, also has the effect of reducing retail display (Moore & Young, 1991).

The formation of MMb is accelerated by all conditions that cause denaturation of the globin moiety. Processing conditions that result in a low muscle pH while the muscle temperature is still high (denaturation) damage the enzyme and reduce the ability of meat to return from the brown MMb back to Mb. For example, incubation of *Longissimus dorsi* muscle at 25 °C results in greater MMb-reducing activity compared to incubation at 10 °C, and the activity is lost when the meat is incubated at 37 °C (Bekhit *et al.*, 2001); retail colour stability is therefore reduced.

Fluid loss due to muscle-lattice shrinkage also has an effect on colour (Rees *et al.*, 2003). In essence, when the pH falls quickly while the muscle is still at a high temperature in the period soon after slaughter, the protein filaments that produce contraction are disrupted or denatured and 'shrink'. This results in a reduced amount of space within the protein lattice and the water is expelled as drip. This mechanism also results in an increase in the paleness of the meat. This is because the reduced lattice spacing within the muscle causes more light to be reflected from the meat surface, imparting a whiter/paler appearance (Unruh *et al.*, 1986). Overall, therefore, inappropriate processing conditions that involve a rapid pH fall while temperatures are still high can give rise to a pale muscle appearance that also causes large amounts of drip, and, because of less reductase enzymes shelf life is reduced.

2.3.2 Water-binding capacity

Water-binding capacity (WBC) is a general term referring to the ability of a defined sample to retain intrinsic or extrinsic fluids under specified conditions (Fennema & Reid, 2008). Lean muscle contains about 75% water. The other main components include protein (19%), lipids and fat (2.5%), carbohydrates (1.2%), vitamins and minerals (2.3%) (Huf-Lonergan & Lonergan, 2005). The water in the muscle is found in the myofibrils, between the myofibrils themselves, between the myofibrils and the sarcolemma, between muscle cells and between muscle bundles (Offer & Cousins, 1992).

Water is a dipolar molecule and is attracted to proteins by means of charges. By description, bound water is water that resides in the area of non-aqueous constituents, thereby lowering mobility and increasing resistance to freezing. True bound water is only a very small fraction of the water present in the muscle (0.5 g per gram of protein). These water molecules are tightly bound to proteins, and during the onset of rigor mortis, there is little change in the amount of bound water (Offer & Knight, 1988; Huf-Lonergan & Lonergan, 2005).

Entrapped or immobilised water is another fraction that exists within the muscle (Fennema & Reid, 2008). These water molecules are either retained by the steric effects and/or through attraction to the bound water. This water is not bound to any proteins, but is held within the structure of the muscle. This water does not flow free in early post-mortem muscle from the muscle, but can be removed through drying and can be converted to ice crystals during freezing. The rigor process and the conversion of muscle to meat can affect this water. Upon alteration of muscle structure and the lowering of the pH, this water can escape as purge (Offer & Knight, 1988).

Free water is water that flows from the tissue unrestricted. There are weak surface forces that keep this water within the meat. This water is not readily seen in pre-rigor meat, but can be seen when entrapped water is expelled from the structures where it is found after the onset of rigor (Fennema & Reid, 2008; Huf-Lonergan & Lonergan, 2005).

Entrapped water is of importance in the conversion of muscle to meat. A reduction in this water is unwanted by processors. The amount of entrapped water retained is influenced by the net charge of myofibrillar proteins, structure of the muscle cells and myofibrils and the amount of extracellular space within the muscle itself (Fennema & Reid, 2008; Offer, 1991).

The amount of water lost is influenced by muscle pH, ionic strength and oxidation, which affect the ability of the myofibrillar proteins and myofibrils and muscle cells to entrap water. Post-mortem glycolysis leads to a lowering of the pH. As the major muscle proteins reach their iso-electric point (pH = 5.4) the net charge of the protein becomes zero, meaning the number of positive and negative charges on the protein becomes equal. The charges within the protein are attracted to each other and this results in a reduction of water attracted and held by the protein. Since opposite charges repel, as the net charge of the proteins that make up the myofibril reach zero, repulsion of structures within the myofibril is reduced allowing those structures to pack more closely together. The end result is a reduction of space within the myofibril, resulting in or drip. Partial denaturation of the myosin head at low temperatures is also thought to be responsible for some of the shrinkage in myofibrillar lattice shrinkage (Offer, 1991).

The interaction of pH and temperature in the pre-rigor muscle is a key factor influencing the WBC (Warner *et al.*, 1997). Conditions of low pH and high temperatures are particularly hostile to protein integrity and accelerate the denaturation process. Some muscle-protein denaturation will invariably occur during the post-mortem period because of the fall in pH associated with the development of rigor. However, the extent of denaturation during the pre-rigor pH fall increases with temperature and becomes particularly severe when high temperatures persist as the muscles near rigor mortis (Offer & Trinick, 1983).

While the pre-rigor period is the critical phase in determining how much water will be lost from the meat, it is the post-rigor loosening of the microstructure that allows the water to become mobilised and to manifest itself as drip. This movement of the water through the meat to collect as drip takes time (Honikel *et al.*, 1981). In beef, the full expression of water lost as drip occurs somewhere between 30 to 60 days post-mortem, while drip loss from lamb can increase to up to 12 weeks of chilled storage. Drip lost during ageing tends to correlate with drip lost during retail display and cooking (Simmons, unpublished).

Accelerated pH decline and low ultimate pH are related to the development of low water-holding capacity and high purge losses (Scopes, 1964). With a rapid pH decline while the muscle temperature is still high, denaturation of many proteins, including the water-binding proteins, occur. The most severe case is found in PSE, commonly found in pigs that have inherited the Malignant hyperthermia gene (Fujii *et al.*, 1991). During the development of rigor, the diameter of muscle cells decrease (Swatland & Belfry, 1985) and the sarcomeres shorten (Honikel *et al.*, 1986). This reduces the space available for water within the myofibril, thereby forcing it out as drip or purge. The amount of drip will also increase if meat is frozen and then thawed. This is largely due to the effect of ice-crystal formation, which both denatures cell proteins and physically damages the cell, with the effect of both decreasing water binding and accelerating the loss of purge (Lawrie, 1998).

When meat is cooked, an unavoidable and usually substantial shrinkage occurs due to contraction of the collagen component of meat. Such shrinkage physically expels fluid, the magnitude of which can reach 40% by weight. In addition, the shrinking process also results in some toughening of the product, a process which shows three phases as the cooking temperature increases: an initial phase beginning at about 40 °C and levelling off at approximately 55 °C, a second phase between 60 °C and 80 °C and a third phase above 80 °C (Davey & Gilbert, 1974). The increases in toughness correlate with changes in the distribution of water in the meat and its shrinkage, first laterally and then longitudinally (Bendall & Restall, 1983). Shrinkage upon

cooking is determined by the method, time and temperature of cooking. The basic principles of water binding still remain the same, but due to the extremities, the cooking loss is much higher than in the case of drip loss. The high temperatures lead to protein denaturation and thus a considerably lower water-binding capacity (Lawrie, 1998).

2.3.3 Tenderness

Tenderness is rated by consumers as the most important factor contributing to meat quality (Wood *et al.*, 1998). However, tenderness is most difficult to define. According to Hwang & Thompson (2001b), tenderisation is the generalised term for the process that leads to improvement in tenderness and can, in reality, only be measured post-rigor. A measure of tenderness is the subjective consumer appreciation of the meat, and a high score is desirable. An objective measure of tenderness is the force required to shear a standardised piece of meat, with low shear values being desirable (Hwang *et al.*, 2003). The overall impression of tenderness to the palate includes texture and involves three aspects: firstly, the ease of penetration of the meat by the teeth; secondly, the ease with which the meat breaks into fragments and thirdly, the amount of residue left after chewing (Weir, 1960).

There are a number of factors influencing tenderness, and these can be broadly split into two categories: direct animal contribution and processing factors. Direct animal factors include species, breed, age and sex (Lawrie, 1998). However, the most important feature of direct animal contribution is the ultimate pH (Watanabe *et al.*, 1996), determined by the level of glycogen in the muscle at the time of slaughter and by pre-slaughter growth rates, which influence the behaviour of the tenderising enzymes after slaughter (Lawrie, 1998). However, neither of these factors can be influenced by the abattoir.

It is generally agreed that slaughter and subsequent carcass processing accounts for between 60% to 70% of tenderness variability, while contributions from the animal itself and cooking make up the remaining 30% to 40% in approximately equal amounts (Johnston *et al.*, 2001). Generally, the management of tenderness by processing is defined by two features: the degree of muscle shortening and the degree of enzyme-induced tenderisation, commonly referred to as ageing or maturation (Chrystall & Daly, 1996). A discussion of these factors will follow in a later segment.

2.4 Processing options related to pH and temperature interaction

There are many biochemical and structural events that take place in the first 24-hour period after the animal is slaughtered and the muscle is converted to meat. This period greatly affects meat tenderness and other meat-quality characteristics and is dependent on the temperature-, time- and pH interaction. Muscle pH and temperature interact continuously during rigor development, as they impact on both the physical shortening (Tornberg, 1996) and proteolytic enzyme activity (Dransfield *et al.*, 1992). To understand this interaction, the process of rigor mortis needs to be explained first.

After exsanguination, glycolysis proceeds without oxygen and produces lactic acid as a result of anaerobic glycolysis. The lactic acid builds up, and this causes the pH to drop. Meat enters rigor when permanent cross bridges are formed between the actin and myosin, called actomyosin. When the pH reach values of 5.7-5.8 rigor normally starts (Hannula & Puolanne, 2004). Rigor can be split up into two phases. During the first, the delay phase, the muscle is still extensible because there is still ATP available to bind with Mg^{2+} on

the myosin, which is the essential step in dissociating myosin from actin and allow the muscle to relax. Creatine phosphate contributes to sustaining ATP levels during this phase, but its depletion slows the phosphorylation of ADP to ATP. This is the signal for the start of the onset phase of rigor. Because there is little ATP available to dissociate the actomyosin bonds, muscles cannot relax and therefore become inextensible (Aberle *et al.*, 2001)

2.5 Cold and rigor shortening

The interaction between chilling rate and pH decline has two extremes and is identified as cold shortening and paler meat with low water-binding capacity, similar to the conditions of PSE meat. Cold shortening is a phenomenon that leads to the contraction and subsequent shortening of sarcomeres of skeletal muscle due to a cold-induced release of calcium from the sarcoplasmic reticulum.

The relationship between sarcomere length and cold shortening to toughness was demonstrated by Herring *et al.* (1965) who showed a direct relationship of sarcomere length to fibre diameter and toughness. Their theory is that as the sarcomere becomes more contracted, there is an increase in fibre diameter due to the overlapping of filaments over each other, resulting in tougher meat. Shortening of the sarcomeres during rigor is temperature dependent (Locker & Hagyard, 1963). Thus, the temperature of the muscle as it enters rigor becomes an important factor in predicting the tenderness of meat. These two authors were the first to find a minimum shortening (10%) at a temperature between 14 °C and 19 °C, which correlates with minimum meat toughness (Tornberg, 1996). They found that cold shortening occurs at temperatures between 0 °C and 14 °C (up to 50%); they also found that heat- or rigor shortening occurs when muscle temperatures are above 20 °C (up to 30%).

Locker & Hagyard (1963) specified that the phenomenon of cold shortening occurs when muscle temperature declines to below 14 °C before the onset phase of rigor mortis has started. Temperature and pH relationships at the moment of onset of rigor can be considered the most significant factors that influence the degree of cold shortening (Hannula & Puolanne, 2004). It is widely recognised that this contracture is ATP dependent and its extent will thus decrease as the muscle pH decreases (Honikel *et al.*, 1983). When muscle temperature is reduced to between 0 °C and 15 °C before the onset phase of rigor, the sarcoplasmic reticulum cannot function properly and is unable to bind calcium ions, which leaves the sarcoplasm with a high concentration of calcium. Davey & Gilbert (1974) showed that there is an increase in the concentration of calcium ions in the myofibrillar region as the temperature drops from 15 °C to 0 °C and that, with an increase of free calcium ions, there is an increase in the shortening of the sarcomere. Because there is still ATP left in the muscle, muscle contraction occurs, causing the filaments to slide over each other, thereby shortening the sarcomere length. According to Aberle *et al.* (2001), the sarcoplasmic reticulum is least functional at an internal temperature of 1-2 °C.

Muscle types vary in their potential to cold shorten, with red being more susceptible than white (Bendall, 1973). The sarcoplasmic reticular system of pre-rigor muscle is stimulated to release calcium ions by the attainment of temperatures below 15 °C, enhancing the contractile actomyosin ATPase. It may be postulated that the re-absorption of these ions could be more readily affected by the type of muscle where the sarcoplasmic reticular system is relatively well developed. It is shown that the system is more extensively elaborated in white muscles than in red muscle. It has furthermore been shown that the rate of uptake of

calcium ions by the sarcotubular system of white muscle is greater than that of red muscle, that inorganic phosphate ions substantially enhance calcium ion uptake in both types and that the cold induced release of calcium ions from the sarcotubular is largely suppressed by relatively small concentrations of inorganic phosphate (phosphate binds with Ca^{2+} to form insoluble calcium phosphate; this happens predominantly in the sarcoplasmic reticulum, because P_i is free to diffuse through the membranes to the highest Ca^{2+} concentration. By reducing the sarcoplasmic reticulum Ca^{2+} concentration, less is released. This is one of the explanations for reduced force of contraction in fatigued muscle *in vivo*. Since the rate of production of inorganic phosphate during post-mortem glycolysis is considerably greater in white muscle than in red muscle (probably as a result of higher initial creatine phosphate levels; the only other significant source of P_i is ATP, but if too much ATP is degraded to release P_i , the fibres goes into rigor, so this is unlikely to be significant in the early stages), it may be assumed that a major factor in the absence of cold shortening in the former type is the attainment of a relatively high concentration of inorganic phosphate early on post-mortem. It would be expected, therefore, that the enhancement of contraction would be less easily suppressed in red muscle and would readily lead to marked intermigration of myosin and actin filaments, thus shortening, as is observed (Lawrie, 1998). The relationship between temperature and pH has led to the generalisation that that cold shortening can be avoided as long as the temperature is kept above 10 °C until the pH is below 6. There is no way in which to completely prevent rigor and the shortening of sarcomeres; however, there are ways to reduce the extent and toughening effects of this process before, throughout and after slaughter (Savell *et al.*, 2005). When slow cooling is combined with a rapid decline in pH, which is normally associated with high levels of ES, it will give rise to a low muscle pH at high temperatures. This condition leads to adverse meat-quality attributes, mostly due to the denaturation of muscle protein which gives rise to a pale colour and a low water-binding capacity similar to that normally associated with PSE. The PSE condition can lead to shortening of the sarcomeres, which gives rise to the term rigor shortening (Pike *et al.*, 1993). Rigor shortening occurs when muscle fibres are maintained at elevated temperatures resulting in a rapid depletion of ATP, followed by the myosin head binding irreversibly to actin and the early onset of rigor. High temperature shortening starts at a pH in the region of 6.2 correlates with the time at which ATP begins to decline rapidly and continues until the end of glycolysis (Marsh, 1954). In contrast, cold shortening occurs too early to be related to ATP concentration. Also, the effects of rigor shortening on objectively measured shear-force values are smaller and less consistent when compared to cold shortening (Locker & Daines, 1976). In contrast, sensory panels have shown significant increases in toughness in rigor-shortened beef, although the toughening mechanism does not match that of cold shortening (Hertzman *et al.*, 1993). Thus the pre-rigor temperature-pH environment determines the quality attributes of tenderness, colour and water-binding capacity. Within the extremes lie a range of processing options that can produce a spectrum of distinctive quality attributes that can be obtained by manipulating the pH decline with regard to the cooling rate by means of ES (Simmons *et al.*, 2008).

2.6 The effects of temperature at the time of rigor on the calpains

Calpains are calcium-activated proteases with an optimum activity at neutral pH. In skeletal muscle, the calpain system consist of at least three proteases, μ -calpain, m-calpain and skeletal muscle-specific calpain, calpain 3 and an inhibitor of μ - and m-calpain, calpastatin (Goll *et al.*, 2003). An important characteristic of μ - and m-calpain is that they undergo autolysis in the presence of calcium. Autolysis reduces the Ca^{2+} requirement for the half maximal activity of μ - and m-calpain. Calpastatin is the endogenous specific inhibitor

of μ - and m-calpain (Koochmaraie & Geesink, 2006). Several isomers of this protein exist, but the predominant form in skeletal muscle contains four calpain-inhibiting domains (Lee *et al.*, 2000). Calpastatin requires calcium to bind and inhibit calpains and is also a substrate for the calpains that can be degraded in the presence of calcium (Koochmaraie & Geesink, 2006).

The effect of rigor temperature on the shear force value was first thought to be optimum at 15 °C due to minimal shortening that occurs at this temperature. However, Devine *et al.* (1999) showed with beef that when shortening is prevented by tight wrapping, mimicking skeletal restraint, with an increase from 15 °C to 35 °C in temperature at rigor attainment, that shear force was greater at high rigor mortis temperatures. These differences continued with ageing. Hwang & Thompson (2001a) measured calpain activity for muscle entering rigor at different temperatures. Their results indicate that μ -calpain activation occurs earlier in muscle entering rigor at a high temperature (38 °C), which results in tougher meat. However, with a slow rigor (15 °C) muscles had higher μ -calpain levels 24 hours post-mortem. At a constant rigor temperature of 35 °C, almost 80% of the μ -calpain activity was lost during rigor development, while only about 20% of activity was lost during rigor development when exposed to a constant temperature of 15 °C (Wahlgren *et al.*, 1997). Simmons *et al.* (1996) measured calpain activity throughout the rigor process and showed that calpain activity remained constant at all temperatures up to a pH of approximately 6.2, when the activity started to decrease. Conditions of low pH and high temperature are known to denature the contractile proteins which are more stable at rigor mortis (Offer, 1991). Such conditions, in conjunction with greater autolysis of calpains at high temperatures (Dransfield *et al.*, 1992), would explain how ageing enzymes are reduced in effectiveness so that shear force increases and the ageing potential is reduced. Hwang & Thompson (2001b) found under *in situ* conditions that the most tender beef meat, after 14 days of ageing, was attained at temperatures from 29 °C to 30 °C at a pH of 6.0.

2.7 Diverse market requirements

Cold shortening and rigor shortening can be classified as the extremes of processing. In between lies a field for different processing options. Simmons *et al.* (2006) uses a predictive model to demonstrate different processing options. For example, meat that is going to be frozen or that is destined for retail sale within a few days of slaughter needs accelerated proteolytic activity to ensure the meat is tender when frozen or purchased. The opposite is chilled meat that is exported (via refrigerated boat, for example) and has up to six weeks before it reaches its destination. Thus, a stable product with a slower rate of tenderisation for this long period is needed. Therefore, a product either being frozen or for retail sale within a few days after slaughter requires a fast onset of rigor; this could be achieved with a high level of ES of the carcass combined with a slow cooling rate. This allows the carcass to undergo a period of high-temperature conditioning. In contrast, the process for a six-week chilled product requires a slow rigor onset with minimal ES and a fast rate in temperature decline. This will reduce the probability of excessive degradation in the meat structure, minimise purge losses by avoiding denaturation and help maintain colour stability after the end of the storage period. However, in a commercial scenario, the optimum balance needs to be obtained between these sometimes conflicting requirements.

The control of post-mortem pH and temperature decline can be managed to provide precise meat quality outcomes. However, in commercial conditions, this is difficult to achieve. Due to the differences in muscle locations and size, there will always be differences in the rate of cooling of different muscles. The rate of pH

fall between animals and the response to ES is also variable, as is shown by Simmons *et al.* (1997). This leads to the option of hot boning (processing the carcass in the pre-rigor state) the carcass and thus improving control over chilling.

2.8 Electrical stimulation

2.8.1 Historical overview

Using ES to increase meat tenderness is not a new idea. Benjamin Franklin remarked in 1749 that, "Killing turkeys electrically, with the pleasant side effect that it made them uncommonly tender, was the first practical implication that had been found for electricity" (Stiffler *et al.*, 1999). In 1951, Harsham & Deatherage filed for a patent on ES for tenderising of meat. Its application to commercial practise was not considered seriously until 1973 (Lawrie, 1998), when first New Zealand and then Australia used it to avoid the toughness that results from cold-induced shortening. The major requirement in the New Zealand situation was to accelerate the onset of rigor mortis so that it occurred at temperatures above those likely to result in cold-induced shortening before the meat was frozen; this applied to meat from both sheep (Chrystall & Hagyard, 1975) and cattle (Davey & Gilbert, 1975). For small lambs, the improvement was dramatic ensuring that the majority of rapidly-frozen meat was acceptable, especially without further ageing. For larger cattle, where conditions which induce cold shortening may not always be encountered, the question of whether or not it is necessary to use ES is not clear, if the assumption is made that reduction of cold-induced shortening is the main aim.

ES of beef carcasses was first applied to prevent the toughening effect of cold shortening (Carse, 1973) by accelerating the onset of rigor at a higher and more optimal temperature in the muscle (Crystal & Devine, 1978). However, with later studies, it was found that ES could accelerate post-mortem tenderisation (Savell *et al.*, 1981) by enhancing the rate of proteolysis stimulated by the release of Ca^{2+} at a higher temperature (Hwang *et al.*, 2003) and also by physically disrupting the structure of the muscle fibre (Takahasi *et al.*, 1987).

2.8.2 Mechanism and effect of electrical stimulation on pH decline

When the muscles of freshly slaughtered animals are electrically stimulated, they contract. There is an associated increase in the biochemical reactions in the muscle cells leading to an accumulation of lactate and resulting in an immediate drop in the muscle pH (ΔpH) (Chrystall & Devine, 1985). Electrical activation of the muscle membranes triggers Ca release from the sarcoplasmic reticulum, triggering the contractile actomyosin ATPase and muscle contractions. Muscle contractions result in a higher rate of ATP breakdown and this leads to a faster post-mortem glycolysis. The Ca^{2+} ions also enhance the activity of phosphorylase, which provides the substrate for the increased rate of post-mortem glycolysis (Chrystall & Devine, 1985).

The current causes the muscles to contract thereby increasing the rate of glycolysis, with a subsequent ΔpH which can range from 0.6 pH units at 35 °C to 0.018 units at 15 °C (Chrystal & Devine, 1980). Following the immediate pH fall, there is an additional acceleration of glycolysis leading to a higher rate of pH fall ($d\text{pH}/dt$) and subsequent early rigor development (Chrystall & Devine, 1978) (see Figure 2). However, this effect tends to be associated with high-voltage stimulation systems and is not necessarily triggered by low voltage stimulation systems (Kastner *et al.*, 1993). In non-stimulated beef (*m. sternomandibularis*) the energy of

activation of the glycolytic process is 40–45 kJ/mol, whereas that for stimulated muscle approaches 70 kJ/mol. This high energy of activation in both cases means that any cooling of the muscles will markedly increase the time for attainment of rigor mortis with a larger effect in stimulated muscle (Chrystall & Devine, 1980). Studies by Jeacocke (1977) characterises this temperature effect and show that for non-stimulated muscle the rate of glycolysis is faster at higher temperatures and fall to a minimum at 12 °C and then increase again. This latter increase arises because of the extra ATP required for cold contracture. When muscle is maintained at a constant temperature, the increased rate of pH fall after stimulation seems to occur with all stimulation parameters and even occurs as a consequence of electrical stunning (Devine *et al.*, 1984).

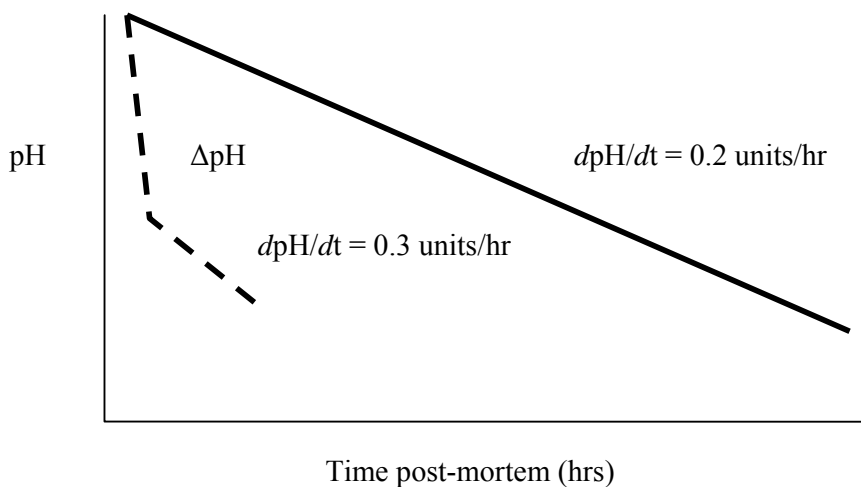


Figure 2 Example of the pH drop in stimulated (- - -) and non-stimulated (—) carcasses (Adapted from Chrystall & Devine, 1985).

Post-mortem delay before stimulation can have a large effect on the response to ES. Muscle temperature can fall, greatly reducing the ΔpH or glycolysis can have progressed so that the pH has fallen, reducing the ΔpH that can be achieved and the nervous system can decay and become unresponsive so that its stimulation cannot elicit any muscle response. It has been shown that the ΔpH produced by any given stimulation decreases as pre-stimulation pH decreases (Chrystall & Devine, 1978). Muscle type has an influence on ΔpH as shown by Devine *et al.* (1984) in beef muscles where the fast twitch *Cutaneous trunci*, largely composed of white muscle fibres, gives high values for ΔpH whereas in the slow twitch *Masseter*, composed of red fibres, there is neither a distinct ΔpH nor an acceleration of $d\text{pH}/d\text{t}$. It was reported by Swatland (1981) that within the *Sternomandibularis*, fast fibres with strong ATPase and weak succinic dehydrogenase (SHD) reactions showed most glycogenolysis in response to ES, while slow fibres with weak ATPase and strong SDH showed the least.

Muscles also differ in their optimum response frequency, where *Longissimus dorsi* is more responsive to 14 pulses per second than 40 pulses per second (Chrystal & Devine, 1978) and the opposite has been shown for beef *Semimembranosus* (Bouton *et al.*, 1980). For any given muscle, the pH responses will be governed

by the electrical characteristics (Simmons *et al.*, 2008). The effect of these characteristics will be discussed in a later section.

2.9 Effects of electrical stimulation on quality characteristics

2.9.1 Colour

There have been numerous studies on the effects of ES on colour and colour stability. ES affects colour in beef, but the extent of the changes it induces depends on the chilling rate and the muscle type (Strydom *et al.*, 2005). In general, ES associated with a higher rigor temperature produces a paler colour, as noted by King *et al.* (2004) on cabrito (Boer goat cross kids) carcasses with high voltage ES increasing both the lightness (L*) and the yellowness (b*) of the *Longissimus thoracis*, due to increased protein denaturation and myofibrillar lattice shrinkage (Offer & Trinick, 1983). According to Rees *et al.* (2003) higher L* values are not a result of protein denaturation, but coincide with the lower muscle pH that increases the free water at the cell surface; this results in an increased reflectance, giving the meat the lighter appearance. However, muscle colour is unaffected when treated with low voltage ES (King *et al.*, 2004). Another reason for the higher L* value following ES could be due to the looser fibre structure that can lead to a higher amount of light being scattered and deeper oxygen penetration (Unrah *et al.*, 1986).

Meat tends to be redder after high (Eikelenboom *et al.*, 1985) and low voltage (Unrah *et al.*, 1986; Sleper *et al.*, 1983; Eikelenboom *et al.*, 1985) ES in beef and Shaw *et al.* (2005) found that sheep carcasses which had undergone high and low voltage ES, compared to the non-stimulated carcass muscles, had redder and less dark muscles the next morning. This effect is due to damage to the enzyme systems responsible for oxygen consumption, reducing the oxygen consumption rate and thus the higher concentrations of MbO in the surface meat layer (Ledward, 1992). This observation is confirmed by Lawrie (1998) who also claims that the brighter red colour can also be attributed to the fast pH decline. This results in the carcasses reaching their iso-electric point much sooner, thereby “opening up” the structure and easing the oxygenation of Mb.

In certain muscles, for example the *Semimembranosus* in beef, ES is associated with increased MMB formation and a resulting loss of colour stability (Lawrie, 1998). Colour stability, when defined as the rate of MMB accumulation on the surface layer, is generally reduced by ES (Wiklund *et al.*, 2001). Electrical stimulation produced a significant loss in display life (hours of Minolta a* value > 12) 1 week post-mortem in electrically stimulated carcasses. After 3 weeks of ageing, the control samples had reduced colour stability, but by this time, the differences between non-stimulated and stimulated meat had disappeared. This result (Wiklund *et al.*, 2001) suggests that ES accelerates the loss of MMB reductase activity, so that after a week, MMB accumulation is accelerated. This action equilibrates after three weeks with non-stimulated meat, and the effect is then lost. Unrah *et al.* (1986) found that on day one steaks was brighter red, but at day five the control (non-stimulated) was redder. A more open muscle structure and the enzymes important to muscle colour are degraded because of the temperature and pH conditions, which may account for the increased rate of colour deterioration during display (Sleper *et al.*, 1983). Van Laack & Smulders (1990), however, found the opposite: that ES increased the display life. They postulated that the more open structure of the muscle may allow more oxygenation and thus increased redness. Similarly, when stimulation is omitted, the tighter structure would limit oxygen diffusion and thus reduce the layer of the red MbO. Consequently, the MMB layer beneath the oxymyoglobin will be nearer to the surface. Moore & Young (1991) stated that with

no ageing, stimulated display life was longer in lamb, but with ageing for two weeks, this advantage disappeared. Freezing and thawing adversely affect colour display and is worsened by ES. This may be possible due the structural damage caused by ES being exacerbated by the structural damage of freezing and thawing (Moore & Young, 1991).

2.8.2 Water-binding capacity

Water-binding capacity refers to the ability of meat to retain water. Water is held in the spaces between the thick and thin filaments of the muscle cell post-mortem (Lawrie, 1998). Lateral shrinkage of the myofibrils causes the fluid that has accumulated between fibres and fibre bundles to be expelled; it is drained by gravity, forming a drip (Offer & Cousins, 1992). A small proportion of the water in muscle is also held by electrostatic attraction between the proteins. The number of the reactive groups that binds water changes with the loss of ATP, the drop in pH, proteolysis and protein denaturation post-mortem (Bond *et al.*, 2004). Conditions of low pH and high temperatures in post-mortem muscle reduce the water-binding capacity of meat, an effect attributed to muscle denaturation, especially myosin. This can occur due to a fast pH drop or during a normal drop combined with a slow chilling rate (Offer & Knight, 1988). ES, by accelerating pH decline, contributes to reduced water-binding capacity, but the extent is determined by the chilling rate.

Li *et al.* (2006) stated that low voltage ES appeared to increase the evaporative loss of conventionally cooled carcasses, where carcasses were rapidly chilled, it lessened the evaporative loss. The drip losses for *Longissimus* steaks from electrical stimulated carcasses did not differ significantly from those that did not undergo stimulation thus indicating that low voltage ES had no effect on the weight loss of the steaks after vacuum packaging. However, Eikelenboom *et al.* (1981) found that there is a one percent increase in drip loss during vacuumed storage from one to seven days post-mortem. Low (Li *et al.*, 2006) and high voltage (Eikelenboom *et al.*, 1981; Strydom *et al.*, 2005) ES increases the cooking loss of *Longissimus* steaks from the conventionally chilled carcasses, but King *et al.* (2004) found no differences, possibly due to rapid pre-rigor chilling. Li *et al.*, (2006) state that ES decreases the water-holding capacity, probably due to denaturation of hydrophilic proteins.

2.8.3 Tenderness

Tenderisation is the generalised term for the process that leads to improved tenderness of meat; in reality, this can only be measured post-rigor. The measurement of tenderness can be influenced by the amount of intramuscular fat and connective tissue present. Shear force is the objective scale which is used to measure tenderness in meat, with lower shear values representing the more desirable meat (Hwang *et al.*, 2003). Electrical stimulation generally lowers shear-force values.

2.8.3.1 Possible reasons for increased tenderness

There are three possible mechanisms which may alter the rate of tenderisation; reduced cold-induced shortening, the enhancement of the rate of proteolysis and alteration of protein structure (Hwang *et al.*, 2003). The first theory is associated with the prevention of cold shortening. The second is based on the phenomenon that electrically stimulated carcasses release proteolytic enzymes sooner and that they work faster, compared to those in non-stimulated carcasses; this is due to the higher carcass temperature. The

third theory is based on electron micrographs which show that muscles from electrically-stimulated carcasses reveal structural damages (Stiffler *et al.*, 1999).

There is a potentially powerful association between the physical disruption of the myofibrillar complex and the increased tenderness in meat (Ho *et al.*, 1996). Takahashi *et al.* (1987) show that stimulation with a 60 Hz waveform produce areas of supercontractions and myofibrillar stretching. Their theory is based on the observation that 60 Hz caused only a small pH decline following stimulation, with a greater tenderising effect than a 2 Hz waveform with a larger pH decline. They conclude that, since the 2 Hz did not cause any physical disruption, that tenderisation occurs due to structural damages rather than a faster pH decline. It is unclear whether this is directly as a result of the physical disruption as such, or whether the disruption facilitates ageing in other ways. Contracture bands are not a direct consequence of electrical current passing through the muscle, but rather due to the super-contraction caused through localised excessive calcium-ion release from the sarcoplasmic reticulum. It is possible that it is the presence of this extra calcium which facilitates the increased tenderisation (Hwang *et al.*, 2003). However, Simmons *et al.* (2008) state that ES needs to produce highly exaggerated levels of free intercellular calcium levels compared to those produced during normal physiological muscle activity *in vivo*, otherwise, exercising would have limited health benefits.

The increase in tenderness due to disruption is a reasonable finding, as the historical reason for the use of ES has been to facilitate the prevention of cold shortening. Cold shortening can be measured at temperatures up to 25 °C (Honikel *et al.*, 1983) with an increase in shortening as the temperature declines and with a minimum shortening between 14 °C and 19 °C when the muscle enters rigor. However, persistent toughness only occurs when shortening is more than 20% of the resting sarcomere length (Hertzman *et al.*, 1993). The general rule that the temperature must be above 10 °C before the muscle pH has dropped below 6 has been used as a measure to prevent cold shortening (Lawrie, 1998). In commercial situations, cold shortening is almost impossible, with severe chilling conditions normally not found in commercial practice needed to induce shortening (Simmons *et al.*, 2008). However, it has also been shown that the applied stimulation treatment improved tenderness under circumstances where the carcasses have not suffered from cold shortening (Hwang *et al.*, 2003).

Individual fibres enter rigor sequentially; thus, those muscles entering rigor earlier and at higher temperatures will initially experience faster tenderisation than those that enter rigor later and at lower temperatures. Rigor occurs when each individual fibre runs out of ATP, rather than across all muscles at the same time (Jeacock, 1984). Using the process of ES, by the time full rigor mortis is attained, more fibres will have aged to a higher degree than those of non-stimulated carcasses. When combined with the higher temperatures, the early rigor mortis ensures a significant difference in the tenderness of meat (Jeacock, 1984; Davey & Gilbert, 1975). The stimulated and non-stimulated carcasses will reach the same level of tenderness, but the stimulated carcasses reach it earlier (Chrystall & Daly, 1996). Sometimes, the slow ageing rate, caused by a slow pH decline and rapid chilling, can be misinterpreted as cold shortening, as shown by Simmons *et al.* (1999) where non-stimulated *Longissimus dorsi* took 30 days to reach acceptable tenderness scores; however, the persistent toughness normally associated with cold shortening did not occur.

Low pH conditions coupled with high temperatures prior to rigor mortis are known to denature the contractile proteins which are stable after rigor mortis; such conditions pre-rigor can also result in the reduced activity of other proteins – such as calpains (Offer, 1991). This statement is supported by the observation that calpain activity remain constant at all temperatures throughout the rigor process until a pH of approximately 6.2 is reached; at this stage, their activities decrease at a faster rate with higher temperatures (Simmons *et al.*, 1996). In conjunction with this, the known greater autolysis of calpains at high pre-rigor temperatures (Dransfield *et al.*, 1992) and their prolonged exposure at these elevated temperatures would reduce their effectiveness, resulting in reduced tenderness and reduced ageing (tenderness) potential. It can be expected that ES could worsen this situation, with muscles entering rigor mortis at high temperatures (Devine *et al.*, 2006).

The temporary rise in free Ca^{2+} that occurs after ES may be an important factor in the activation of calpains and subsequent tenderisation. It may also be responsible for the ultra-structural changes which result from supercontraction nodes and can affect the tenderisation process in addition to those factors that interact with the levels of pH_u (Hwang *et al.*, 2003)

The sum of all these effects, rapid ageing of muscle fibers resulting in early rigor; ageing at elevated temperatures; temporary rises of calcium; possible ultra-structural changes; and avoidance of significant autolysis of calpains, is that they all interact to produce a window of optimal conditions that result in the quickened tenderisation phenomenon found to a greater extent in electrically stimulated muscles (Devine *et al.*, 2006).

2.8.3.2 Shear-force values

ES tends to decrease the shear-force values compared that of non-stimulated meat one to two days post-mortem (Uytterhaegen *et al.*, 1992; White *et al.*, 2006; Toohey *et al.*, 2008); however, the degree of decrease depends on the parameters of the ES administered. As time progresses, the differences between electrical and non-stimulated samples decrease and will disappear completely if sufficient ageing time is allowed. However, if carcasses receive excessive amounts of stimulation, shear-force values are higher in the stimulated carcasses versus the non-stimulated ones, as shown by Geesink *et al.*, (2001). They found that at day one, there are no differences in shear-force values, but after seven days of ageing, stimulated carcasses are significantly tougher. This is supported by Koh *et al.* (1987) and Unruh *et al.* (1986) who noted that the effects of excess stimulation can be attributed to denaturation of muscle structural proteins and enzymes. Heat shortening occurs if rigor is attained at high carcass temperatures (Locker & Hagyard, 1963) and leads to a reduced capacity to tenderise, due to the permanent shortening of the sarcomeres, which has been shown to correlate positively with an increase in toughness (Herring *et al.*, 1965).

A study by Hwang & Thompson (2001a) on steers and heifers, showed a significantly lower shear-force score and a slower pH decline when stimulation was applied 40 minutes after stunning compared with 3 minutes after stunning. There was a significant interaction between the type of stimulation and the time of stimulation post-mortem for adjusted tenderness scores. The high voltage stimulation after 3 minutes resulted in tougher meat. Thus, a very rapid pH decline resulted in a small increase of meat toughness which was probably due to rigor shortening. A higher level of calpastatin post-stimulation at 3 minutes coincided with a higher shear-force value compared to stimulation at 40 minutes. If there is a rapid pH fall resulting

from stimulation within 2 minutes of slaughter, after 3 days of ageing at 4 °C the meat is not as tender as it is when stimulation takes place 30 minutes after slaughter (with rigor mortis at approximately 15 °C). However, both are more tender than non-stimulated meat. After 14 days ageing, all the meat becomes tender, but the meat stimulated at 30 minutes is still the most tender. Temperature records show that meat stimulated at 30 minutes attains rigor mortis at close to 15 °C. This suggests that when rigor mortis is attained at 15 °C, the muscles will be the most tender (Wahlgren *et al.*, 1997).

Polidori *et al.* (1999) notes that low voltage ES significantly reduces shear-force values for *Longissimus*, but not for *Semimembranosus* muscles. This indicates that different muscles react differently to ES. Li *et al.* (2006) show that the shear-force values correlate with post-stimulation pH and temperature at various time intervals. Thus, due to different cooling rates and composition of fibre types, muscles will react differently to ES.

Davel *et al.* (2003) note no significant differences in shear-force values between stimulated and non-stimulated muscles when tested with the Warner-Bratzler shear-force compression test on *Longissimus dorsi* *et lumborum* samples of Dorper sheep with 2 permanent incisors (AB class; age of animal according to SA carcass classification system) and similar fatness. There were, however, decreases in the variances between the standard deviations, indicating that there were less variations in tenderness in electrical stimulated carcasses compared to non-stimulated carcasses; there is a positive effect on reducing the variation in meat tenderness, thus improving meat quality.

2.8.3.3 Sarcomere length

ES had no effect on sarcomere length in lambs with the callipyge phenotype (Kerth *et al.*, 1999) or crossbred lambs with no permanent incisors (Toohey *et al.*, 2008). There were, however, differences between the different muscles, with the muscles with longer sarcomere lengths obtaining lower Warner-Bratzler shear-force values and higher tenderness scores from a trained sensory panel (Kerth *et al.*, 1999). This may have been as a result of the chilling regime, as it was not severe, with an average chilling temperature of 6.7 °C. Although the trend showed that stimulated meat had a longer mean sarcomere length than non-stimulated meat, under more severe chilling regimes, the advantage of stimulation would be expected to be greater. This assumption is supported by White *et al.* (2006). Hot-boned *semimembranosus* muscle subjected to ES exhibited significantly longer sarcomeres than hot-boned, non-stimulated *semimembranosus* muscles where either slow or fast chilling was used. The effect of ES on sarcomere length is expected to be seen only if cold shortening is a risk, and thus by lowering the pH to below 6 before the temperature is 10 °C. However, over-stimulation may result in shorter sarcomeres (Geesink *et al.*, 2001). Thus, the temperature-pH interaction is responsible for sarcomere length, and ES could be used to manipulate this environment.

2.8.3.4 Proteolysis

Proteolysis affects all muscle proteins, including connective tissue, but its outcome is different if it affects the contractile proteins actin and myosin rather than the structural or cytoskeletal proteins (Ferguson *et al.*, 2000). The term proteolysis is used in this chapter to refer to the degradation of structural proteins that precedes tenderisation.

Early post-mortem ES is usually used to increase meat tenderness and promote the activity of some endogenous proteolytic enzymes, including μ -calpain. In myofibrils, titin is a very long filamentous protein that extends from the Z-line, while nebulin comprises inextensible filaments that are closely associated with the thin filaments. The protein, α -actinin is an integral Z-line protein and desmin the major component of intermediate filaments that associate with Z-lines. The degradation of some of these structural proteins has been implicated for the loss of myofibrillar integrity, thus increasing meat tenderness (Ho *et al.*, 1996).

The activity of protease enzymes, most notably the calpains, has been shown to be sensitive to the prevailing pH and temperature of the meat (Dransfield, 1994) and since ES alters the post-mortem temperature and pH relationship, it is reasonable to expect that proteolysis would also be affected. The degradation of myofibrillar proteins during the post-mortem period has been studied using a number of techniques, including the fragmentation of myofibrils (Olson *et al.*, 1976), determination of 'free' amino acids (Field & Chang, 1969), measurement of protein solubility (Claeys *et al.*, 1994) and gel electrophoresis (Olson *et al.*, 1977). However, studies evaluating the effect of ES on proteolysis are conflicting.

The appearance of a 30 kDa component, a result of troponin-T degradation has been considered a good indicator of post-mortem tenderisation in muscle (Yates *et al.*, 1983) and is therefore used as an indicator of proteolysis (Ho *et al.*, 1994). SDS – PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) assays reveal that protein profiles are similar in rapid- and slow-chilled muscle, indicating that the rate of proteolysis is the same, irrespective of whether the meat was cold shortened or not (White *et al.*, 2006). Park *et al.* (2003) found that higher pre-rigor temperatures ($36\text{ }^{\circ}\text{C} > 15\text{ }^{\circ}\text{C} > 5\text{ }^{\circ}\text{C}$) induced a faster rate of troponin-T degradation in beef muscles irrespective of sarcomere length. However, these authors segmented the muscles into strips to allow for the temperature regimes to be more effective than those that comprise chilling of whole muscles as used by the other authors. Conflicting results have been reported for the effects of ES on the degradation of troponin-T. Ducastaing *et al.* (1985) report that 30 kDa and 32 kDa subunits appear earlier in stimulated beef muscle (four hours post-mortem). Geesink *et al.* (1994) report that stimulation for 64 seconds increased the appearance of the 30 kDa subunit in beef *Longissimus dorsi*, but after 21 days there were no difference with the control. Stimulation for 8 seconds resulted in no difference with the control. However, Pommier (1992) and Sonaiya *et al.* (1982) found that stimulation had no effect on troponin-T degradation.

According to Ho *et al.* (1996), intact nebulin and titin both disappear slightly faster in electrically stimulated myofibrils when 8% gels are taken over a period. A ~1200-kDa band present at day 0 also decreases faster in electrically stimulated samples. Western blots prepared from 15% gels of non-stimulated and electrically stimulated samples were labelled with polyclonal anti- α -actinin. ES and post-mortem time do not alter the α -actinin content in myofibrils. This is in direct conflict with a similar study by Ho *et al.* (1997) who found no effect on the rate of degradation. The only variable being the breed of animals used (Angus X Jersey versus Brahman X Simmental). Rhee *et al.* (2000) found that the degradation rate of titin and nebulin was increased in native Korean bulls (Hanwoo) with stimulation and conditioning at higher temperatures. Hopkins & Thompson (2001a,b) found that stimulation increased the degradation rate of a protein designated as M1, but had no effect on the 30 kDa subunit, while Geesink *et al.* (2001) reported that stimulation had no effect on degradation rate. Both these studies were done on sheep carcasses.

Ducastaing *et al.* (1985) reported that there was an increased myofibrillar protein solubility when measured three days post-mortem compared with non-stimulated muscle, while Geesink *et al.* (2001) reported a faster increase in solubility. This differs from the findings of Gilbert *et al.* (1984) and Den Hertog-Meischke *et al.* (1997) who found no differences between stimulated and control samples.

Sonaiya *et al.* (1982) reported that the myofibrillar-fragmentation index was increased in *Longissimus dorsi*, *Semimembranosus* and *Triceps brachii* muscles that were stimulated, but Geesink *et al.* (1993) found that stimulation only increased the rate of the myofibrillar fragmentation index in *Longissimus dorsi* but not in the *Semimembranosus* muscle. There are many conflicting results, but the general feeling is that ES does have a significant effect on proteolysis; however, the extent is regulated by the severity of the chilling regime.

2.8.3.5 Calpain and calpastatin activity

Calpains are calcium-activated proteases with an optimum activity at neutral pH. In skeletal muscle, the calpain system consists of at least three proteases, μ -calpain, m-calpain and skeletal muscle-specific calpain, calpain 3, and an inhibitor of μ - and m-calpain, calpastatin (Goll *et al.*, 2003). An important characteristic of μ - and m-calpain is that they undergo autolysis in the presence of calcium. Autolysis reduces the Ca^{2+} requirement for half maximal activity of μ - and m-calpain (Koochmaraie & Geesink, 2006). ES promotes the release of Ca^{2+} from the sarcoplasmic reticulum and hydrolysis of ATP (Ducastaing *et al.*, 1985). These two factors may increase the Ca^{2+} concentration in the cell which may, in turn, activate calpains that cause partial degradation of myofibrils by removing α -actinin from the Z-line, with the subsequent hydrolysis of troponin-T, troponin-C, C protein, tropomyosin, connectin and desmin (Dayton *et al.*, 1976).

The application of either ES (Ducastaing *et al.*, 1985; Uytterhaegen *et al.*, 1992) or high temperature during the early post-mortem period (Whipple *et al.*, 1990) has been shown to cause an increase in μ -calpain activity and the consequent improvement in tenderness. However, when ES is combined with a very slow chilling regime, it has led to an increase in toughness (Geesink *et al.*, 2001) due to the earlier exhaustion of μ -calpain at the higher carcass temperatures, thus causing less ageing potential during chillier storage (Dransfield *et al.*, 1992; Simmons *et al.*, 1996).

Ducastiang *et al.* (1985) demonstrated that when carcasses are stimulated 30 minutes post-mortem (550 V, 60 Hz, 2 min), the activity of μ -calpain at 4 hours post-mortem is reduced by 80% and that activity directly after stimulation was 10-15% higher than in the non-stimulated controls. However, Hwang & Thompson (2001a) showed in pre- and post stimulation samples that there was approximately a 30% and 15% decrease in μ -calpain and calpastatin activities respectively, irrespective of the type of stimulation. The m-calpain level did not change until 24 hours post-mortem. High voltage stimulation (800 RMS V of continuous alternating polarity of bi-directional half sinusoidal pulses of 10 ms width, 14.3 pulses per second) resulted in significantly lower calpastatin levels, and there was a tendency towards lower levels of μ -calpain after stimulation ($p = 0.11$) than for low voltage stimulation (70 peak V of uni-directional square wave pulses of 7 ms width, 14.3 pulses per second).

Post-stimulation calpastatin levels were also affected by the time of stimulation post-mortem, whereby high and low voltage ES at 3 minutes (HV3 and LV3) resulted in a significantly higher level than high- and low

voltage ES at 40 minutes (HV40 and LV40). However, HV40 and high voltage ES at 60 minutes (HV60) post-mortem had similar levels of μ -calpain and calpastatin pre- and post-stimulation. At 24 hours post-mortem, all stimulation treatments resulted in similar levels of calpastatin that were significantly lower than the control sides. There was a significant time \times stimulation type interaction on the μ -calpain level at 24 hours, in that the HV3 treatment lowered μ -calpain levels more than the other treatments. The non-stimulated control sides showed a trend towards a higher residual level of μ -calpain compared to the LV40 treatment ($p = 0.06$). They indicated that the activation of the calpain system during stimulation was quite likely linked to the type of stimulation system. These results imply that the application of high-voltage stimulation possibly causes greater acceleration of the autolysis and proteolytic activity of μ -calpain and calpastatin during stimulation (Hwang & Thompson, 2001a).

At one hour post-mortem, Uytterhaegen *et al.* (1992) found no difference in activity due to high voltage stimulation (600 V, 50 Hz, 2 mins) within one hour of death, but by twenty-four hours post-mortem, there was a significant reduction in m-calpain and calpastatin activity. Geesink *et al.* (1994) showed that low voltage stimulation (85 V, 14 Hz) within five minutes of exsanguination for eight seconds had no effect on m-calpain and calpastatin activity at one-and-a-half hours, whereas stimulation for sixty-four seconds caused a significant decrease in activity for both. However, Dransfield *et al.* (1992) indicated a threshold effect of pH on protease activity, such that at a pH greater than 6.2 activities of both the μ -calpain and calpastatin were static, whilst at the lower pH, there was an accelerated decrease in both. The role of temperature at the time of sampling in the contrary results, as found in the literature cannot be ruled out.

2.8.3.6 Calcium levels

Low voltage ES leads to an increase of free calcium ions in the cytosol post-stimulation (Hopkins & Thompson, 2001c). Thus at the same temperature, stimulated muscle will be exposed to higher levels of free calcium ions, and this could be reflected by increased proteolysis. The 'free' Ca^{2+} concentration in post-mortem muscle is correlated with different indicators of proteolysis (Hopkins & Thompson, 2001c). It is also feasible that low- and high-voltage stimulation may have different effects on the flow of Ca^{2+} ions into the cytosol from the sarcoplasmic reticulum. Related to this is the response of different muscles to the various stimulation procedures which have been attributed to fibre-type variation (Devine *et al.*, 1984).

Another variable in these biochemical interactions is the production of phosphate as a consequence of the conversion of ATP to ADP. It has been noted that Ca^{2+} ions precipitate with phosphate and that available Ca^{2+} therefore decreases as muscle becomes fatigued (Kabbara & Allen, 1999). The speed of this precipitation may impact the pool of 'free' Ca^{2+} ions and affect the activation of the calpains. In relation to this, it has been demonstrated that, as the frequency of stimulation increases, the concentration of phosphate in muscles increases, which would have a direct impact on the extent of Ca^{2+} precipitation (Giannesini *et al.*, 2003).

2.8.3.7 Ageing

Ageing refers to the process where meat becomes tender over a time and involves the specific degradation of structural proteins (Hwang *et al.*, 2003). The process is due to proteolytic enzymes called calpains. Ageing starts after the completion of rigor, and at elevated meat temperatures, the process is faster than at lower temperatures (Dransfield *et al.*, 1992). Such is not the case in normal commercial operations where

lower temperatures are used in transportation, cutting and display. In fact, full ageing may not be realised with commercial operations at temperatures of around 4 °C in less than one week (Devine *et al.*, 2006).

In an effort to examine the temperature effects independent of the rate of pH fall, the calpain and calpastatin activity was compared at equivalent pH values in muscles that had been held at three different but constant pre-rigor temperatures (Simmons *et al.*, 1996). This work showed that at the completion of rigor, μ -calpain, the enzyme that has been most closely linked with meat tenderisation, was substantially depleted in muscle held at 35 °C; but levels in muscle held at 15 °C, and to a lesser extent at 25 °C, were largely unchanged from the levels found at slaughter. In this work, Tenderometer measurements at rigor showed that the 35 °C samples had lower shear force scores than the muscles maintained at 15 °C and 25 °C. In contrast, samples held at 15 °C and 25 °C had lower shear force values than the 35 °C samples after 4 days. These effects are characteristic of the heat-shortening phenomenon and show how the enzymes involved in tenderisation are affected by temperature: at high temperatures (+35 °C), the enzymes are very active and produce tender meat very quickly, but they also lose their activity very quickly. In effect, they 'burn out'. Hence meat subjected to these processing conditions does not attain the very low shear-force values required for high-value, high-quality markets. At lower temperatures, the balance is shifted towards a longer but slower period of enzyme activity; because the enzymes remain active for longer and due to less burnout, the meat reaches much lower shear-force values, as long as the meat is given enough time to age.

ES accelerates the rate of glycolysis and thus the onset of rigor, so that, as the muscle enters rigor at a high temperature, the meat commences to age rapidly at these high temperatures, and thus it would be consumer ready earlier. Polidori *et al.* (1999) noted that the ageing of stimulated carcasses improves tenderness; they become even more tender than non-stimulated ageing carcasses. It is clear that both ES and ageing significantly improve the tenderness of meat. However, with an increase in aging time, there is a decrease in the difference of the shear force between stimulated and non-stimulated meat (Strydom *et al.*, 2005).

2.9 Electrical Immobilisation

This invention relates to carcass immobilisation and has been devised particularly but not solely (could also be used as an low voltage ES system) for the immobilisation of freshly-slaughtered carcasses in an abattoir situation. Immobilisation is used in the animal processing industry as an aid to worker safety (Simmons *et al.*, 2006).

Freshly slaughtered carcasses still have active nervous systems for some minutes after death, and a violent reflex action may be initiated by even a small stimulus such as moving a limb into a new position, small knife, incisions etc. Depending on species, the nerves can remain alive and active for periods in excess of five minutes. This nerve action can trigger spontaneous and/or continuous violent movement, which is potentially dangerous to anyone in close proximity, particularly when sharp implements are being used (Anon, 2008).

Head-only electrical stunning meets the requirement for instantaneous and sustained loss of consciousness that allows exsanguination to be the primary cause of death. However, the epilepsy triggered by a head-only stun cause severe convulsions that need to be managed both to limit risks to operators and to maintain high throughputs. The standard procedure used in New Zealand plants for both sheep and beef slaughter has been to follow the electrical stun with a period of electrical immobilisation during the bleeding procedure to

suppress convulsive activity and thus allow the workers to safely undertake further workup on the carcass (Simmons *et al.*, 2006).

The two most common forms of carcass immobilisation are by mechanical restraint, where limbs are physically pinned to prevent movement and electrical restraint, which uses an applied electrical energy to override nerve function and control the muscles (Anon, 2008).

Electrical immobilisation in the early stages of post-mortem acts primarily on the nerves while they are still alive (and it is only the live nerves that generate potentially dangerous movement). In order to hasten work on the carcass, immobilisation of the nerves is often applied almost immediately after death. For this purpose, an electrical current based on the standard low-voltage stimulation parameters of 90 V and 10 ms pulses delivered at 15 Hz has been the standard system, as these voltages are considered sufficiently safe to allow operation without safety barriers (Simmons *et al.*, 2006).

According to Simmons *et al.* (2006), this low-frequency waveform has been borrowed from ES procedures and endures because it has proven to be effective at suppressing convulsive activity. There has been little regard for this method's implications on pH decline in immobilised carcasses. However, not surprisingly, a waveform developed for the purpose of stimulation is particularly effective at reducing muscle pH, often beyond the optimal levels for meat quality. In addition, the stimulation effect of immobilisation can interact with subsequent carcass stimulation after dressing to produce adverse effects on meat quality (Simmons *et al.*, 2006). Effectively suppressing post-stun convulsions, while also controlling effects on pH decline, are therefore important for managing meat quality during processing.

There is, however, an alternative system that has been developed by Simmons *et al.* (2006). The use of stimulation waveforms based on short pulse durations (around 100 μ s), combined with frequencies up to 2000 Hz has been found to produce effective and persistent suppression of convulsive activity while generating only marginal levels of pH decline. In an objective demonstration of the effects of a high frequency immobilisation waveform compared to the conventional 15 Hz waveform, the responsiveness of sheep carcasses was measured immediately after stunning and exsanguination, by hanging the carcass on a load cell. Carcass responsiveness was measured at 15 second intervals during the course of a 60 second immobilisation protocol using either 100 μ s pulses at 2000 Hz or 10 ms pulses at 15 Hz. At each 15 second interval, measurements were made of carcass response to two test stimuli; one that would trigger muscle contraction through either direct stimulation of the muscle or indirectly through the innervations of the muscle, and the other that would only elicit a response through stimulation of the peripheral nervous system. Before each pair of test pulses, the carcass stimulation was stopped for 3 seconds to allow for full relaxation. Their results show that both the high frequency and low frequency waveforms cause a rapid loss of responsiveness to the nerve test pulse, and this reflects the loss of spontaneous physical movement in the carcass following the immobilisation procedure. However, the responses to the muscle test pulse were distinctly different: whereas the low frequency immobilisation produced a rapid loss of responsiveness, the high frequency immobilisation produced only a marginal change. The implication is that the loss of responsiveness to the 10 ms pulses reflects muscle fatigue and an associated drop in muscle pH, and separate experiments have shown that this immobilisation protocol regularly reduces the pH of the *Longissimus thoracis et lumborum* to below 6.4. In contrast, the high frequency waveform has little effect on

muscle responsiveness, and this is reflected in a muscle pH that is typically greater than 6.8. Toohey & Hopkins (2007) illustrated the effect of high frequency immobilisation versus no immobilisation (2000 Hz, 400 V, 150 µs pulse width) on the meat quality of sheep. The results of the study showed that stimulation had no significant effect on pH decline, sarcomere length, shear-force values, cooking losses or meat colour based on L*, a* and b* values.

Electrical immobilisation after stunning plays an important role in ensuring operator safety and maintaining high throughput levels. The conventional system, based on low frequency waveforms, often results in excessive pH decline during and following stimulation, particularly when immobilising large cattle that subsequently cool very slowly (Simmons *et al.*, 2006).

2.10 Classification of Electrical inputs

There are a number of methods by which ES can be applied and many different possible electrical specifications. Regardless of the species, stimulation can be applied immediately after slaughter or at any point in time thereafter until the muscles become unresponsive. The time until muscles fail to respond is related to the natural rate of glycolysis and the voltage being applied, the duration of the stimulation and the type of response expected (Chrystall & Devine, 1985).

Most commercially used ES systems employ the conveying rail as ground, and a live electrode makes contact with some other part of the body or carcass. The most basic systems employ a live electrode clip which is manually applied and is grounded either by the rail or by an earth electrode hooked to the tail of the animal. However, as the voltage increase more sophistication is needed, due to safety concerns that accompany the higher voltages (Chrystall & Devine, 1985).

The electrical characteristics of the waveforms are often poorly described. Voltages range the most from very low (< 50 V) to high (> 200 V) and can be described in various ways. The value specified might be that of the peak voltage, or the root mean square (RMS) voltage. In some cases, the average voltage over the total time is used. The RMS is the effective value or the heating capacity of the waveform. The RMS value is calculated as follow:

Equation 1 Calculation of the root mean square voltage.

$$V_{\text{RMS}} = V_{\text{peak}} \times \text{duty cycle of waveform}$$

Many different waveforms have been used; some have been well researched and described, whereas others seem to be merely used based on the equipment available. The waveforms can be either uni-polar or bipolar and applied as discrete pulses or pulse trains. The following figures (Figure 3 – Figure 7) are set out to illustrate the meaning of the different terms to describe voltages and waveforms.

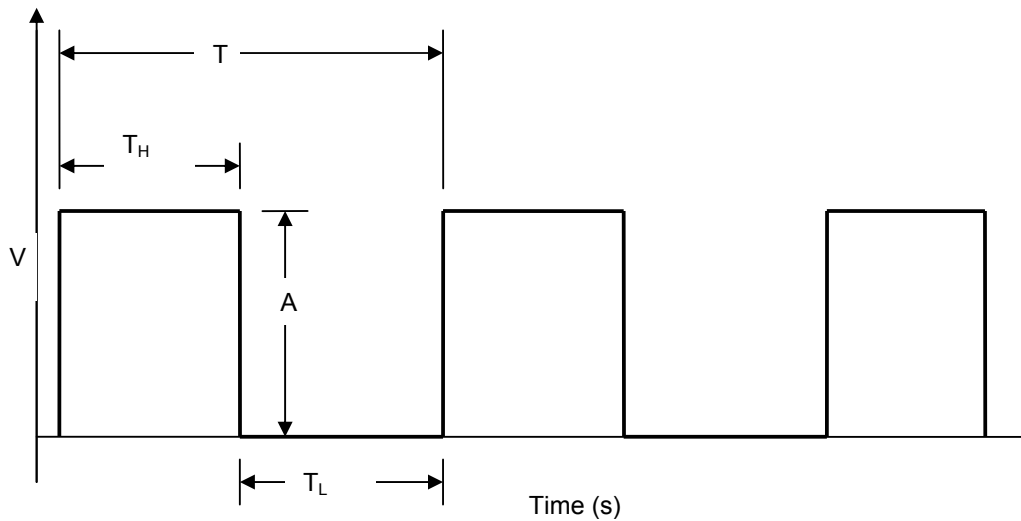


Figure 3 Square waveform of an electrical impulse with an indication of amplitude (A), the time, high (TH) and low (TL), for the period (T) of the pulse (Devine *et al.*, 2004)

A square or rectangular waveform is characterised by flat maximum and minimum levels, fast rising and fast falling edges and right angled square corners. The amplitude (A) of a rectangular waveform is a measure of the distance between the minimum and maximum levels (the peak-to-peak value). Amplitude is most often expressed in units of volts, although units of current and power can be useful at times. The period (T) of a rectangular waveform is the time required to complete one full cycle. The period is measured in units of seconds. The period of a rectangular waveform can be further broken down into two phases: time high (TH) and time low (TL). The duty cycle (D) of a square waveform is the ratio of time high to the total period. The duty cycle of a waveform has no units and is normally expressed as a percentage where:

Equation 2 Calculation of the duty cycle of a waveform.

$$D (\%) = (TH/T) \times 100$$

Rectangular waveforms are sometimes used for regulating the amount of power applied to a load. The higher the duty cycle, the greater the amount of power applied to the load. It is important because it relates to peak- and average power in the determination of total energy output. The frequency of a waveform is given by:

Equation 3 Calculation of frequency from the period of a waveform.

$$F = 1/T$$

However, there is no relationship between frequency and the duty cycle of a rectangular waveform.

In Figure 4 there are 50 sinusoidal cycles per second (100 half sine wave pulses) with the peak and RMS voltages indicated. The pulses in Figure 5 are obtained by cutting out half of the sinusoidal pulses, which in this case gives 10 ms duration pulses, 14.3 pulses per second with the same peak voltage (Chrystall & Devine, 1985). The pulse width (mark) and space between pulses give the mark-to-space ratio used to specify a single repetitive cycle, with the polarity of pulses and the number of cycles per second required to

complete the description. The waveforms in figures 5 and 6 both have the same period (1/F) and peak amplitude, but have different shape characteristics (Devine et al., 2004). The waveform in Figure 7 is commonly used in the USA, with intermittent on and off times of a 50 Hz waveform.

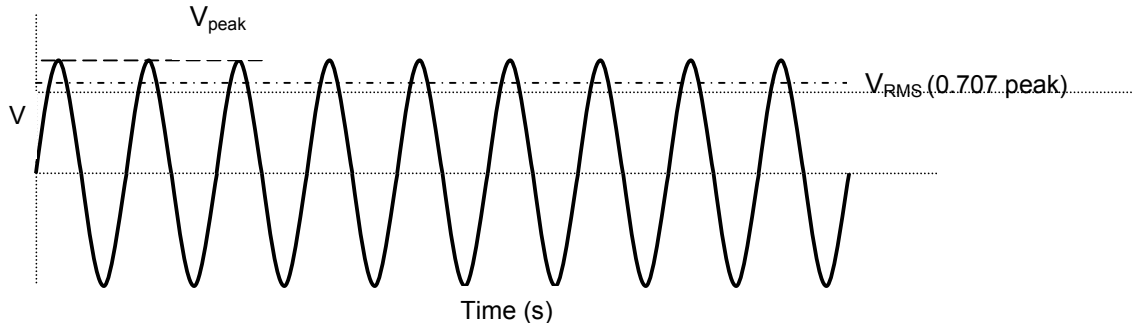


Figure 4 50 Hz alternating current waveform

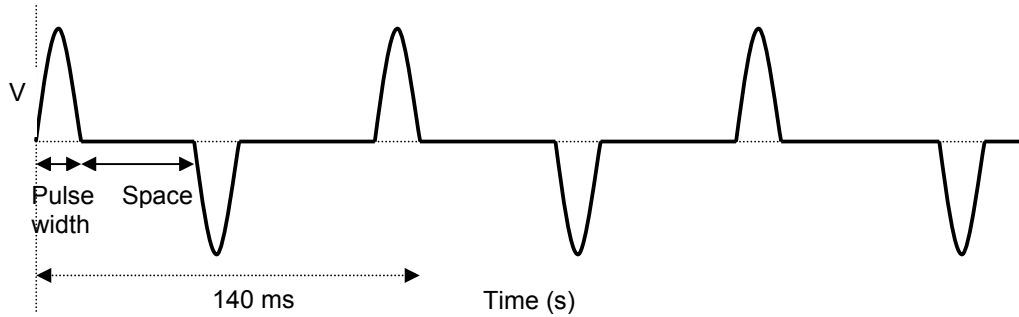


Figure 5 Modification of the 50 Hz waveform to produce a 14.3 Hz half sine waveform

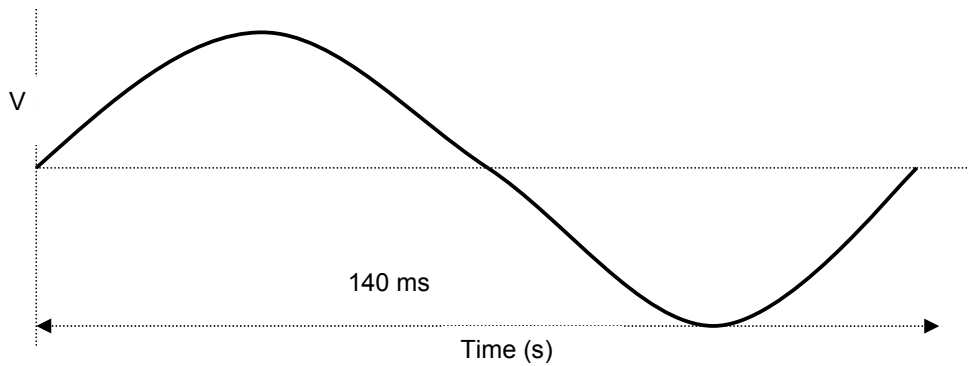


Figure 6 7 Hz alternating current waveform

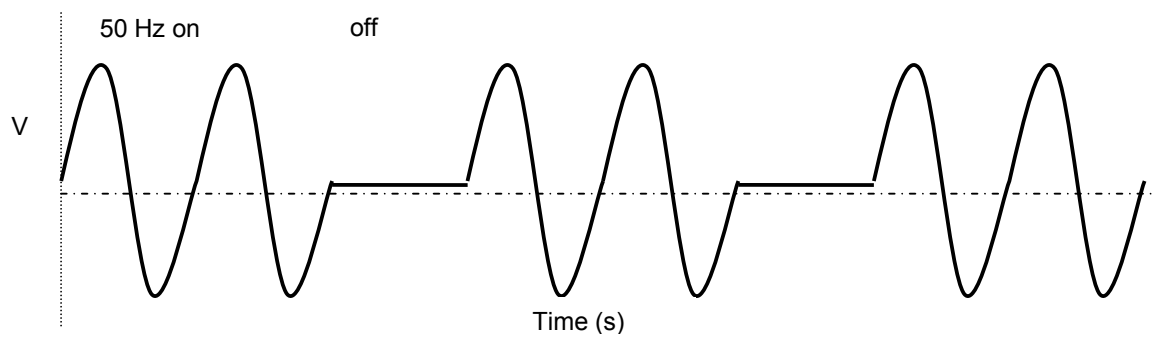


Figure 7 Burst of 50 Hz waveform. Common on off times are 2 s on: 2 s off

Muscle force is controlled by the number of motor units activated and the amount of force generated in the units. The muscle force is thus the work done by the muscle and is therefore related to the pH decline of the muscle (Riener & Quintern, 1997). For any given muscle, the pH response will be governed by the electrical characteristics of voltage, such as pulse frequency, shape and polarity. In general, the higher the current (at a constant resistance, current increases with an increase in voltage), the greater the response will be. This response will be asymptotic to some maximal value; however, continually increasing the current will not lead to a continuing increase in the effect (Chrystall & Devine, 1978). For all of the species tested, the pulse shape does not seem to be a critical factor, with equivalent responses being achieved with 10 ms duration half-sine wave pulses and 5 ms duration square-wave pulses at the same frequency (Chrystall & Devine, 1978).

2.10.1 Pulse frequency

Muscle force can be controlled by modulating pulse frequency. The pulse frequency can exert a profound effect because of the interaction with muscle type and response frequency. If a single muscle is considered, the magnitude of ΔpH is maximal with a pulse rate of between 9 and 16 per second. At frequencies between 10 and 20 pulses per second, ΔpH values are, respectively, 40% and 75% greater than at 50 and 100 pulses per second (Crystall & Devine, 1978). Simmons *et al.* (2008) stated that an additional effect needs to be accounted for when considering stimulation frequency. They stated that a frequency of 50 Hz produces a higher peak contraction force, while the response decays much faster, an effect that cannot be solely attributed to the higher metabolic demand of greater force generation. According to the authors, the area under the curve – a measure of the work done by the muscle related, therefore to the pH decline – is 35% less in the 50 Hz stimulation compared to the 15 Hz (Figure 8). This is only applicable if stimulation is done for long enough. A possible explanation of this phenomenon can be attributed to accumulation of extracellular potassium in response to the rapid rate of stimulation that renders it refractory to further stimulation (Juel, 1988; Cairns *et al.*, 1995). With the use of intermittent stimulation waveforms (2s on/2s off) the short rest periods allow the membrane to recover, and an effective decline in pH is possible at high frequencies. Ultimately, the muscle becomes unresponsive due to metabolic fatigue and extreme pH decline (Simmons *et al.*, 2008).

Different muscle types have different responses to frequencies because the rate at which the Ca^{2+} is reabsorbed differs. Fast-twitch muscle has a better developed sarcoplasmic reticulum so that it can reabsorb Ca^{2+} faster and produce full fusion at a higher frequency. The ΔpH (drop in pH during ES) should include the variable of the duration of the stimulation when different frequencies are compared: at low frequencies, the

Δ pH will be the same but require more time than is usually allowed for during the stimulation process, whether experimental or commercial. What isn't usually discussed or understood is why the Δ pH decreases at the higher frequencies. Simmons *et al.* (2008) have proposed that this is due to membrane fatigue rather than metabolic fatigue; accordingly, responsiveness is lost due to K^+ accumulation at membrane level (Juel, 1988; Cairns *et al.*, 1995). With the use of intermittent stimulation waveforms (2s on/2s off) the short rest periods allow the membrane to recover and an effective decline in pH is possible with high frequencies. Ultimately, the muscle becomes unresponsive due to metabolic fatigue and extreme pH decline (Simmons *et al.*, 2008). Muscles also differ in their optimum response frequency, where *Longissimus dorsi* is more responsive to 14 pulses per second than to 40 pulses per second (Chrystal & Devine, 1978) and the opposite has been shown for beef *Semimembranosus* (Bouton *et al.*, 1980).

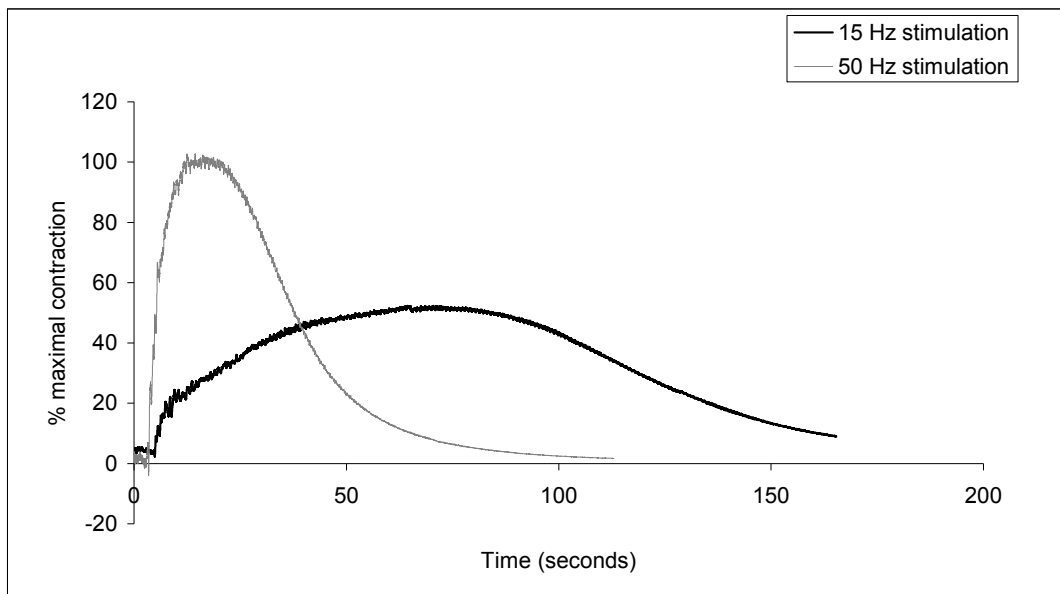


Figure 8 Muscle contraction response with 15 and 50 Hz ES (adapted from Simmons *et al.*, 2008)

2.10.2 Pulse width and amplitude

There is a distinct interaction between pulse width and amplitude. If the pulse amplitude is held constant while reducing the pulse width, the response will weaken and eventually disappear altogether (Aston, 1991). Pulse-amplitude modulation is, in effect, equivalent to pulse-width modulation, since an increase in either recruits more motor units (Crago *et al.*, 1980) and thus a higher muscle force is generated with an increase in pH decline. Thus, with an increase in amplitude, pulse width can be decreased without any effect on contraction and *vice versa*, the limit being a pulse width that requires such a large voltage that tissue damage occurs through ohmic heating. The relationship can be described by the classical pulse strength-duration curve, which defines the threshold for producing an electrically-induced stimulus (Figure 9) (Simmons *et al.*, 2008).

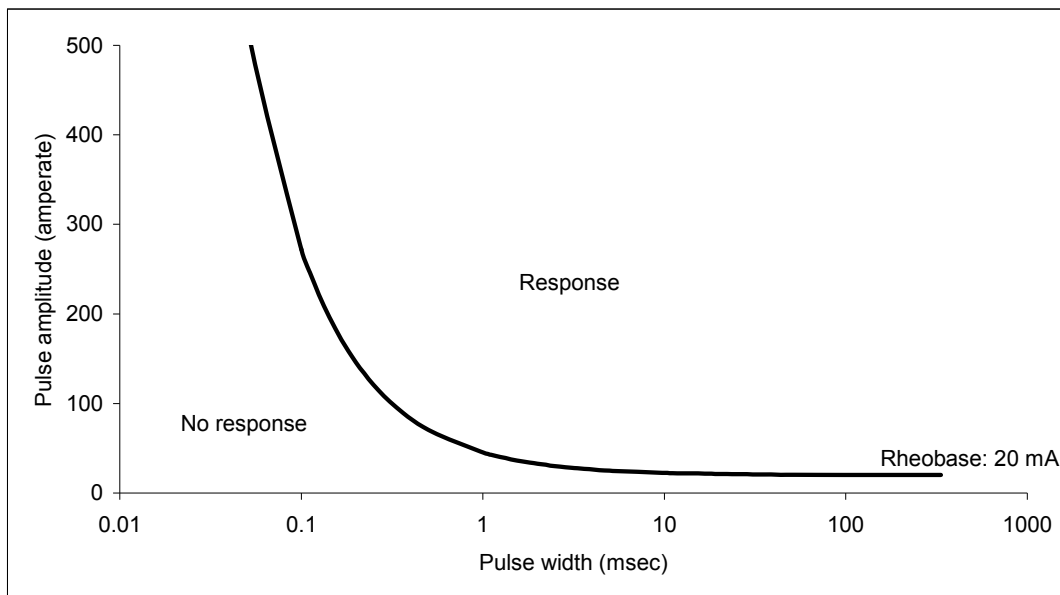


Figure 9 Muscle response curve to ES (adapted from Simmons *et al.*, 2008)

Simmons *et al.* (2008) used measurements of muscle pressure to put values to the relationship. They found that in carcasses stimulated immediately after slaughter, the muscle response is insensitive to pulse width and that the threshold current amplitude needed for a response is very low (< 50 mA). Furthermore, the response (increase in force per unit per increase in stimulation magnitude) was very steep, reaching a maximum at less than 100 mA. However, continuous stimulation caused the stimulation to decay rapidly, signalling responses mediated by the nervous system. However, there are steep contrasts when the response is measured in the carcass after dressing (20-30 minutes). There is a more defined force increase in response to increased stimulus strength. The wide distribution of threshold voltage reflects the range of fibre diameter, the main determinant of an electrically-induced depolarisation threshold for both nerve and muscle tissue (Daly, 2005). The importance of pulse width becomes more evident as the carcass becomes more fatigued and the pH falls. The response threshold increases as the muscle becomes more fatigued and wider pulse widths are needed to maintain an effective response (Simmons *et al.*, 2008).

2.10.3 Pulse polarity

Pulse polarity has not received much attention although it was indicated that for low-voltage stimulation the polarity of pulses influences the magnitude of ΔpH and the movement of the carcass during stimulation. The greatest responses are obtained when the cranial end of the animal is positive in relation to the rest of the animal (Chrystall & Devine, 1985).

2.11 Physiological response to stimulation

The physiological characteristics of muscle define its contraction response. Electrical activation of muscle membranes triggers Ca^{2+} release from the sarcoplasmic reticulum, triggering the contractile actomyosin ATPase and muscle contractions. Muscle contractions result in a higher rate of ATP breakdown and this leads to a faster post-mortem glycolysis. The Ca^{2+} ions also enhance the activity of the phosphorylase, which provides the substrate for an increase in the rate of post-mortem glycolysis (Lawrie, 1998). Fibre type composition of the muscle will have an effect on calcium release and reuptake after stimulation and the

properties of the associated force generated (Lawrie, 1998; DeLuca *et al.*, 1982). Muscle type has an influence on ΔpH as shown by Devine *et al.* (1984) in beef muscles where the fast twitch *Cutaneous trunci*, largely composed of white muscle fibres, gave high values for ΔpH whereas the slow twitch *Masseter*, composed of red fibres, had neither a distinct ΔpH nor an acceleration of $d\text{pH}/dt$. It was reported by Swatland (1981) that within the *Sternomandibularis*, fast fibres with strong ATPase and weak SHD reactions showed most glycogenolysis in response to ES, while slow fibres with weak ATPase and strong SDH showed the least. White muscles have a greater response to ES than red muscles, because anaerobic fibres respond more readily. The higher rate of glycogenolysis in fast-twitch muscles is caused by a high content of phosphorylase, greater activation of phosphorylase and a higher content of creatine phosphate. The effects of ES on meat are not limited to the actual period of stimulation, but persist afterwards, perhaps because of changes in the sarcoplasmic reticulum. ES causes swelling of the sarcoplasmic reticulum, transverse tubules and mitochondria, together with autolytic ultrastructural changes. Increased binding of glycolytic enzymes to actin filaments may also be involved. Red and white muscles also differ in the way that temperature affects the activity of the sarcoplasmic reticulum (Lawrie, 1998).

In his review, Daly (2005) stated that there are two considerations when assessing the responses of excitable tissues to an applied electrical waveform: pulse duration and pulse frequency. Reducing pulse duration increases the voltage threshold needed to trigger an action potential, but this threshold is also defined by the physical characteristic of the target cell: cells with low internal electrical resistance, primarily as a consequence of large cell size (or, in the case of neurones, cell diameter) are able to respond to shorter pulses than cells with high internal resistance. Peripheral motor neurones, for this reason, have the lowest threshold. In contrast, although muscle fibres can have large diameters, the dense packing of contractile proteins produce a high internal electrical resistance and these cells have, by comparison, a much higher threshold. The consequence of these response features means that appropriate choice of waveform frequencies can be used to selectively stimulate specific responsive tissues, and the use of very short pulse durations will permit stimulation of motor neurones without direct stimulation of skeletal or cardiac muscle. The electrical parameters used to produce a maximal muscle response in a whole carcass will involve pulse durations of 2–10 ms and 500–1000 peak V, while stimulation of motor neurones with minimal effects on muscle tissues can be accomplished with pulse durations around 0.1 ms and 100–200 V. The second important attribute of the stimulation waveform is the pulse frequency. With increasing frequencies, the interval between pulses becomes less than the time needed for responsive cell membranes to recover from the induced action potential: in other words, the pulses encroach on the partial refractory period, which both fails to trigger an action potential but also delays recovery of the membrane to the responsive state. The consequence of this is that the responses of the cell to high frequency stimulation becomes intermittent and generates only a low frequency of responses. In peripheral motor neurones, with an action potential duration of, typically, around 1 ms, a reduction in muscle activation becomes particularly evident when frequencies exceed 1000 Hz and decreases monotonically as the frequency increases above this level.

Most reported investigations refer to applied voltage, despite widely differing procedures for making contact. The voltages should be of sufficient magnitude to overcome resistance effects at the electrodes and ensure a saturation flow of current through the musculature (Chrystall & Devine, 1980). Crystall *et al.* (1980) suggest that muscles not lying directly in the current pathway may not benefit equally from ES. Current follows the path of least resistance from the positive to the negative electrode. Devine *et al.* (1984) suggest that muscle

response to ES is probably dependent on the muscle fibre type, because muscle exhibits a wide range of metabolic and functional characteristics. Muscles consisting mainly of slow-twitch, oxidative fibres are reported to be relatively insensitive to electric fields with strength lower than 2 V/cm (Houlier *et al.*, 1980).

Traditionally, the application of ES is divided into either low- or high voltage stimulation. Low voltage ES (< 100 V peak) is used right after exsanguination while the nervous system is still active and in such a way facilitates muscle contraction (Lawrie, 1998). High voltage ES is normally applied after dressing with voltages of up to 1143 V peak. The muscle is either stimulated directly or by impulses from the nervous system (Lawrie, 1998). However, some intermittent- or medium-voltage stimulation (Shaw *et al.*, 2005) that lead to the relative benefits of meat quality and operator safety have been reported.

2.12 Conclusion and Objectives

Different results are reported by authors that have experimented with the application of ES. These contradictions can mainly be ascribed to the different experimental methods and conditions of application.

Electrical stimulation has been shown to enhance certain quality characteristics of meat such as lean colour and tenderness. The packager, retailer and consumer therefore could benefit if ES is used as an integral part of the process of converting muscle into meat. Electrical stimulation could be used on the carcasses of older animals to reduce such variations in tenderness that may result from age, nutrition, animal history and stress during slaughter. Electrical stimulation seems to be an important application in modern abattoirs, and with the use of rapid chilling, it is beneficial to the supplier and consumer.

The objective of this study is to quantify the effect of different EI and stimulation procedures, frequencies and pulse widths on the meat quality of beef. Abattoirs are combining electrical stunning with EI as a procedure to minimise the convulsive activity induced by stunning so as to maintain high throughput speeds. There is very little data available on the effect of EI when combined with ES on meat quality. This study will make use of two different immobilisation frequencies (high – 800 Hz and low – 15 Hz) combined with either high (1040 V) or medium (300 V) voltage ES to determine the effect on meat quality. The hypothesis is that high frequency immobilisation improves meat quality when combined with either high- or medium voltage ES (Chapter 3). A second hypothesis is that medium voltage ES improves or has no adverse effects on meat quality in relation to high voltage ES combined with high-frequency immobilisation (Chapter 3).

In Chapter 4, utilising the results from Chapter 3, the EI waveform (HFI) is standardised, as is the ES (MVS) with the frequency being modulated. In Chapter 5, the pulse width of the waveform is changed to optimise the ES system.

This study undertakes to illustrate that there are alternative electrical parameters to voltage that can be used to change the pH decline of beef muscles, and by changing frequency and pulse width, subtle changes in the meat quality can be induced. Since every abattoir is different, due to layout, chiller space and cooling regime, these electrical parameters need to be modulated to optimise an ES system without expensive modification to the whole system. By quantifying the meat-quality attributes that develop from different processing conditions it is hoped that the knowledge gained will assist in defining appropriate specifications for practical application.

2.13 References

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ELECTRICAL IMMOBILISATION AND STIMULATION OF BEEF CARCASSES AND ITS EFFECT ON MEAT QUALITY

Abstract

Electrical immobilisation (EI) is used to control animal movement after electrical stunning, while electrical stimulation (ES) is used to induce rapid tenderisation. These two interventions are frequently used together. The objective of this investigation is to evaluate the combination of these two techniques on the meat quality of beef muscles. Forty Holstein steers were electrically stunned (head only, 2 A, 50 Hz, 2 s) and slaughtered, after which low (14.3 Hz, 90 V peak, 10 ms pulse duration; LFI) or high frequency (800 Hz, 110 V peak, 0.2 ms pulse duration; HFI) EI (20 seconds) was applied within two minutes of killing. After carcass dressing, high voltage (1140 V peak, 10 ms pulse duration, 14.3 Hz; HVS) or mid-voltage (300 V peak, 14.3 Hz, 1 ms pulse duration; MVS) ES was applied. Meat-quality measurements were made from the *Longissimus dorsi* (LD) and *Semimembranosus* muscles after 1, 5 and 9 days of storage at 0°C. LFI HVS produced significantly greater drip during storage and shear force values (storage drip = 3.30±0.223%; shear force = 102.9±4.5N) when compared to the HFI HVS (storage drip = 2.45±0.261%; shear force = 5.2±4.0%) or HFI MVS (storage drip = 2.60±0.178%; shear force = 4.2±4.2N) in the LD. LFI HVS ($a^* = 20.79 \pm 0.31$; chroma = 22.92±0.33) and LFI MVS ($a^* = 20.24 \pm 0.27$; chroma = 22.23±0.30) had a redder and more vividly bloomed colour than HFI HVS ($a^* = 19.71 \pm 0.33$; chroma = 21.49±0.37) and HFI MVS ($a^* = 20.00 \pm 0.27$; chroma = 21.98±0.31). MTT (*Tetrazolium Salt*) assay correlated linearly ($r = -0.63$ and -0.73) with a^* values at 24 hours post-mortem after allowing 3 hours of bloom.

3.1 Introduction

The two main determinants of post-slaughter processing outcomes are the rates of pH- and temperature decline (Simmons *et al.*, 2006). Muscle pH and temperature interact continuously during rigor development so as to affect both the muscle contracture (Tornberg, 1996) and proteolytic enzyme activity (Dransfield *et al.*, 1992). However, the pH can be manipulated independently of temperature through electrical stimulation, and this gives rise to the opportunity to manipulate meat-quality outcomes. The effects of electrical stimulation on meat quality has been well documented in reviews (Cross, 1979; Polidori *et al.*, 1996; Hwang *et al.*, 2003). Electrical stimulation tends to decrease shear force values as compared to non-stimulated meat (Strydom *et al.*, 2005), but the extent of this effect depends on the amount of electrical input applied. There are three possible mechanisms which may alter the rate of tenderisation: reduced cold-induced shortening, enhancement of the rate of proteolysis and alteration of protein structure (Hwang *et al.*, 2003). The first theory is associated with the prevention of cold shortening. The second theory is based on the phenomenon that electrically-stimulated carcasses release proteolytic enzymes sooner and that these work faster than those in non-stimulated carcasses, this is due to the higher carcass temperature. The third theory is based on electron micrographs that reveal that muscles from electrically-stimulated carcasses show structural damage (Stiffler *et al.*, 1999). As ageing progresses, the differences between electrical and non-stimulated

samples decrease (Strydom *et al.*, 2005) and can disappear completely if sufficient time is allowed (Chrystall & Daly, 1996) and cold-induced contraction of the muscle is avoided.

Meat tends to be redder after high (Eikelenboom *et al.*, 1985) and low voltage (Unrah *et al.*, 1986; Sleper *et al.*, 1983; Eikelenboom *et al.*, 1985) ES, compared to the non-stimulated carcass muscles. This effect is due to the damage done to the enzyme systems of the mitochondria responsible for oxygen consumption, a reduced oxygen consumption rate and the resulting higher concentrations of oxymyoglobin in the surface meat layer (Ledward, 1992). This observation is confirmed by Lawrie (1998) who also claims that the brighter red colour can also be attributed to the fast pH decline. This results in the carcasses reaching their isoelectric point much sooner, thereby "opening up" the structure and easing the oxygenation of myoglobin. Colour stability, when defined as the rate of metmyoglobin accumulation on the surface layer, is generally reduced by ES (Wiklund *et al.*, 2001). The result found by Wiklund *et al.* (2001) suggests the ES accelerates the loss of metmyoglobin reductase activity so that, after a week, metmyoglobin accumulation is accelerated. This action equilibrates after three weeks with non-stimulated meat, and the effect is then lost. Unrah *et al.* (1986) found at day one that steaks were brighter red, but at day five the control (non-stimulated) was redder. A more open muscle structure and the enzymes important to muscle colour are degraded because of the temperature and pH conditions and may account for the increased rate of colour deterioration during display (Sleper *et al.*, 1983). Van Laack & Smulders (1990), however, found the opposite: that ES increased the display life. They postulated that the more open structure of the muscle may allow more oxygenation and thus increased redness. Similarly, when stimulation is omitted, the tighter structure would limit oxygen diffusion and thus reduce the layer of the red oxymyoglobin. Consequently, the metmyoglobin layer beneath the oxymyoglobin will be nearer to the surface.

Fluid loss due to muscle-lattice shrinkage has an effect on colour (Rees *et al.*, 2003). In essence, when the pH falls quickly in the period soon after slaughter while the muscle is still at a high temperature, the protein filaments that produce contraction are disrupted or denatured and 'shrink'. This results in a reduced amount of space within the protein lattice, and the water is expelled as drip. This mechanism also results in an increase in the paleness of the meat. This is because the reduced lattice spacing within the muscle causes more light to be reflected from the meat surface, imparting a whiter/paler appearance (Unrah *et al.*, 1986). Overall, therefore, inappropriate processing conditions that involve a rapid pH fall while temperatures are still high can give rise to muscle that is pale in appearance, loses large amounts of drip and, because of less reductase enzyme, has a reduced shelf life.

Electrical immobilisation is used to suppress post-stun convulsion triggered by electrical stunning. Electrical stunning is becoming popular due to the requirements set out for a Halal slaughter (Simmons *et al.*, 2006). The epilepsy triggered by an electrical stun causes severe convulsions that need to be suppressed to ensure the safety of workers and to maintain high throughput levels in an abattoir. The traditional method of immobilisation was the use of a low voltage electrical stimulation unit of 90 V at 15 Hz as it is considered safe for workers to work on carcasses under these conditions. These units led to sufficient suppression of the convulsive activity, but also led to a rapid pH decline, which could have adverse effects on meat quality, especially if the cooling rate is slow. Effectively suppressing post-stun convulsions, while also controlling the effects on pH decline are therefore important for managing meat quality during processing (Simmons *et al.*, 2006). Simmons and co-workers (2006) developed stimulation waveforms based on short pulse durations

combined with frequencies up to 2000 Hz, which were found to produce effective and persistent suppression of convulsive activity while generating only marginal levels of pH decline. Toohey & Hopkins (2007) also illustrated the effects of high-frequency immobilisation (2000 Hz, 400 V, 150 μ s pulse width) versus no immobilisation on the meat quality of sheep. The results of their study showed that immobilisation had no significant effect on pH decline, sarcomere length, shear force values, cooking losses or meat colour based on L*, a* and b* values.

If carcasses receive excessive amounts of stimulation, shear force values after ageing are higher in the stimulated carcasses compared to non-stimulated carcasses, especially when chilling is slow (Geesink *et al.*, 2001; Koh *et al.*, 1987). In addition, excess stimulation can reduce water-holding capacity (Eikelenboom *et al.*, 1985) and colour stability (Unrah *et al.*, 1986). The effects of excess stimulation can be attributed to the coincidence of high temperatures and low muscle pH, conditions known to denature muscle structural proteins (Offer, 1991) and accelerate the autolysis of calpains (Simmons *et al.*, 1996). Severe rigor contractions also occur if rigor is attained at a high carcass temperature (Hertzman *et al.*, 1993), and this can be expected to affect the capacity of meat to tenderise.

As mentioned, abattoirs combine electrical stunning with electrical immobilisation as a procedure to maintain high throughput speeds. However, the effects of these two electrical procedures on the meat-quality attributes of cattle are still poorly understood. The purpose of this experiment was therefore to determine the effects of two electrical immobilisation and stimulation treatments on the tenderness, water-binding capacity and colour stability of two beef muscles. The objective is to prove that HFI improves meat quality when combined with either HVS or MVS. Another objective is to prove that MVS improves or has no adverse effects on meat quality in relation to HVS combined with HFI. By quantifying the meat quality attributes that develop from different processing conditions, it is hoped that the knowledge gained will assist in defining appropriate specifications for practical application.

3.2 Materials and methods

3.2.1 Animals and treatments

The trial consisted of two days of kill. Forty Holstein steers of the same age for each kill were electrically stunned (head only, 4 Amps, 50 Hz, 3 seconds), followed by a throat cut and thoracic stick. Carcasses were electrically immobilised (EI) using either low (LF) (14.3 Hz, 90 V for 20 seconds) or high frequency (HF) (800 Hz, 200 V for 20 seconds) EI within two minutes of slaughter. After carcass dressing, electrical stimulation (ES) was applied using either a high voltage (HVS) (1040 V, 14.3 Hz) or a medium voltage (MVS) (300 V, 14.3 Hz, 1 ms pulse duration).

Thereafter, the carcasses were placed in the chiller (40 minutes) and were cooled down in two phases. The first phase involved keeping the temperature at 8 °C for the first 8 hours, and then at 0 °C until 24 hours post-mortem. After 24 hours the *Longissimus dorsi* (LD) and *Semimembranosus* (SM) muscles were cold boned and used for meat quality assessment at days 1, 5 and 9 following chilled storage at 0 °C. In the first kill, the LD and SM muscles were used, while in kill two only the LD was used for meat quality assessment. A section of loin consisting of the LD and overlaying subcutaneous fat from the 12th rib to the lumbar–sacral junction was removed from the left side of each carcass. The piece of loin weighing approximately 1.5 kg was subdivided into three portions with the samples being assigned to days 1, 5 and 9 of assessment, such

that each day had the same amount of pieces from the cranial, sacral and caudal end of the LD. The SM was sliced into three longitudinal portions and the centre slice taken for assessment. The slice of SM, weighing about 2.5 kg, was subdivided into three portions being randomly assigned to days 1, 5 and 9 of assessment that each day had the same amount of pieces from the cranial, sacral and caudal end of the SM. The meat-quality attributes evaluated included shear force, drip loss during retail display and vacuum-packed storage, cooking loss, water-binding capacity, colour during retail display, MTT assay and myofibrillar density. The treatments were the same for both kills.

3.2.2 pH and temperature measurements

The pH measurements were taken at 0.5, 1.5, 2.5, 3.5 and 24 hours post-mortem with a handheld meter (Orion 3 Star portable with a ionade pH-electrode probe, model IJ44) while the temperature decline was taken from the LD and between the femur and SM by means of a temperature-data logger measuring every 15 minutes. Data loggers were placed in the respective muscles as they entered the chiller at 40 minutes post-mortem.

3.2.3 Shear force and cooking loss

Shear force evaluation of tenderness was done according the method of Simmons *et al.* (2000) measured using a Digital Tenderometer™. The samples of the LD and SM, approximately 400 g to 500 g, were cooked in a water bath at 100 °C in weighted plastic bags until the internal meat temperature reached 75 °C as measured by an Ebro TFX 392 temperature meter. The portions were then removed and immediately chilled on ice. The basic principle in assessing meat tenderness is to determine the force required to shear through a 10 x 10 mm square cross-section sample at right angles to the fibre axis. Ten bites per sample were sheared using a triangular-shaped tooth using a Tenderometer (G2 Digital) and used to give an average shear force value in kilogram force which was converted to Newton (1 kgf = 9.8 N). The temperature of the samples was ±1 °C.

Before cooking, each muscle sample was weighed. After cooking, the drip was removed. The following day, the cooled samples were dried using paper towels to remove excess moisture. Afterwards, the samples were weighed again. The cooking loss was measured as the difference between the sample mass before and after cooking and was expressed as a percentage of the starting mass (Simmons *et al.*, 2000).

Equation 2.1 Determination of cooking loss percentage

$$\text{Cooking loss \%} = (\text{weight before cooking} - \text{weight after cooking}) / \text{weight before cooking} \times 100$$

3.2.3 Drip loss (retail and storage)

Fresh samples of about 500 g were vacuum packed in bags (Sealed Air, Tufflex®) and stored at 0 °C for either 5 or 9 days. Before vacuuming, the samples were dried of surface wetness and weighed. When the samples were taken out, they were again dried and weighed. Drip was measured as the difference between the sample mass before and after storage and expressed as a percentage of the starting mass (Simmons *et al.*, 2000).

Equation 2.2 Determination of drip loss percentage during cold storage

$$\text{Storage drip loss \%} = (\text{weight before vacuumed storage} - \text{weight after storage}) / \text{weight before storage} \times 100$$

The samples for drip during retail display life were weighed and placed in trays and overwrapped with oxygen permeable film (Progressive Enterprises, W/O ID 838485, Roll 404). The trays were placed in a chiller (Skope, B550PH2CX:V48JF) at 5 °C to mimic retail storage. After display, the samples were dried and weighed. The display times varied for the different time points of assessment, with day one having ten days of retail display display life, day five having nine days of retail display shelf life and day nine having eight days of retail display shelf life, respectively. The drip was measured as the difference between the sample mass before and after and expressed as a percentage of the starting mass (Simmons *et al.*, 2000).

Equation 2.3 Determination of drip loss percentage during retail display

$$\text{Retail drip loss \%} = (\text{weight before retail display} - \text{weight after retail display}) / \text{weight before retail display} \times 100$$

3.2.4 Water-binding capacity

The water-binding capacity (WBC) of the muscles was determined by the press method where 500 mg fresh meat sample (24 hours) was placed on a filter paper (Whatman #2) sandwiched between two Perspex plates and pressed at a standard pressure (588 N) for 1 minute. The WBC was calculated by determining the ratio of meat area and the liquid area after compression. The measurement was taken in duplicate. Photos were taken of the two areas that were then measured by means of the Image J 1.41 computer package (Trout, 1988).

3.2.5 Colour and colour stability

Steaks, of roughly 20 mm thick, were cut from the LD and SM and placed on a tray (vertex plix 810) and overwrapped with covering film (Progressive Enterprises, W/O ID 838485, Roll 404). The trays were placed in a chiller (Skope, B550PH2CX:V48JF) to mimic retail storage. Three colour measurements were carried out across the individual sample surfaces on top of the covering film, and mean values were used for statistical analysis. Non-bloomed colour was measured after cutting, and bloomed colour (day 0) was taken three hours later. Colour measurements were taken at roughly the same time each day thereafter until the meat was visually unacceptable. For time point 1, 5 and 9 (day of assessment) the days of retail display were 10, 9 and 8 days respectively.

Colour measurements followed the CIELab (CIE, 1986) colour convention, where the three fundamental outputs are L*, a* and b*. L* is lightness on a scale of 0 (all light absorbed) to 100 (all light reflected); a* spans from +60 (red) to -60 (green) and b* spans from +60 (yellow) to -60 (blue). Chroma (saturation) was calculated as $(a^{*2} + b^{*2})^{1/2}$, and hue angle as $\tan^{-1} b^*/a^*$. Colour was measured using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing Inc.) calibrated against a white tile (L* = 93.30, a* = 0.3158 and b* = 0.3322). The aperture was 8 mm, and illuminant D65 and 10 ° observer were used.

3.2.6 MTT (Tetrazolium Salt) Assay

MTT is used to measure the reduction of a specific tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to measure the total reductive capacity of meat. The assay was done according to Berridge & Tan (1993) as modified by Simmons *et al.* (2008).

Two grams of meat with no connective tissue or fat was homogenised in 12 ml homogenisation buffer (250mM sucrose (BDH 10274413), 1mM EDTA (disodium salt, J.T. Baker 8993-01), 10mM KCl (J.T. Baker 3040-01), adjusting the pH of the buffer to 5.3, using KOH and homogenized for 20 seconds at 9000 rpm. 75 µl homogenate was added to tubes (three replicates plus one blank per sample). A 200 µl homogenisation buffer was added to the blank tube and 200 µl 0.25 mg/mL MTT solution (tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Applichem A2231, 0001), to the three replicate tubes. The tubes were incubated in a dark room for 2 hours and then spun in a centrifuge (Jouan CR3i multifunction) at 4000 g for 1 minute. The supernatant was removed and 1.25 ml DMSO (Scharlau SU0153 reagent grade) was added to the pellet which was then vortexed until it had completely dissolved. The tubes were incubated for 1 hour at room temperature and spun by centrifuge at 4000 g for 1 minute (Jouan CR3i multifunction). The supernatant (formazan MTT) absorbance was read by a spectrometer using the Ocean Optics Inc. Ultra Fluorescence Kit (LS – 1 Tungsten Halogen Light source, Gated Fluorescence Spectrometer USB 4000, Spectrasuite software) at 505 nm against the DMSO blank for the first^t kill. In the second kill, the absorbance was read by a spectrophotometer (Floustar) at 495 nm against the DMSO blank.

3.2.7 Myofibrillar density assay

The myofibrillar density indicates the degree of protein denaturation; the higher the myofibrillar density value, the greater the amount of denaturation. The myofibrillar samples were prepared according to the MTT assay. 500 µL of MTT homogenate (2 g meat in 12 mL MTT homogenisation buffer, homogenised 20 s at 9000 rpm) was centrifuged at 4000 g for 2 minutes, after which, the supernatant was removed and the pellet suspended in 500 µL MTT homogenisation buffer. The density marker beads were hydrated overnight in 1 ml MQ water. DMB-1 (blue) and DMB-3 (green) vials were used. The density was recorded, as this varies with each batch. 15 µL myofibrillar sample and 5 µL each of blue and green density marker beads were added into the tube and were mixed by inversion. Samples were centrifuged at 18 000 g for 30 minutes at room temperature. Samples were removed from the centrifuge, and the distance from the bottom of the meniscus to each marker bead and myofibril layer was measured (Dobbie *et al.*, 2004).

The density was calculated using the following equations:

$$\% \text{ Travel (position of myofibrils as a \% of the distance between blue and green)} = (\text{myofibril line} - \text{blue bead line}) / (\text{green bead line} - \text{blue bead line})$$

$$\text{Density of myofibrils} = \text{stated density of green} - \text{stated density of blue} \times \% \text{ travel}$$

3.2.8 Statistical analysis

A general linear model analysis of variance (ANOVA) was conducted using MINITAB[®] Release 14 statistical software. Shear force measurements, drip losses, cooking loss, water binding capacity, oxygen consumption rate (MTT assay) and myofibrillar density were analysed, using a mixed model with main effects being kill

(day 1 and day 2), stimulation treatment (HFI HVS, HFI MVS, LFI HVS and LFI MVS), time point (1,5 and 9 days post-mortem) and muscle (LD and SM). Colour and colour stability were analysed using a mixed model with main effects for kill (1 and 2), stimulation treatment (HFI HVS, HFI MVS, LFI HVS and LFI MVS), day of retail display (non-bloomed to day 10) for each time point (1, 5 and 9 days post-mortem) and muscle (LD and SM). Differences between main effects were determined with Tukey's 95% test for difference.

3.3 Results

3.3.1 pH decline

The amount of variation (coefficient of determination; R^2) explained by the model in the ANOVA for the pH decline was 87.37%. The day of kill had an effect ($p = 0.040$) on the pH decline of the LD. The data was therefore split, and each day of kill was analysed separately. The amount of variation (coefficient of determination; R^2) explained by the model for kill 1 was 91.24%. The mean values for pH decline during kill 1 are shown in Table 1. There was an interaction ($p = 0.0001$) between treatment and time of pH measurement on the pH values for kill 1. After 0.5 hours post-mortem, treatments with LFI combined with either HVS or MVS had lower ($p < 0.05$) pH values compared to HFI HVS and HFI MVS. LFI HVS was also lower ($p < 0.05$) than LFI MVS. After 1.5 hours post-mortem, differences continued, with LFI HVS still having the lowest pH, which differed ($p < 0.05$) from HFI MVS and LFI MVS. LFI HVS had the most rapid pH decline, but when ultimate pH was measured at 24 hours post-mortem, there were no differences ($p > 0.05$) between treatments.

The amount of variation (coefficient of determination; R^2) explained by the model for kill 2 was 87.75%. The mean values for pH decline during kill 2 are shown in Table 1. There was an interaction ($p = 0.0001$) between treatment and time of pH measurement on the pH value for kill 2. After 0.5 hours post-mortem, treatments with LFI combined with either HVS or MVS had lower ($p < 0.05$) pH values compared to HFI HVS and MVS. After 1.5 hours post-mortem, differences continued with LFI HVS having the lowest pH, which differed ($p < 0.05$) from HFI HVS and HFI MVS. LFI HVS had the most rapid pH decline, but when ultimate pH was measured at 24 hours post-mortem, there were no differences ($p > 0.05$) between treatments.

Table 1 Mean pH values of beef *Longissimus dorsi* at various stages post-mortem for kill 1 and 2 after electrical stimulation.

pH	Kill	Treatment				SEM
		HFI HVS	HFI MVS	LFI HVS	LFI MVS	
pH _{0.5}	1	6.43 ^a	6.48 ^a	5.95 ^c	6.06 ^b	0.041
	2	6.40 ^a	6.36 ^a	6.06 ^b	5.98 ^b	0.035
pH _{1.5}	1	5.78 ^{bc}	5.90 ^a	5.71 ^c	5.88 ^{ab}	0.019
	2	6.05 ^a	6.02 ^{ac}	5.90 ^b	5.93 ^{bc}	0.017
pH _{2.5}	1	5.62 ^{ab}	5.70 ^a	5.59 ^b	5.71 ^a	0.016
	2	5.76 ^a	5.77 ^a	5.69 ^a	5.75 ^a	0.021
pH _{3.5}	1	5.52 ^a	5.60 ^{ab}	5.55 ^{ab}	5.62 ^b	0.013
pH ₂₄	1	5.46 ^a	5.45 ^a	5.47 ^a	5.45 ^a	0.014
	2	5.62 ^a	5.54 ^a	5.54 ^a	5.59 ^a	0.014

^{a,b} Values with a different subscript within a row differ significantly ($p < 0.05$)

3.3.2 Temperature

The temperature declines in the *Longissimus dorsi* and *Semimembranosus* for kill 1 and 2 are depicted in Figure 1. The *Longissimus dorsi* cooled down more rapidly than the *Semimembranosus*.

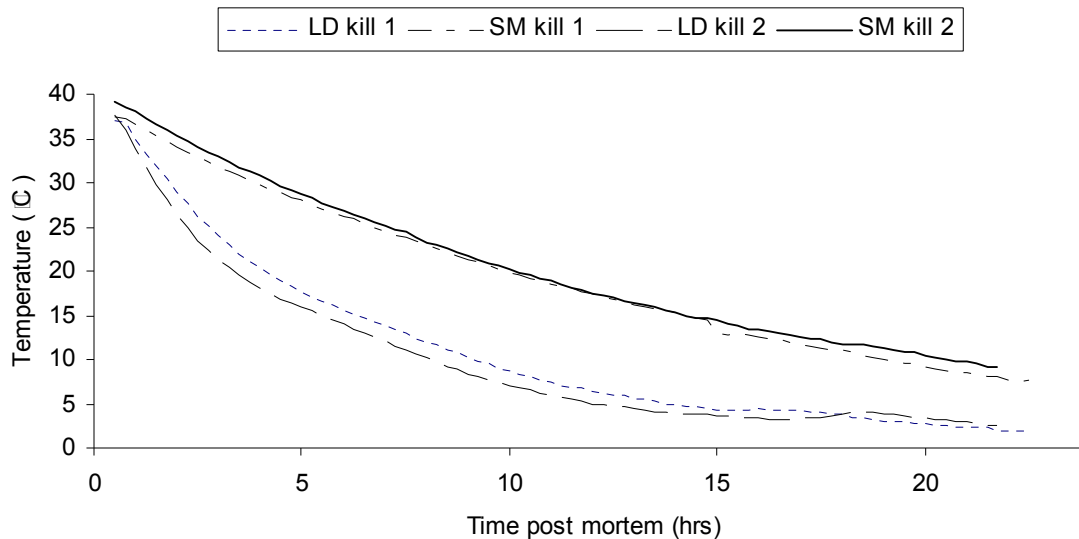


Figure 10 Temperature decline in the *Longissimus dorsi* and *Semimembranosus* of beef for kill 1 and 2.

3.3.3 Shear force

The amount of variation (coefficient of determination; R^2) explained by the model in the ANOVA was 47.45%. The day of kill had no effect ($p = 0.268$) on shear force values, and the data was therefore pooled for further statistical analyses. There were also no interactions between muscle \times time point \times treatment ($p = 0.147$), treatment \times time point ($p = 0.179$), but there were interactions between muscle \times treatment ($p = 0.028$) and

muscle × time point ($p = 0.0001$) on the shear force values. Mean shear force values are and depicted in Figure 2 for the interaction of muscle and time point. From the data, it is evident that the LD had higher ($p < 0.05$) shear force than the SM for day 1 of assessment, but at days 5 and 9, the LD had lower ($p < 0.05$) shear force values than the SM. The shear force values decreased ($p < 0.05$) in the LD as the days of assessment increased (Day 1 > Day 5 > Day 9), however, in the SM the shear force values did not decrease ($p > 0.05$) with time post-mortem.

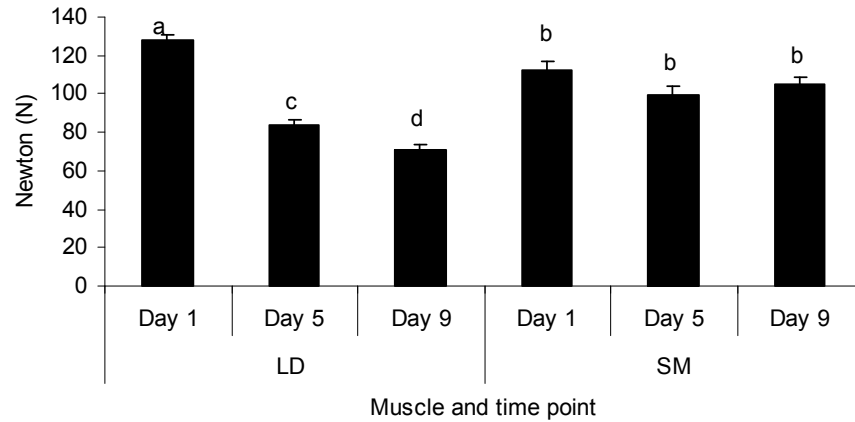


Figure 11 Mean shear force values for the interaction between beef muscles (*Longissimus dorsus* and *Semimembranosus*) and time point (days 1, 5 and 9)

The mean values for the interaction between muscle and stimulation treatments are depicted in Figure 3. In the LD, stimulation with LFI HVS had higher ($p < 0.05$) shear force values than HFI HVS; however, in the SM there were no difference ($p > 0.05$) between stimulation treatments.

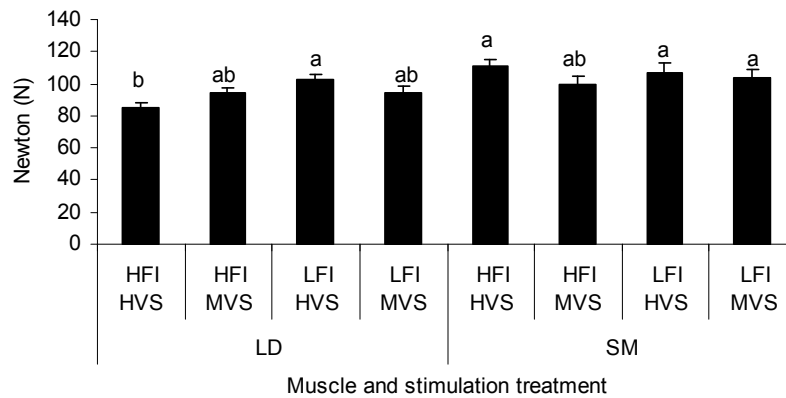


Figure 12 Mean shear force values in Newton for the interaction between beef muscle (*Longissimus dorsus* and *Semimembranosus*) and stimulation treatment (HFI HVS, HFI MVS, LFI HVS and LFI MVS)

3.3.4 Cooking loss

The day of kill had an effect ($p = 0.0001$) on the cooking loss. The data of the different days of kill were therefore separated, and a GLM ANOVA was performed on the data, with the model explaining 59.89% of the variation for kill 1. There were no interactions for muscle \times time point \times treatment ($p = 0.230$), treatment \times time point ($p = 0.419$) and muscle \times treatment ($p = 0.110$). However, muscle \times time point ($p = 0.011$) had an interaction on the cooking loss percentage for kill 1 (see Figure 4). The cooking loss percentage was higher ($p > 0.05$) for time points day 1 and 5 compared to day 9 of the LD, while in the SM there were no difference ($p > 0.05$) between time points. The SM had higher ($p < 0.05$) cooking losses for all the time points when compared to the time points in the LD. Stimulation treatment influenced ($p = 0.0001$) the cooking loss percentage, with HFI HVS (32.32 ± 0.404) and HFI MVS (33.00 ± 0.400) having a higher cooking loss than LFI HVS (30.66 ± 0.463) and LFI MVS (29.93 ± 0.411).

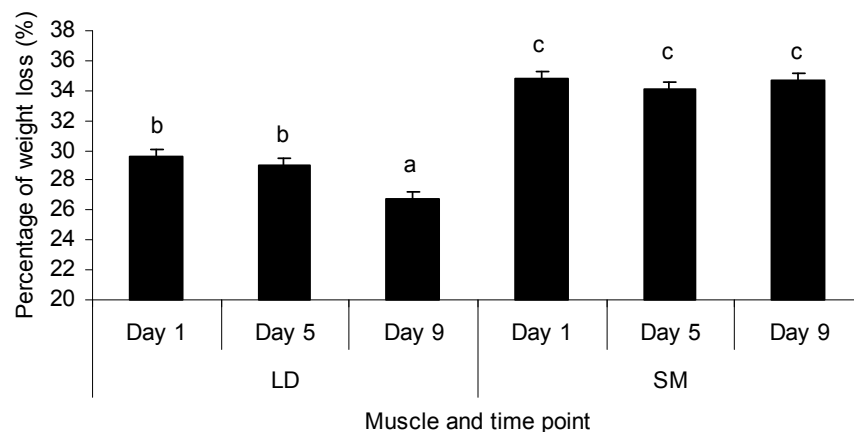


Figure 13 Mean cooking loss % for beef for the interaction between muscle (*Longissimus dorsis* and *Semimembranosus*) and time point (days 1, 5 and 9) for kill 1

The data of the second day of kill was analysed by means GLM ANOVA, with the model explaining only 15.26% of the variation for cooking loss percentage in the LD. The main effects were the time point and stimulation treatment. There was no interaction for time point \times treatment ($p = 0.980$) on the cooking loss percentage. Stimulation treatment influenced ($p = 0.001$) the cooking loss percentage, with HFI HVS and LFI HVS having a higher ($p < 0.05$) cooking loss than LFI MVS (Figure 5). Time point ($p = 0.728$) had no effect on the cooking loss percentage.

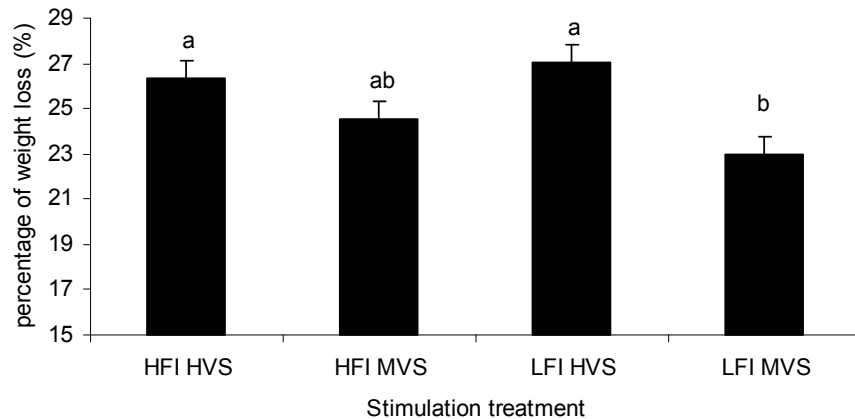


Figure 14 Mean cooking loss % for the different stimulation treatments (HFI HVS, HFI MVS, LFI HVS and LFI MVS) for the *Longissimus dorsi* of kill 2

3.3.5 Drip loss

As day of kill ($p = 0.526$) had no effect on the drip loss percentage during retail display, the data was pooled and analysed, with the amount of variation (coefficient of determination; R^2) explained by the ANOVA being 58.33%. There were no interactions between muscle \times time point \times treatment ($p = 0.281$), treatment \times time point ($p = 0.234$) or muscle \times treatment ($p = 0.157$), but there was an interaction between muscle \times time point ($p = 0.0001$) on the drip loss percentage during retail display. Stimulation treatment had no effect on drip loss percentage during retail display. Day 1 had higher ($p < 0.05$) drip loss than day 5 and 9 for both muscles. The SM had higher ($p < 0.05$) drip losses than the LD for the same time points (Table 2).

As the day of kill ($p = 0.090$) had no effect on the drip loss percentage during cold storage, the data was pooled and analysed with the amount of variation (coefficient of determination; R^2) explained by the ANOVA being 58.49%. There were no interactions between muscle \times time point \times treatment ($p = 0.171$) or treatment \times time point ($p = 0.582$), but there were interactions between muscle \times time point ($p = 0.014$) and muscle \times treatment ($p = 0.0001$) on the drip loss percentage during cold storage. The SM had higher ($p < 0.05$) drip losses during cold storage than the LD for both ageing periods. The amount of drip loss did not differ ($p > 0.05$) between day 5 and 9 for the LD, but there was a difference ($p < 0.05$) in the SM (Table 2). There were no difference ($p > 0.05$) between stimulation treatments in the LD, while in the SM LFI HVS and LFI MVS had a higher ($p < 0.05$) drip loss than HFI HVS and HFI MVS (Table 3). There were no differences ($p > 0.05$) between the LD and SM for stimulation with HFI HVS and HFI MVS, but LFI HVS and LFI MVS had higher ($p < 0.05$) drip loss in the SM than in the LD.

The collective drip-loss percentage was analysed, and the amount of variation (coefficient of determination; R^2) explained by the ANOVA was 62.17%. The collective drip-loss percentage was analysed, and the day of kill ($p = 0.077$) had no effect on drip loss. There were no interactions between muscle \times time point \times treatment ($p = 0.057$), but there were interactions between treatment \times time point ($p = 0.008$), muscle \times time point ($p = 0.001$) and muscle \times treatment ($p = 0.0001$) on drip loss. The collective drip loss was higher ($p < 0.05$) at day 5 and 9 than day 1 for both muscles, the only difference being that in the SM day 9 had a higher ($p < 0.05$) drip loss than day 5. The SM had higher ($p < 0.05$) drip loss than the LD at the same time

points (Table 3). LFI HVS had a higher ($p < 0.05$) drip loss than HFI HVS in the LD, while in the SM LFI HVS and LFI MVS had higher ($p < 0.05$) drip loss than HFI HVS and HFI MVS. HFI HVS had a higher ($p < 0.05$) drip loss than HFI HVS in the LD. The SM had higher ($p < 0.05$) drip loss than the LD for all the stimulation treatments.

Table 2 Retail display, cold storage and collective drip loss percentage values (mean \pm se) for the interaction between time point (days 1, 5 and 9) and muscle (*Longissimus dorsi* and *Semimembranosus*) of beef.

Time point (d)	Retail display drip loss (%)	Drip loss during cold storage (%)	#Collective drip loss (%)
LD			
1	1.32 \pm 0.079 ^b	n/a	1.32 \pm 0.079 ^a
5	0.89 \pm 0.049 ^a	2.77 \pm 0.157 ^a	3.67 \pm 0.180 ^b
9	0.78 \pm 0.045 ^a	3.03 \pm 0.176 ^a	3.81 \pm 0.206 ^b
SM			
1	3.1 \pm 0.169 ^d	n/a	3.1 \pm 0.169 ^b
5	1.49 \pm 0.100 ^{bc}	3.63 \pm 0.245 ^b	5.12 \pm 0.237 ^c
9	1.78 \pm 0.127 ^c	4.87 \pm 0.393 ^c	6.65 \pm 0.389 ^d

^{a,b} Values with a different subscript within a column differ significantly ($p < 0.05$)

#Note that retail and cold storage drip loss percentages were added. Different volume to surface cut areas will affect the collective drip loss

Table 3 Retail display, cold storage and collective drip loss percentage values (mean \pm se) for the interaction between stimulation treatments (HFI HVS, HFI MVS, LFI HVS and LFI MVS) and muscle (*Longissimus dorsi* and *Semimembranosus*) of beef.

Stimulation treatment	Retail display drip loss (%)	Drip loss during cold storage (%)	Collective drip loss (%)
LD			
HFI HVS	0.89 \pm 0.060 ^a	1.63 \pm 0.229 ^a	2.55 \pm 0.251 ^a
HFI MVS	0.92 \pm 0.057 ^a	1.74 \pm 0.199 ^{ab}	2.66 \pm 0.200 ^{ab}
LFI HVS	1.13 \pm 0.088 ^a	2.20 \pm 0.251 ^{ab}	3.37 \pm 0.240 ^{bc}
LFI MVS	1.04 \pm 0.084 ^a	2.18 \pm 0.260 ^{ab}	3.22 \pm 0.258 ^{abc}
SM			
HFI HVS	1.96 \pm 0.164 ^b	1.66 \pm 0.233 ^{ab}	3.63 \pm 0.233 ^c
HFI MVS	2.32 \pm 0.232 ^b	2.47 \pm 0.376 ^b	4.79 \pm 0.279 ^d
LFI HVS	2.16 \pm 0.239 ^b	3.86 \pm 0.720 ^c	6.02 \pm 0.567 ^e

HVS

LFI

MVS 2.05±0.192^b 3.87±0.365^c 5.91±0.522^e

^{a,b} Values with a different subscript within a column differ significantly ($p < 0.05$)

#Note that retail and cold storage drip loss percentages were added. Different volume to surface cut areas will affect the collective drip loss

3.3.6 Water-binding capacity

The amount of variation (coefficient of determination; R^2) explained by the ANOVA was 65.74%. The day of kill ($p = 0.110$) had no effect on the WBC. There were no interactions for muscle \times time point \times treatment ($p = 0.213$), muscle \times treatment ($p = 0.984$). However, muscle \times time point ($p = 0.0001$) and treatment \times time point ($p = 0.030$) had an interaction on the amount of expressible water, or inversely, water binding capacity. As shown in Figure 6 the SM had a higher ($p < 0.05$) percentage expressible water, or lower WBC, than the LD for all the time points of assessment. There was an increase ($p < 0.05$) in the amount of expressible water from day 1 to days 5 and 9 of assessment in the LD; however, in the SM, day 1 had a higher ($p < 0.05$) percentage expressible water than day 9.

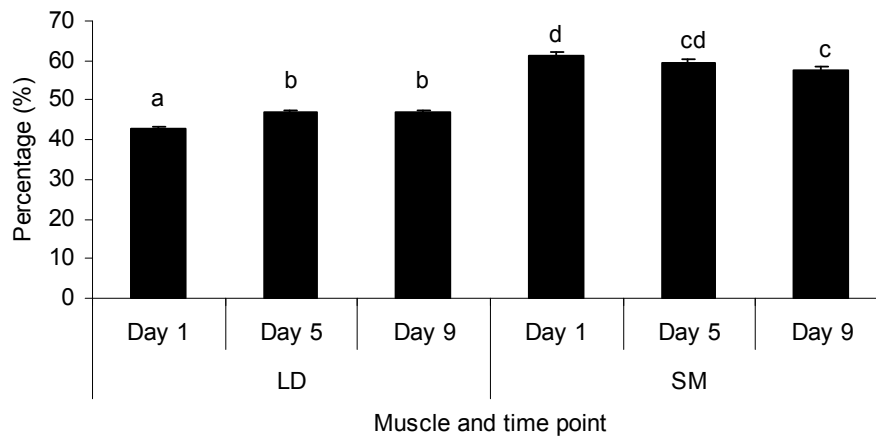


Figure 15 Mean percentage expressible water for the interaction between beef muscle (*Longissimus dorsi* and *Semimembranosus*) and time point (days 5 and 9)

The data for the interaction between muscle and stimulation treatments are shown in Figure 7. LFI MVS for day 9 had the lowest percentage expressible water, which differed ($p < 0.05$) from the other treatments at day 9.

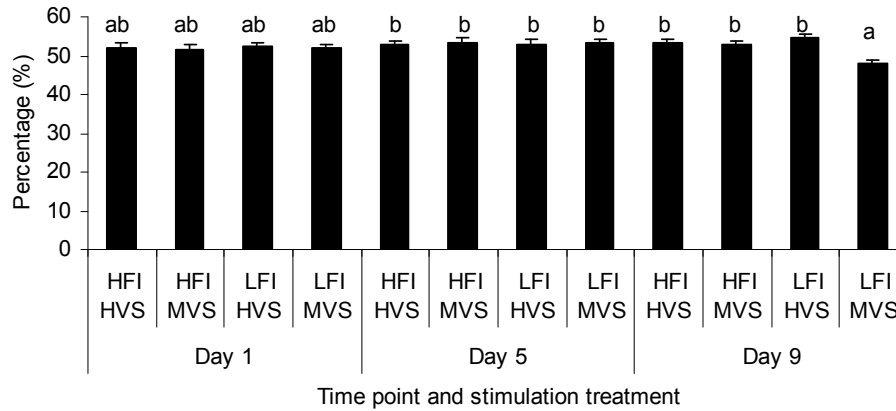


Figure 16 Mean percentage expressible water for the interaction between time points (days 1, 5 and 9) and stimulation treatments (HFI HVS, HFI MVS, LFI HVS and LFI MVS) of beef

3.3.7 Colour

The mean values for L^* of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the LD are shown in Figure 8. There were no difference between days of kill ($p = 0.120$; $p = 0.605$; $p = 0.146$) for the respective time points. At day 1 ($p = 0.965$; time point 1), day 5 ($p = 0.605$; time point 2) and day 9 ($p = 0.146$; time point 3) of assessment, there was no interaction between retail day and treatment; however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) had effects on the L^* values during retail display of the LD. At day 1, LFI HVS (40.50 ± 0.089) and LFI MVS (40.44 ± 0.089) had higher ($p < 0.05$) mean chroma values compared to HFI MVS (40.00 ± 0.089). HFI HVS (40.16 ± 0.089) did not differ ($p > 0.05$) from any of the other treatments. At day 5, LFI HVS (40.92 ± 1.05) had a higher ($p < 0.05$) mean L^* value compared to the other treatments. LFI MVS (40.42 ± 1.05) had a higher ($p < 0.05$) mean L^* value compared to HFI MVS (39.87 ± 1.05). HFI HVS (40.06 ± 1.05) did not differ ($p > 0.05$) from HFI MVS or LFI MVS. At day 9, LFI HVS (41.83 ± 1.11) had a higher ($p < 0.05$) mean L^* value compared to the other treatments. LFI MVS (41.41 ± 1.11) had a higher ($p < 0.05$) mean L^* value compared to HFI MVS (40.91 ± 1.11) and HFI HVS (40.80 ± 1.11).

The L^* values at days 1, 5 and 9 of assessment increased ($p < 0.05$) from the non-bloomed state to a bloomed state. There was an increase in L^* values for all stimulation treatments as the days of retail display progressed for time point 1 of assessment, while at the subsequent time points (day 5 and 9), the values stayed constant.

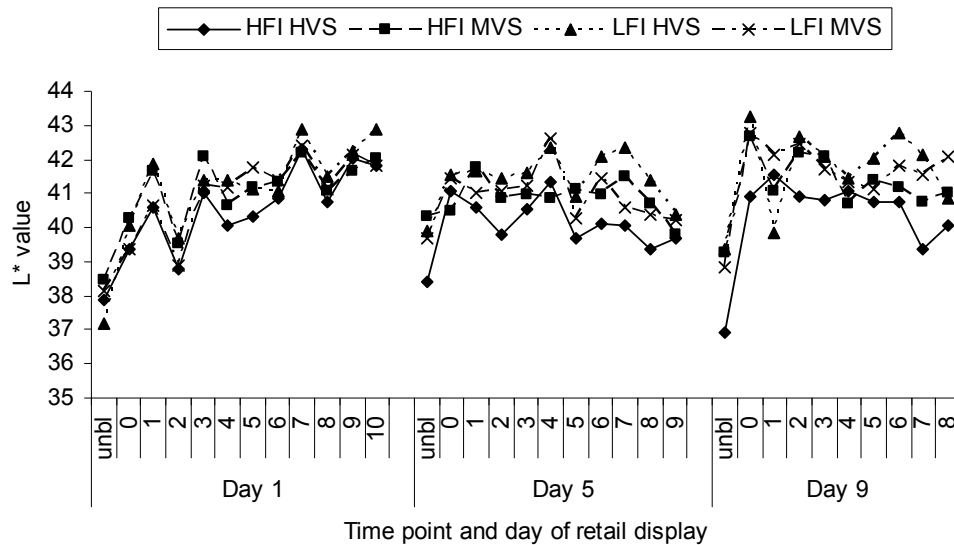


Figure 17 Change in L* value of beef *Longissimus dorsi* during retail display for the time points days 1, 5 and 9

The mean values for L* of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the SM are shown in Figure 9. At day 1 ($p = 0.567$; time point 1) and day 5 ($p = 0.586$; time point 2) of colour assessment, there was no interaction between retail day and treatment; however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) had effects on the L* values during retail display of the SM. At day 1, LFI HVS (43.72 ± 0.183) had the highest mean L* values during retail display, which differed ($p < 0.05$) from the other stimulation treatments. HFI MVS (43.12 ± 0.142) had a higher ($p < 0.05$) mean L* value than HFI HVS (41.91 ± 0.142) and LFI MVS (41.60 ± 0.142). There was no difference ($p > 0.05$) between HFI HVS and LFI MVS. At day 5, LFI HVS (44.14 ± 0.179) and HFI MVS (44.15 ± 0.139) had higher ($p < 0.05$) mean L* values during retail display compared to HFI HVS (42.96 ± 0.139) and LFI MVS ($42.79 \pm 0.0.139$). There were no difference ($p > 0.05$) between HFI HVS and LFI MVS.

At day 9 (time point 3) of colour assessment there was an interaction ($p = 0.0001$) between retail day and stimulation treatment on the L* values during retail display of the SM. There were no differences ($p > 0.05$) between treatments at days of retail display from non-bloomed to day 7. At day 8 of retail display, HFI HVS and LFI HVS had higher ($p < 0.05$) L* values compared to LFI MVS.

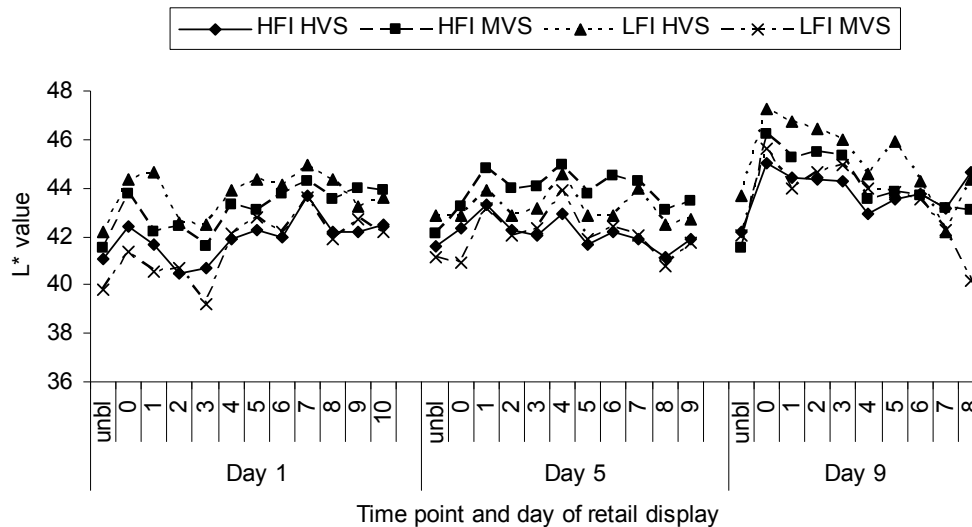


Figure 18 Change in L* value of beef *Semimembranosus* during retail display for the time points days 1, 5 and 9

The mean values for a* of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the LD are shown in Figure 10. There were no difference between the days of kill ($p = 0.997$) on the a* values during retail display.

At day 1 (time point 1) of assessment, there was an interaction ($p=0.0001$) between retail day and stimulation treatment on the a* values during retail display of the LD. There was a difference between treatments for non-bloomed colour, where LFI HVS had a higher ($p < 0.05$) a* value than HFI HVS. At day 0 (bloomed colour) of retail display, LFI HVS was higher ($p < 0.05$) than HFI HVS and HFI MVS. At day 8 of retail display, LFI HVS and LFI MVS had higher ($p < 0.05$) a* values than HFI HVS.

At days 5 ($p = 0.135$; time point 2) and 9 ($p = 0.237$; time point 3) of assessment, there was no interaction between retail day and treatment; however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) had effects on the a* values of the LD during retail display. LFI HVS (18.74 ± 0.120) had a higher ($p < 0.05$) mean a* value than the other treatments. At day 5, HFI MVS (17.88 ± 0.120) and LFI MVS (17.79 ± 0.120) had higher ($p < 0.05$) a* values than HFI HVS (17.31 ± 0.120). At day 9, LFI HVS (19.37 ± 0.115) and LFI MVS (19.22 ± 0.115) had higher ($p < 0.05$) mean a* values than the other treatments. HFI MVS (18.81 ± 0.115) had a higher ($p < 0.05$) a* value compared to HFI HVS (17.70 ± 0.115). The a* values from the non-bloomed state to the bloomed state had increased ($p < 0.05$) for days 1, 5 and 9. There was a gradual decrease in a* values for all stimulation treatments as the days of retail progressed.

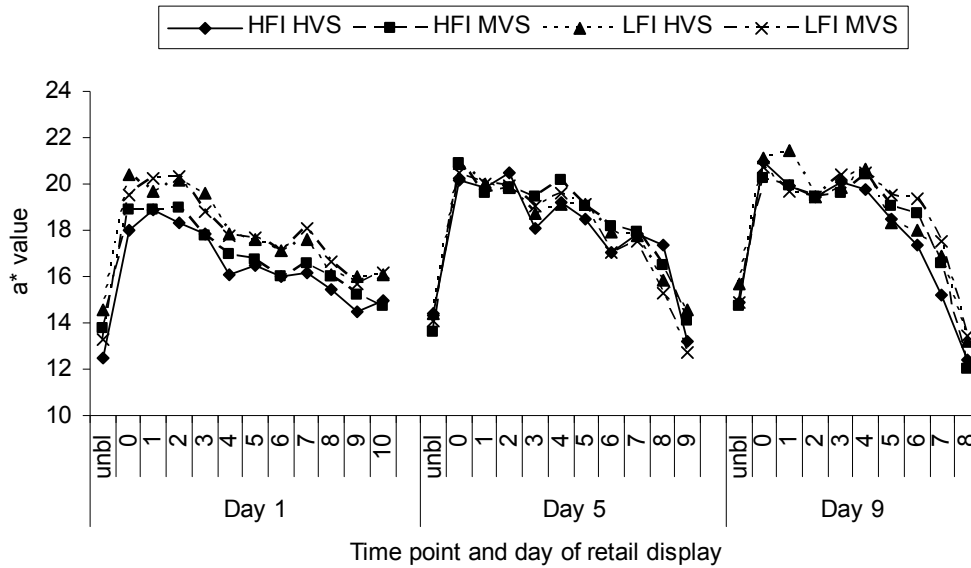


Figure 19 Change in a* value of beef *Longissimus dorsi* during retail display for time points days 1, 5 and 9.

The mean values for a* of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the SM are shown in Figure 11. At day 1 (time point 1) of colour assessment, there was an interaction ($p=0.004$) between retail day and stimulation treatment on the a* values during retail display of the SM. There were no differences ($p > 0.05$) between treatments at days of retail display except for day 1 of display where LFI HVS had a higher ($p < 0.05$) a* value than HFI HVS.

At day 5 ($p=0.134$; time point 2) of colour assessment, there was no interaction between retail day and treatment; however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) had effects on the a* values during retail display of the SM. LFI MVS (18.58 ± 0.101) had a higher ($p < 0.05$) mean a* value than HFI HVS (18.48 ± 0.101) and HFI MVS (18.42 ± 0.101). LFI HVS (18.86 ± 0.131) did not differ ($p < 0.05$) from any of the other treatments. At day 9 (time point 3) of colour assessment, there was an interaction ($p = 0.0001$) between retail day and stimulation treatment on the a* values during retail display of the SM. There were no differences ($p > 0.05$) between treatments at days of retail display except for day 8 of display where HFI HVS had lower ($p < 0.05$) a* values compared to the other stimulation treatments. The a* values increased ($p < 0.05$) from the non-bloomed to bloomed state at days 1, 5 and 9, which was followed by a gradual decline for all stimulation treatments as the days of retail progressed.

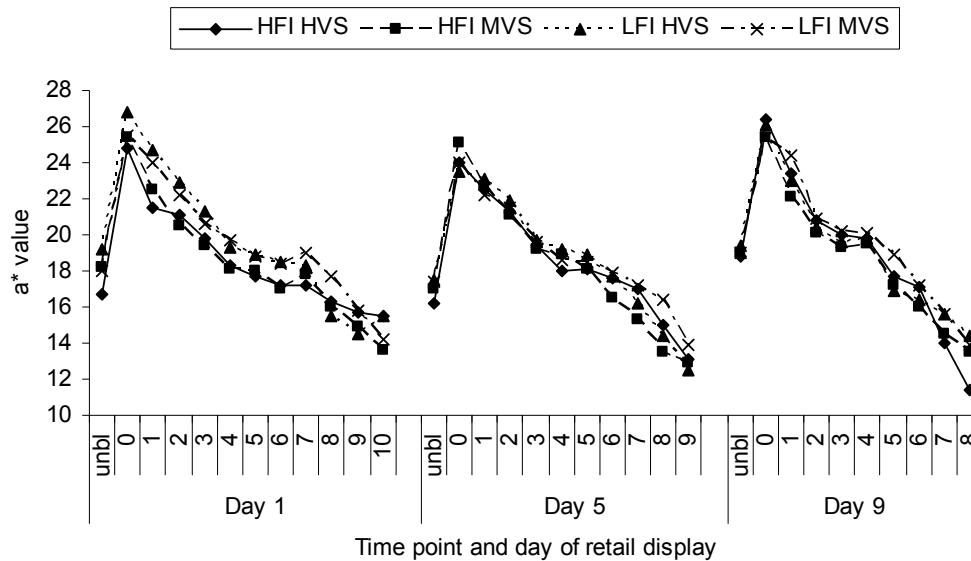


Figure 20 Change in a* value of beef *Semimembranosus* during retail display for time points days 1, 5 and 9

The mean values for b* of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the LD is shown in Figure 13. At day 1 (time point 1) of assessment there was an interaction ($p=0.0001$) between retail day and stimulation treatment on the b* values during retail display of the LD. There was no difference ($p > 0.05$) between treatments for non-bloomed colour. At day 0 (bloomed colour) of retail display LFI HVS was higher ($p < 0.05$) than HFI HVS and HFI MVS, while LFI MVS was only higher ($p < 0.05$) than HFI HVS. At day 8 of retail display LFI HVS and LFI MVS had higher ($p < 0.05$) b* values than HFI HVS. At day 5 ($p = 0.067$; time point 2) and 9 ($p = 0.660$; time point 3) of assessment there was no interaction between retail day and treatment, however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) on the b* values during retail display of the LD. At day 5, LFI HVS (8.52 ± 0.067) had a higher ($p < 0.05$) mean b* value compared to the other treatments. HFI HVS (7.77 ± 0.068), HFI MVS (7.97 ± 0.067) and LFI MVS (7.95 ± 0.067) did not differ ($p < 0.05$). At day 9, LFI HVS (8.80 ± 0.065) and LFI MVS (8.70 ± 0.065) had higher ($p < 0.05$) mean b* values compared to HFI HVS (7.85 ± 0.065) and HFI MVS (8.27 ± 0.065), while HFI MVS was higher ($p < 0.05$) than HFI HVS.

The b* values from the non-bloomed to a bloom state increased ($p < 0.05$) for all of the time points (days 1, 5 and 9). There was a gradual decrease in b* values for all stimulation treatments as the days of retail progressed.

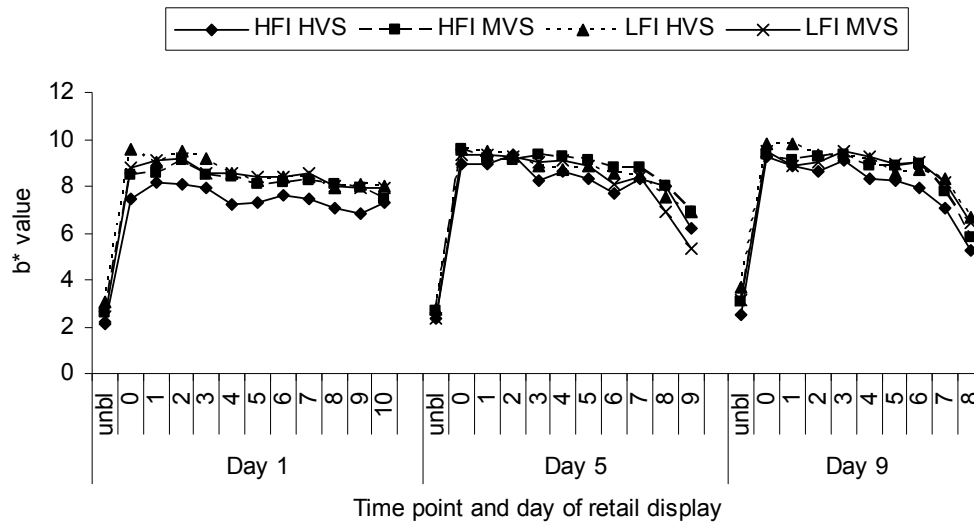


Figure 21 Change in b^* value of beef *Longissimus dorsi* during retail display for time points days 1, 5 and 9

The mean values for b^* values of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the SM are shown in Figure 13. There was no difference between the days of kill ($p = 0.139$).

At days 1 ($p = 0.145$; time point 1), 5 ($p = 0.944$; time point 2) and 9 ($p = 0.716$; time point 3) of colour assessment, there was no interaction between retail day and treatment; however, the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$; only day 1 and 5) both had an effect on the b^* values during retail display of the SM. At day 9, stimulation treatment had no effect ($p = 0.087$) on the b^* value. At day 1, LFI HVS (10.77 ± 0.113) had a higher ($p < 0.05$) mean b^* value when compared to the other treatments. HFI MVS (10.30 ± 0.087) and LFI MVS (10.08 ± 0.092) had lower ($p < 0.05$) b^* values than LFI HVS and higher ($p < 0.05$) values than HFI HVS (9.46 ± 0.087). At day 5, HFI HVS (9.68 ± 0.074) had a lower ($p < 0.05$) mean b^* value compared to the other treatments. HFI MVS (10.09 ± 0.074), LFI HVS (10.20 ± 0.096) and LFI MVS (10.09 ± 0.078) did not differ ($p > 0.05$) from each other. The b^* values from the non-bloomed to a bloomed state increased ($p < 0.05$). There was a gradual decrease in b^* values for all stimulation treatments as the days of retail progressed.

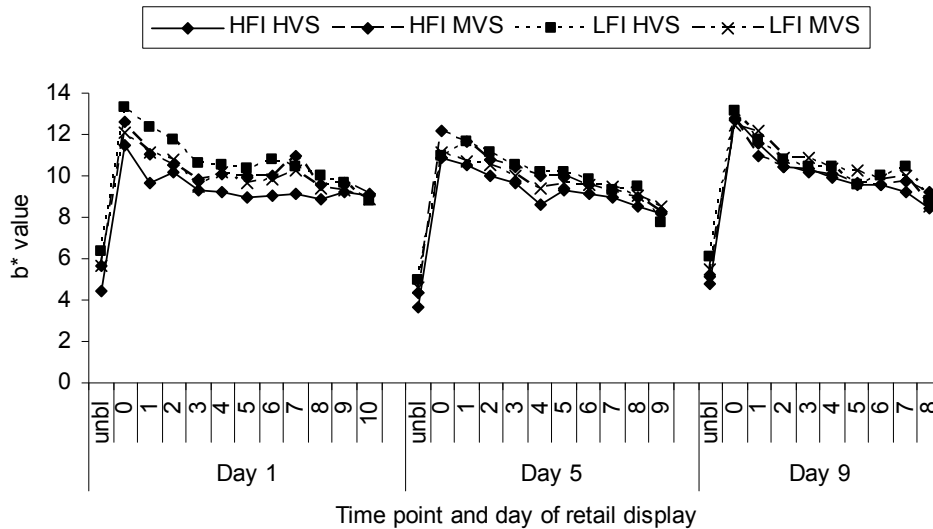


Figure 22 Change in b^* value of beef *Semimembranosus* during retail display for time points days 1, 5 and 9

The mean values for hue angle of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the LD are shown in Figure 14. There were no difference between the different kills ($p = 0.182$) on the hue angle of the LD.

At day 1 (time point 1) of colour assessment, there was an interaction ($p = 0.002$) between retail day and stimulation treatment on the hue angle during retail display of the LD. LFI HVS had a higher ($p < 0.05$) hue angle than HFI HVS for the non-bloomed colour of the LD. There were no differences ($p > 0.05$) between treatments at any of the days of retail display except at day 0, 9 and 10 where LFI HVS had a higher ($p < 0.05$) hue angle than HFI HVS.

At day 5 (time point 2) of colour assessment, there was an interaction ($p = 0.0001$) between retail day and stimulation treatment on the hue angle during retail display of the LD. LFI HVS had a higher ($p < 0.05$) hue angle than HFI HVS for the non-bloomed colour of the LD. There were no differences ($p > 0.05$) between treatments at any of the days of retail display except at day 9 where HFI MVS and LFI HVS had a higher ($p < 0.05$) hue angle than LFI MVS.

At day 9 (time point 3) of colour assessment, there was an interaction ($p = 0.0001$) between retail day and stimulation treatment on the hue angle during retail display of the LD. LFI HVS and LFI MVS had a higher ($p < 0.05$) hue angle than HFI HVS and HFI MVS for the non-bloomed colour of the LD. There were no differences ($p > 0.05$) between treatments at any of the other days of retail display. The hue angle from the non-bloomed to bloomed colour for all treatments increased ($p < 0.05$), followed by a gradual increase from day 0 to 10 for all treatments.

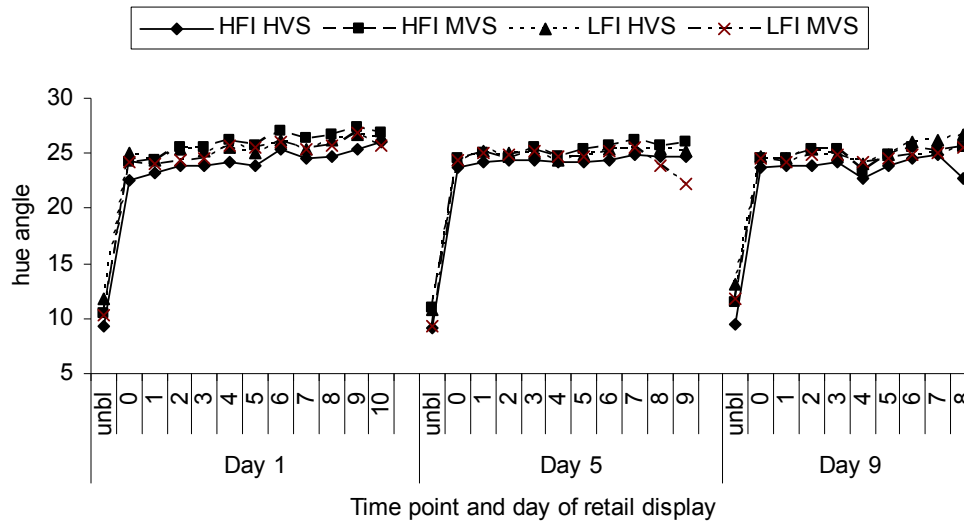


Figure 23 Change in hue angle of beef *Longissimus dorsi* during retail display for time points days 1, 5 and 9

The mean values for hue angle of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the SM are shown in Figure 15. At day 1 (time point 1) of colour assessment, there was an interaction ($p = 0.001$) between retail day and stimulation treatment on the hue angle during retail display of the SM. There were no differences ($p > 0.05$) between treatments at days of retail display from non-bloomed to day 6. On day 8 of retail display, LFI HVS had a higher ($p < 0.05$) hue angle than HFI HVS. At day 5 (time point 2) of colour assessment, there was no interaction ($p = 0.102$) between retail day and treatment; however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) had effects on the hue angle during the retail display of the SM. HFI MVS (29.39 ± 0.151) and LFI HVS (29.04 ± 0.194) had a higher ($p < 0.05$) mean hue angle than HFI HVS (28.12 ± 0.151) and LFI HVS (28.24 ± 0.159).

At day 9 (time point 3) of colour assessment, there was an interaction ($p = 0.0001$) between retail day and stimulation treatment on the hue angle during the retail display of the SM. There were no differences ($p > 0.05$) between treatments at days of retail display from non-bloomed to day 7. At day 8 of retail display, HFI HVS had a higher ($p < 0.05$) hue angle than LFI combined with either HVS or MVS. The hue angle from the non-bloomed to a bloomed colour increased ($p < 0.05$) for all stimulation treatments. There was a gradual increase in hue angle for all stimulation treatments as the days of retail progressed.

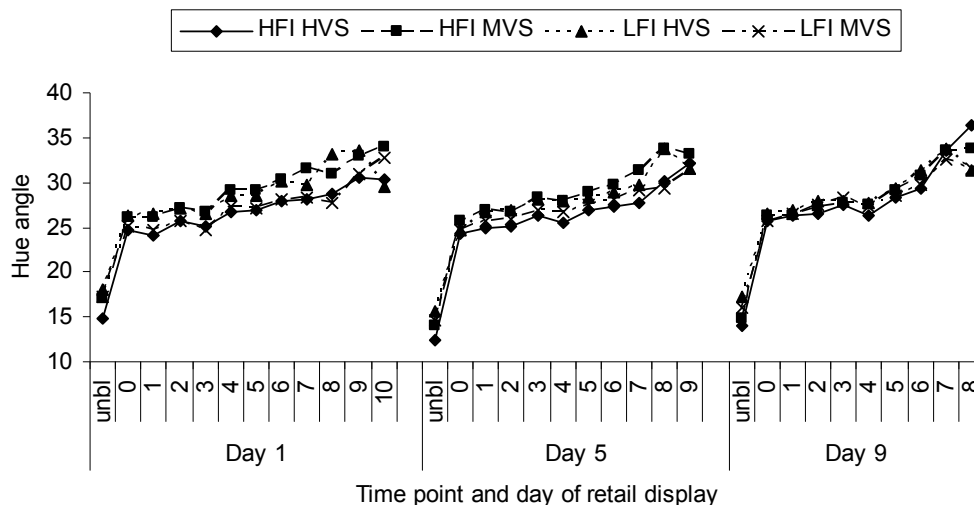


Figure 24 Change in hue angle of beef *Semimembranosus* during retail display for time points days 1, 5 and 9

The mean values for chroma of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the LD are shown in Figure 16. There were no differences between the different days of kill ($p = 0.449$).

At day 1 (time point 1) of assessment, there was an interaction ($p = 0.0001$) between retail day and stimulation treatment on the chroma values during retail display of the LD. There was a difference ($p < 0.05$) between treatments for non-bloomed colour, where LFI HVS had a higher ($p < 0.05$) chroma value than HFI HVS. At day 0 (bloomed colour) of retail display, LFI HVS was higher ($p < 0.05$) than HFI HVS and HFI MVS, while LFI MVS only had a higher ($p < 0.05$) chroma value in comparison with HFI HVS. At day 8 of retail display, LFI with either HVS or MVS had a higher ($p < 0.05$) chroma value than HFI HVS.

At days 5 ($p = 0.142$; time point 2) and 9 ($p = 0.319$; time point 3) of assessment, there was no interaction ($p = 0.142$) between retail day and treatment; however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) an effect on the chroma values during the retail display of the LD. At day 5, LFI HVS (20.64 ± 0.135) had a higher ($p < 0.05$) mean chroma value than the other treatments. LFI MVS (19.55 ± 0.135) and HFI MVS (19.64 ± 0.135) had higher ($p < 0.05$) mean chroma values than HFI HVS (19.04 ± 0.135). At day 9, LFI HVS (20.77 ± 0.185) and LFI MVS (20.88 ± 0.185) had higher ($p < 0.05$) mean chroma values compared to HFI HVS (19.91 ± 0.185) and HFI MVS (20.30 ± 0.185). HFI MVS had a higher ($p < 0.05$) mean chroma value compared to HFI HVS.

The chroma values from the non-bloomed to a bloomed colour increased ($p < 0.05$). There was a gradual decrease in chroma values for all stimulation treatments as the days of retail progressed.

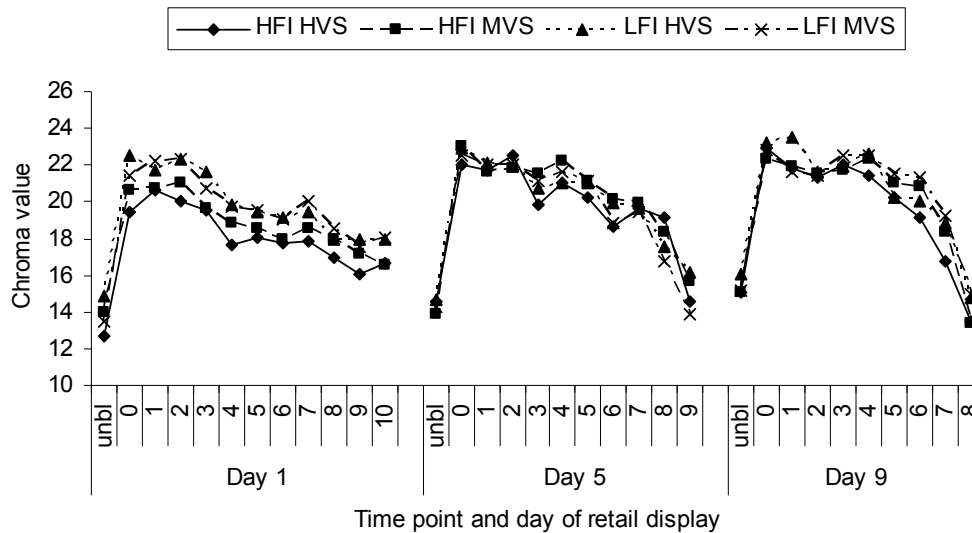


Figure 25 Change in chroma of beef *Longissimus dorsi* during retail display for time points days 1, 5 and 9.

The mean values for chroma of the different treatments for the days of retail display at days 1, 5 and 9 of assessment of the SM are shown in Figure 17. At day 1 (time point 1) of colour assessment, there was an interaction ($p = 0.018$) between retail day and stimulation treatment on the chroma values during the retail display of the SM. There were no differences ($p > 0.05$) between treatments at any of the days of retail display except for day 1 of retail display, where HFI HVS had a lower ($p < 0.05$) chroma value than LFI HVS.

At day 5 (time point 2) of colour assessment, there was no interaction ($p = 0.417$) between retail day and treatment; however, both the day of retail ($p = 0.0001$) and the stimulation treatments ($p = 0.0001$) had effects on the chroma values during the retail display of the SM. LFI MVS (21.56 ± 0.122) had higher ($p < 0.05$) mean chroma values compared to HFI HVS (20.90 ± 0.116) and HFI MVS (20.90 ± 0.116). LFI HVS (21.37 ± 0.149) did not differ ($p > 0.05$) from any of the other treatments. At day 9 (time point 3) of colour assessment, there was an interaction ($p = 0.013$) between retail day and stimulation treatment on the chroma values during the retail display of the SM. The chroma values from the non-bloomed to a bloomed colour increased ($p < 0.05$) for all stimulation treatments. There was a gradual decrease in chroma value for all stimulation treatments as the days of retail progressed.

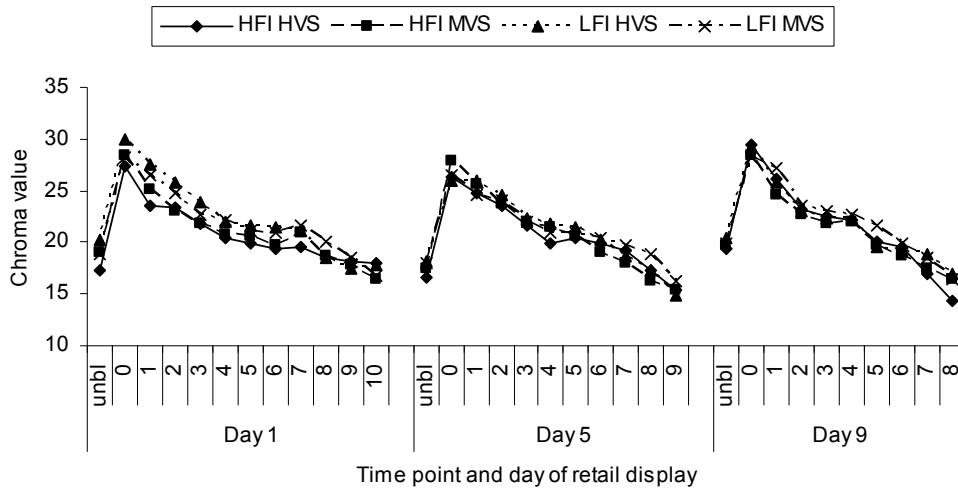


Figure 26 Change in chroma of beef *Semimembranosus* during retail display for time points days 1, 5 and 9

3.3.8 MTT assay

The MTT assay was only done on day 1 (time point 1), and the data is shown in Figure 18 for kill 1. The data for MTT activity for the different days of kill was analysed separately due to slightly different methodologies used in acquiring the data. LFI HVS had a higher ($p < 0.05$) MTT activity than the other stimulation treatments in the LD during kill 1. There were no differences ($p > 0.05$) between treatments in the SM. For all treatments, there was a higher ($p < 0.05$) MTT activity in the LD than in the SM. MTT activity values had a significant ($p = 0.0001$) inverse linear correlation (-0.613) with the a^* values measured from the steaks 24 hours post-mortem after blooming for 3 hours.

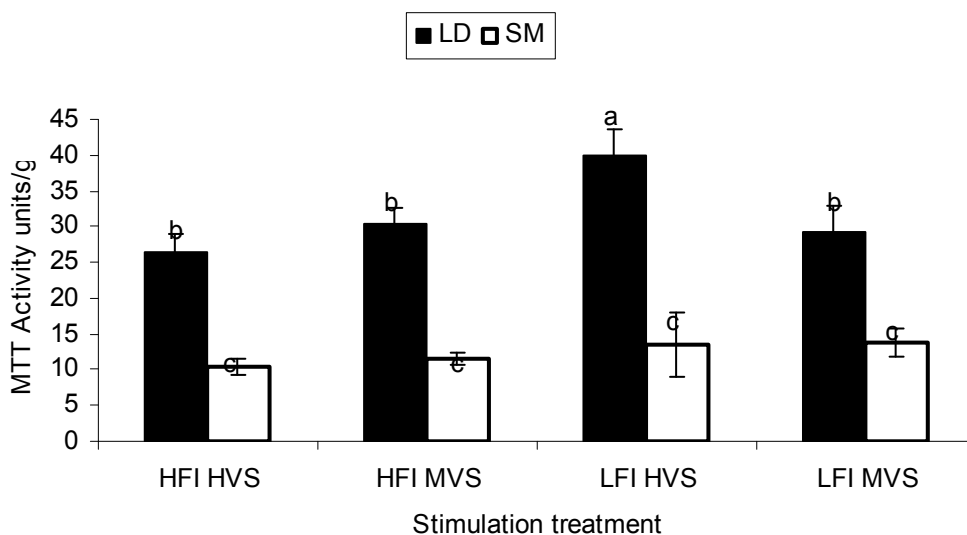


Figure 27 MTT activity (units/g) measuring the oxygen consumption rate of the *Longissimus dorsi* and *Semimembranosus* during kill 1

The MTT assay was done only at day 1 (time point 1) for kill 2. The mean MTT values for kill 2 are shown in Figure 19. HFI HVS and HFI MVS had a higher ($p < 0.05$) MTT activity compared to LFI HVS and LFI MVS in the LD. MTT activity values had a negative correlation (-0.730 ; $p = 0.0001$) with colour ordinate a^* measured from the steaks 24 hours post-mortem after 3 hours of exposure to air (bloom).

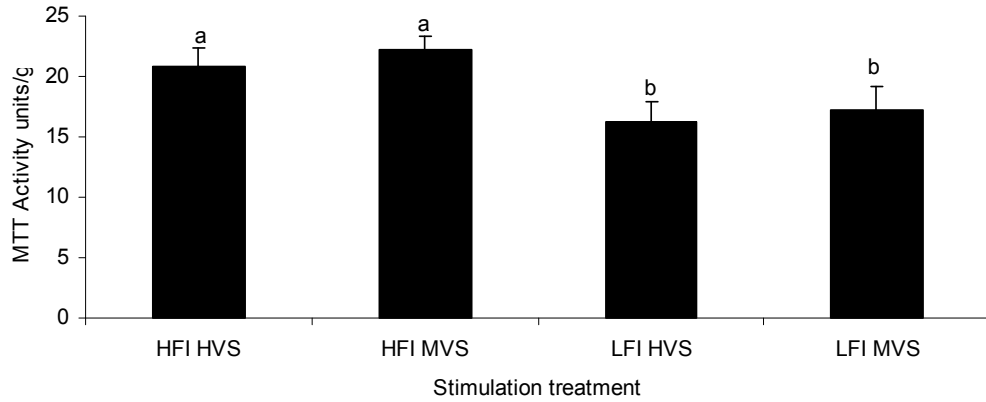


Figure 28 MTT activity (units/g) measuring the oxygen consumption rate of the *Longissimus dorsi* during kill 2

3.3.9 Myofibrillar density

The myofibrillar density was measured only at the day 1 time point of kill 2 as shown in Figure 20. There were no differences ($p > 0.05$) between any of the treatments.

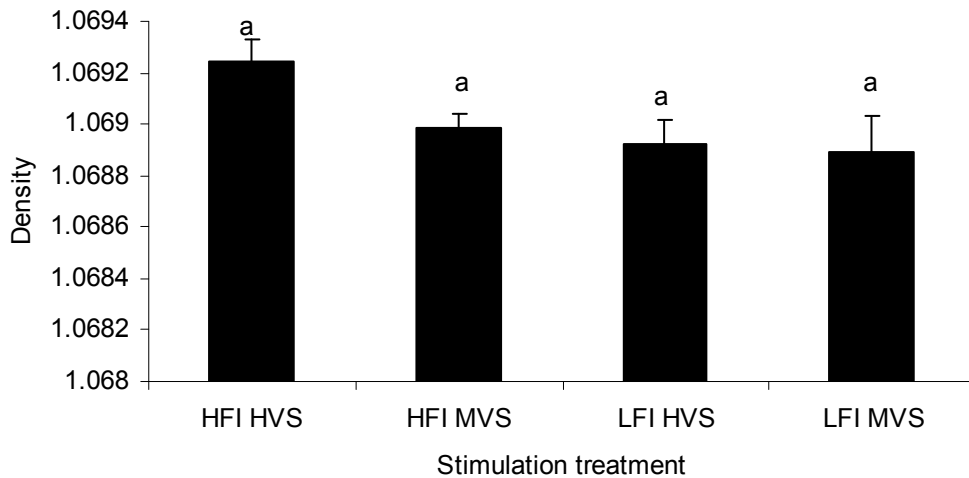


Figure 29 Myofibrillar density measuring denaturation of the *Longissimus dorsi* during kill 2

3.4 Discussion

3.4.1 pH

pH decline was the most rapid when a combination of LFI and HVS was used. The effects of HF and LF immobilisation are clearly evident. LFI was very effective in lowering the pH to such a stage that, after carcass dressing was finished, the pH was around 6. LFI (14.3 Hz, 90 V for 20 seconds) is based on standard low voltage electrical stimulation parameters and would thus be expected to induce a rapid pH decline. Combined with the high temperature of the carcass, the pH drop can be quite huge. However, HFI led to a minimal pH drop during dressing. This can be directly attributed to how the carcasses respond to the electrical parameters, as shown in a review article by Daly (2005). In brief, there are two considerations when assessing the responses of excitable tissues to an applied electrical waveform: pulse duration and pulse frequency. Reducing pulse duration increases the voltage threshold needed to trigger an action potential, but this threshold is also defined by the physical characteristics of the target cell – cells with low internal electrical resistance, primarily as a consequence of large cell size, are able to respond to shorter pulses than cells with high internal resistance. For this reason, peripheral motorneurons have the lowest threshold. In contrast, although muscle fibres can have large diameters, the dense packing of contractile proteins produce high internal electrical resistance, and these cells have, by comparison, a much higher threshold. The consequence of such response characteristics is that the appropriate choice of waveform frequencies can selectively stimulate specific responsive tissues, and the use of very short pulse durations will permit the stimulation of motorneurons without direct stimulation of skeletal- or cardiac muscle. The electrical parameters used to produce a maximal muscle response in whole carcasses will involve pulse durations of 2–10 ms and 500–1000 peak V, while the stimulation of motorneurons with minimal effects on muscle tissues can be accomplished with pulse durations of around 0.1 ms and 100–200 V. The second important attribute of the stimulation waveform is pulse frequency. With increasing frequencies, the intervals between pulses become less than the time needed for responsive cell membranes to recover from the induced action potential; in other words, the pulses influence the partial refractory period, which fails to trigger an action potential but also delays recovery of the membrane to the responsive state. The consequence of this is that the responses of the cell to high frequency stimulation becomes intermittent and generates only a low frequency of responses. In peripheral motorneurons, with an action potential duration of, typically, around 1 ms, a reduction in muscle activation becomes particularly evident when frequencies exceed 1000 Hz and decreases monotonically as the frequency increases above this. HFI thus ensures that immobilisation is caused by membrane fatigue in the motorneurons, ensuring that the pH is kept relatively high, while LFI causes metabolic fatigue by lowering pH.

The effects of MVS and HVS did not have such a big influence on the pH decline when combined with LFI, due to the fact that the muscle was already metabolically fatigued. It has been shown that the Δ pH produced by any given stimulation decreases as pre-stimulation pH decreases (Chrystall & Devine, 1978). There were differences between the LFI with HVS or MVS at 1.5 hours post-mortem. Because the muscle is already fatigued, the threshold for response of the muscle increases, and, according to the muscle response curve, with an increase in amplitude at a constant pulse width, the response generated will increase (Simmons *et al.*, 2008). However, the drop in pH was more when HFI was used. In these treatments, HVS and MVS lowered the pH considerably compared to when it was used in combination with LFI; this is due to the muscle having a higher pH.

3.4.2 Shear force

Electrical stimulation tends to decrease the shear force values when compared to non-stimulated meat 1 to 2 days post-mortem (Uytterhaegen *et al.*, 1992; White *et al.*, 2006; Toohey, *et al.*, 2008). Thus, it should be noted that all stimulation treatments had quite low shear force values. LFI combined with HVS resulted in higher shear force values throughout the ageing period in the LD, an effect that can be attributed to the rapid pH decline ($r = -0.277$; $p = 0.034$) while the carcass temperature is still high. In comparison, stimulation had no effect on the SM. Differences in the effects of the stimulation treatments on the different muscles can be attributed to the overriding effect of the very slow cooling curve of the SM. If carcasses receive excessive amounts of stimulation, as is the case with LFI HVS, shear force values are higher in stimulated carcasses than in non-stimulated carcasses, as shown by Geesink *et al.* (2001). They found that at day 1, there were no differences in shear force values, but after 7 days of ageing, stimulated carcasses were significantly tougher. This is supported by Koh *et al.* (1987) and Unruh *et al.* (1986) and may be due to loss of calpain activity through rapid autolysis (Simmons *et al.*, 1996) or rigor shortening (Hertzman *et al.*, 1993). Heat shortening occurs if rigor is attained at high carcass temperatures (Locker & Hagyard, 1963) and leads to a reduced capacity to tenderise, due to the permanent shortening of the sarcomeres, which has been shown to correlate with an increase in toughness (Herring *et al.*, 1965).

The shear force values of the SM was significantly higher than the LD for days 5 and 9 which is in accordance with the findings of other authors for beef (Belew *et al.*, 2003; Carmack *et al.*, 1995; McKeith *et al.*, 1985) and sheep (Tschirhart-Hoelscher *et al.*, 2006). At day 1, the LD had a higher shear force value than the SM. This difference could be attributed to the slower cooling curve of the SM (Figure 1) and that, at day 1 of assessment, the SM had aged more. However, the SM does not age with regard to the subsequent time points, which may be due to calpain activity loss because of the denaturising conditions.

There are three possible mechanisms which may alter the rate of tenderisation: reduced cold-induced shortening, enhancement of the rate of proteolysis and alteration of protein structure (Hwang *et al.*, 2003). In this trial, no cold induced shortening took place, as the pH was below 6 when a carcass temperature of 10 °C was reached. The theory that stimulation causes tenderisation by means of structural changes has been widely debated and is based on the work of Takahashi *et al.* 1987. The third theory on the effects of electrical stimulation on tenderness is based on accelerated pH decline (Chrystall & Hagyard, 1975; Chrystall & Devine, 1985). This mechanism is based on the principle that the tenderisation process begins at or near rigor mortis, when the ultimate pH is attained. Since electrical stimulation accelerates the onset of rigor mortis, tenderisation begins sooner, and, more importantly, this earlier onset of rigor mortis is associated with higher carcass temperatures, so that the rate of tenderisation is faster than would be the case if rigor mortis is attained later and the carcass temperature is lower. Of course, the carcass temperature would eventually decline to normal chill temperatures, but, by then, the stimulated carcass will have had a head start. The effect of rigor temperature on shear force values was first thought to be optimum at 15 °C due to the minimal shortening that occurs at this temperature. However, Devine *et al.* (1999) showed that when shortening in beef is prevented by tight wrapping, mimicking skeletal restraint, with an increase from 15°C to 35°C in temperature at rigor attainment, shear force was greater at high rigor mortis temperatures. These differences continued with ageing. Hwang & Thompson (2001b) measured calpain activity for muscle entering rigor at different temperatures and found that μ -calpain activation occurs earlier in muscle entering

rigor at high temperatures (38 °C). However, with slow rigor (15 °C), muscles had higher μ -calpain levels at 24 hours post-mortem. At a constant rigor temperature (35 °C) almost 80% of the μ -calpain activity was lost during rigor development, while only about 20% of activity was lost during rigor development when muscle was exposed to a constant temperature of 15 °C (Wahlgren *et al.*, 1997). Simmons *et al.* (1996) measured calpain activity throughout the rigor process, and they showed that calpain activity remained constant at all temperatures up to a pH of approximately 6.2, and then activity decreased. Conditions of low pH and high temperature are known to denature the contractile proteins, which are more stable at rigor mortis (Offer, 1991). Such conditions, in conjunction with greater autolysis of calpains at high temperatures (Dransfield *et al.*, 1992) would explain how ageing enzymes are reduced in effectiveness so that both shear force increases and ageing potential is reduced when rigor is attained at a high temperature.

A study by Hwang & Thompson (2001a) on steers and heifers showed a significantly lower shear force score and a slower pH decline when stimulation was applied 40 minutes after stunning compared to 3 minutes after stunning. The high voltage stimulation after 3 minutes resulted in tougher meat. Thus, a very rapid decline of pH resulted in a small increase in meat toughness; this was probably due to rigor shortening. A higher level of calpastatin post-stimulation at 3 minutes coincided with a higher shear force value compared with the results of stimulation at 40 minutes. If there is a rapid pH fall resulting from stimulation within 3 minutes of slaughter, the meat is not as tender after 3 days of ageing at 4 °C as it would be when stimulation takes place at 30 minutes after slaughter. However, the meat in both cases is more tender than non-stimulated meat. Over time (after 14 days of ageing) all the meat becomes tender, but the meat stimulated at 30 minutes were still the most tender. Their experiment supports our findings, that when rapidly lowering the pH of meat, as in the case of LFI HVS, tenderness is negatively affected (Figure 3).

3.4.3 WBC

The results confirm that early and rapid reduction of muscle pH leads to a lower water-binding capacity. This is particularly noticeable on the drip losses during retail display following LFI, which lowers the pH before dressing and increases the time interval between stimulation and cooling the carcasses in a chiller. Li *et al.* (2006) stated that low voltage electrical stimulation had no effect on drip losses for *longissimus* steaks from electrical stimulated carcasses compared to those that did not undergo stimulation; this indicated that low-voltage electrical stimulation had no effect on the weight loss of the steaks after vacuum packaging. However, Eikelenboom *et al.* (1985) found that there is a one per cent increase in drip loss during vacuumed storage from one to seven days post-mortem. The number of the reactive groups that bind water changes with the loss of ATP, the drop in pH, proteolysis and protein denaturation post-mortem (Bond *et al.*, 2004). Conditions of low pH and high temperatures in post-mortem muscle reduce the water-binding capacity of meat, an effect attributable to muscle denaturation, especially myosin. This can occur due to a fast pH drop or during a normal drop combined with a slow chilling rate (Offer & Knight, 1988).

Cooking losses were higher in the HFI treatments for days 5 and 9 while at day 1, there were no significant differences. Low- (Li, *et al.*, 2006) and high voltage (Eikelenboom *et al.*, 1985; Strydom *et al.*, 2005) electrical stimulation increased the cooking loss of *longissimus* steaks from the conventionally chilled carcasses, but King *et al.* (2004) found no differences, possibly due to rapid pre-rigor chilling (Li *et al.*, 2006). The higher cooking losses of the HFI treatments may be attributable to the higher drip loss which occurred during cold storage with the LFI treatments for these periods. These drip losses during cold storage were significantly

higher for both ageing periods, and, when the samples were cooked, there was a higher amount of fluid expelled due to a higher amount of water bound to the samples of HFI HVS and HFI MVS before cooking started, resulting in the higher cooking losses (Figure 5). This is related to drip losses during retail display, as the losses decreased as days post-mortem increased. It is known that, with ageing, drip increases due to denaturation of the structural proteins. However, in this trial, it decreased; but, due to the increase in drip loss during storage, the overall purge increased.

The data indicate that the amount of expressible water did not differ between the treatments. The problem with this methodology is that, due to a difference in water levels within the muscle at days 5 and 9, it is difficult to compare the treatments with each other. The drip loss during cold storage should have an impact on the amount of expressible water because the initial amount of water within the muscle has changed. Thus, when all the drip losses is considered overall, electrical stimulation causes a reduced water-binding capacity by accelerating the pH decline but the extent may be determined by the chilling rate, as was indicated by Li *et al.*, 2006.

3.4.4 Colour stability

To the naked eye, blooming is perceived as an increase in bright redness (Ledward, 1992). This represents the oxygenation of myoglobin and is influenced by the oxygen-consumption rate of the meat: higher oxygen consumption limits oxygen penetration into the meat and results in less redness (darker meat). The oxygen-consuming machinery of the mitochondria is affected by pH/temperature conditions during processing, as well as by the time elapsed during ageing (Young *et al.*, 1999). Certainly a^* values increase during blooming, but so do b^* values. Therefore, blooming is probably well represented by chroma (Young *et al.*, 1999). The bloomed chroma values were the highest with LFI HVS and the lowest with HFI with either HVS or MVS (Figures 16 and 17). It is evident that LFI had the most rapid pH decline, a condition known to cause denaturation of the cells. This is also evident in the muscles where the SM had a higher bloomed colour, which can be attributed to a slower cooling curve. The oxygen-consuming machinery of the mitochondria is damaged when the pH is low at high temperatures, so that the meat blooms better (Young *et al.*, 1999). However, later effects on colour are adverse. There was an increase in the L^* values as the meat bloomed for all treatments of the LD, with the meat being more reflective when it was stimulated with LFI HVS and LFI MVS 3 hours later. Fluid loss due to muscle-lattice shrinkage has an effect on colour (Rees *et al.*, 2003). In essence, when the pH falls quickly while the muscle is still at a high temperature in the period soon after slaughter, the protein filaments that produce contraction are disrupted or denatured and 'shrink'. This results in a reduced amount of space within the protein lattice, and the water content is expelled as drip. This mechanism also results in an increase in the paleness of the meat. This is because the reduced lattice spacing within the muscle causes more light to be reflected from the meat surface, imparting a whiter/paler appearance (Unruh *et al.*, 1986). Denaturation of the myofibrillar proteins and redistribution of water with the associated increase in reflectance may have caused the increase in L^* values (Figure 8).

In certain muscles, for example the SM in beef, ES is associated with increased metmyoglobin formation and a resulting loss of colour (Lawrie, 1998). The results confirm that that the SM has less colour stability compared to the SM. Muscles of high colour stability have a high resistance to induced metmyoglobin formation, high oxygen-penetration depth, low oxygen-consumption rate, low myoglobin content and low oxidative rancidity. (McKenna *et al.*, 2005). Ledward (1985) found that the temperature at which muscle went

into rigor strongly affected metmyoglobin formation on display. Colour stability, when defined as the rate of metmyoglobin accumulation on the surface layer, is generally reduced by ES (Wiklund *et al.*, 2001). Colour stability during retail display decreased with days post-mortem. The redness (a^* value) of the meat increased rapidly until fully bloomed at day 1 of retail display, but declined steadily as days of retail increased. There are several factors which contributed to this phenomenon. Oxygen-consumption rates decrease with increasing days of retail display for all beef muscles (McKenna *et al.*, 2005). Another factor that has an influence is the amount of ES applied. Meat tends to be redder after high- (Eikelenboom *et al.*, 1985) and low voltage (Unrah *et al.*, 1986; Sleper *et al.*, 1983; Eikelenboom *et al.*, 1985) ES compared to non-stimulated carcass muscles. This effect is due to damage done to the enzyme systems of the mitochondria responsible for oxygen consumption, a reduced oxygen-consumption rate and thus the higher concentrations of oxymyoglobin in the surface meat layer (Ledward, 1992). This observation is confirmed by Lawrie (1998) who also claims that the brighter red colour can also be attributed to the fast decline in pH. This results in the carcasses reaching their iso-electric point much sooner, thereby "opening up" the structure and easing the oxygenation of myoglobin. The result found by Wiklund *et al.* (2001) suggests the ES accelerates the loss of metmyoglobin reductase activity, so that after a week, metmyoglobin accumulation is accelerated. This action equilibrates after 3 weeks with non-stimulated meat, and the effect is then lost. Unrah *et al.*, (1986) found at day 1 that steaks were brighter red, but, at day 5, the control (non-stimulated) was redder. A more open muscle structure and the enzymes important to muscle colour are degraded because of temperature and pH conditions and may account for the increased rate of colour deterioration during display (Sleper *et al.*, 1983). Van Laack & Smulders (1990), however, found the opposite – that ES increased the display life. They postulated that, due to the more open structure of the muscle, it may allow more oxygenation and thus for increased redness. Similarly, when stimulation is omitted, the tighter structure would limit oxygen diffusion and thus reduce the layer of red oxymyoglobin. Consequently, the metmyoglobin layer beneath the oxymyoglobin will be nearer to the surface.

The literature gives conflicting results on the effect of ES on colour stability. Colour stability in electrically-stimulated meat is dependent on two factors: the depth of the oxymyoglobin layer on the surface and the stability of the metmyoglobin reducing enzymes. With an increase in stimulation, a more open structure leads to a deeper oxymyoglobin layer; however, at the same time, the temperature and pH interaction leads to less stable metmyoglobin reductase. There is a conflicting mechanism at work here, which might explain the conflicting results of the literature. It is important to optimise the two conflicting mechanisms affecting colour stability to improve retail display.

The results indicate that LFI with either HVS or MVS tended to have higher mean a^* values with a more vivid colour (higher chroma values) during retail display than the HFI HVS or MVS treatments. The higher mean values do not necessarily indicate a longer display life for the meat after lengthy storage periods. Young *et al.* (1999) showed adverse effects in display life with an increase in storage period and with an increase of temperature of rigor attainment. The oxymyoglobin layer that is formed may be greater in LFI treatments due to the more rapid pH decline. These conditions are also known to denature the reductase enzymes that reduce metmyoglobin back to the red oxymyoglobin. Due to the higher initial a^* values, the average is higher, but they have a more rapid decline as the reductase activity decreases. HFI HVS and HFI MVS treatments had more stable metmyoglobin reductase enzymes and lower oxygen-consumption rates (indicated in the MTT activity of kill 2) that kept the formation of metmyoglobin to a minimum during retail

display, but had less penetration of the oxymyoglobin of the surface layer. For the stimulation LFI with either HVS or MVS, the faster pH decline led to less stable metmyoglobin reductase enzymes; however, the layer of oxymyoglobin that formed with these treatments was deeper on the surface layer.

3.4.5 MTT and Myofibrillar density

Density is a measurement with which to assess the amount of protein denaturation, and MTT is a measurement of the rate of oxygen consumption of meat that correlates with meat-colour development during retail display.

As mentioned, myofibrillar density indicates the degree of protein denaturation; the higher the density value, the greater the extent of the denaturation. Although HFI HVS had the highest density value, the difference is very small, and the degree of denaturation between treatments according to this assay is very small. These results do not correspond well to the other measurements taken throughout the trial, especially the L^* values (inverse linear correlation of - 0.258, $p = 0.113$) and, more specifically, the higher purge losses as the release of fluid from the denatured muscle with LFI HVS or MVS (Figure 20).

MTT is an assay that measures both the amount of linked oxygen reduction (oxygen consumption rate) and cytochrome b5 dependent metmyoglobin reductase activity. The higher the reductase activity, the better the reducing capability (an enzyme-driven process) of the meat (Simmons *et al.*, 2008). When there is a high degree of reducing activity, the metmyoglobin (brown colour) can be reverted back to a purple-red myoglobin state, and thus the fresh red colour of the meat is maintained. The higher the rate of oxygen consumption, the darker the appearance of the meat colour, as oxygen is not able to penetrate into the meat and give meat its bright red colour.

The MTT activity values for kill 1 do not correspond well to what was expected in accordance with the literature (Simmons *et al.*, 2008). LFI HVS had the highest value, which was not expected. The values, however, are low in comparison with work done by Simmons *et al.* (2008). In a repeat experiment, kill 2 had a different result with the LFI with HVS or MVS having lower MTT activity (lower oxygen consumption and reductase activity), as would be expected from the denaturing conditions the samples were exposed to due to a rapid pH decline at high carcass temperatures. This is also evident in muscles where the SM has lower MTT values than the LD, mostly due to the slower cooling rate of the SM (Figure 18).

The MTT value is expected to be closely related to the oxygen-consumption rate of meat (Berridge & Tan, 1993). A reduced oxygen-consumption rate means better oxygenation of the bloomed surface, so MTT values should be inversely related to a^* -values or hue values. Current results (inverse linear correlation coefficient of -0.63 and -0.73) are confirmed by the work of Simmons *et al.* (2008), who found an inverse linear correlation with a correlation coefficient of 0.66 with hue angle.

3.5 Conclusion

The pH-temperature interaction gives rise to an opportunity to produce meat products for diverse markets, whether frozen-, chilled local- or export markets. HFI treatments have a higher pH at 0.5 hours than LFI treatments, allowing for better management of early post-mortem pH by means of HVS and MVS. It is evident that keeping pH high during the dressing of the carcass improves meat quality and offers the

opportunity to optimise processing specifications to produce meat for diverse markets. In addition, it is clear that excessive electrical stimulation, for example when using the standard LFI followed by HVS as is the case in some New Zealand plants, can reduce meat quality, particularly through reduced tenderness and increased purge losses.

The effects of ES and immobilisation on meat colour and retail display life are complex, with complicated mechanisms at work. It is evident that stimulation increases the red bloom colour of meat after cold storage for a number of days. LFI HVS initially resulted in redder meat, but the meat became dark more quickly as the retail days progress. The results of HFI with either HVS or MVS were less red, but the meat had a more stable colour as retail days progressed. Increasing storage times, which is beneficial for tenderness, also has the effect of reducing retail display life. These stimulation treatments would be useful when cold storage time is limited and when periods of storage are extended. It would be advisable not to stimulate the meat in such circumstances, thus increasing the stability of the meat colour. The results indicate that MTT is a good method for predicting oxygen-consumption rates and the stability of the reductase enzyme.

The results indicate HFI improves meat quality when combined with either HVS or MVS and that MVS has no adverse effects on meat quality in relation to HVS combined with HFI. It is therefore essential that HFI is used in abattoirs that use immobilisation to maintain high throughput levels.

3.6 References

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THE EFFECT OF DIFFERENT ELECTRICAL STIMULATION FREQUENCIES ON MUSCLE PH DECLINE AND BEEF TENDERNESS

Abstract

Electrical stimulation (ES) is used on carcasses to induce rapid tenderisation and plays a key role in manipulating the conversion of muscle to meat in modern day abattoirs. Optimisation of the temperature and pH decline is important, as it affects various meat-quality characteristics. The aim of the trial was to evaluate the effect of pulse frequency on pH decline and the subsequent tenderness of the beef. Thirty-six steers were electrically stunned (head only, 2 A, 50 Hz, 2 s) and slaughtered and then electrically immobilised (EI) for 20 seconds using high frequency (800 Hz, 110 V peak, 0.2 ms pulse duration; HFI) within 2 minutes of kill. After carcass dressing, electrical stimulation (ES) was applied, using medium voltage (300 V peak, 1 ms pulse duration; MVS) with either 5 Hz, 15 Hz or 50 Hz. Stimulation with 15 Hz (0.47 ± 0.040) and 5 Hz (0.41 ± 0.045) had a larger pH drop (ΔpH) during stimulation than 50 Hz (0.29 ± 0.027). Shear force measurements and cooking loss percentage were obtained from the *Longissimus dorsi* after 24 hours of chilled storage at 0 °C. There were no difference between the stimulation treatments for shear force (15 Hz = 121.3 ± 3.3 N; 5 Hz = 123.8 ± 7.6 N; 50 Hz = 114.8 ± 7.94 N), while cooking loss was higher in 15 Hz ($28.8 \pm 0.47\%$) than 50 Hz ($25.9 \pm 0.71\%$), which correlated linearly ($r = 0.43$; $p = 0.01$) with ΔpH . By modulating the pulse frequency, the rate of pH decline can be manipulated so as to optimise the pH-temperature interaction in accordance with the specific product produced by the plant without making, wholesale changes to the system.

4.1 Introduction

External electrical activation of the muscle membranes triggers Ca^{2+} release from the sarcoplasmic reticulum, triggering the contractile actomyosin ATPase and muscle contractions. Muscle contractions result in a higher rate of ATP breakdown, and this leads to a faster post-mortem glycolysis. The Ca^{2+} ions also enhance the activity of the phosphorylase, which provides the substrate for the increase in rate of post-mortem glycolysis (Lawrie, 1998) with a subsequent drop in pH (ΔpH) which can range from 0.6 pH units at 35 °C to 0.018 units at 15 °C (Chrystal & Devine, 1980). The pH drop is time dependent and will be influenced by the duration of external stimulation. Following the immediate pH fall, there is an additional acceleration of glycolysis leading to a higher rate of pH fall ($d\text{pH}/dt$) and, subsequently, an early rigor development (Chrystal & Devine, 1978). However, this effect tends to be associated with high-voltage stimulation systems and is not necessarily triggered by low-voltage stimulation systems (Kastner *et al.*, 1993). In non-stimulated beef *Sternomandibularis*, the energy of activation of the glycolytic process is 40-45 kJ/mol, whereas that for stimulated muscle approaches 70 kJ/mol. This high activation energy means in both cases that any cooling of the muscles will markedly increase the time for attainment of rigor mortis, with a larger effect in stimulated muscle (Chrystal & Devine, 1980).

The application of electrical stimulation in sheep and beef carcasses in the processing industry has been erratic around the world; this may be reflection of a general lack of knowledge on how to optimise the technology. The electrical characteristics of the waveforms used are often poorly described. Muscle force is controlled by the number of muscle fibres activated and the amount of force generated in the fibres (Riener & Quintern, 1997). Thus, the muscle force is the work done by the muscle and is therefore related to the pH decline of the muscle (Simmons *et al.*, 2008). For any given muscle, the pH response will be governed by the electrical characteristics of voltage (current), pulse frequency, width, shape and polarity.

Pulse frequency has a great effect on force because of the summation effect; if the Ca^{2+} is not fully reabsorbed between each pulse, it gradually accumulates. Also, as a result, increasing frequency also fuses the twitches, so a full tetanus produces a much higher peak force than an individual maximal pulse. As shown by Devine *et al.* (1984), muscle type has an influence on ΔpH in beef muscles, where the fast-twitch *Cutaneous trunci*, largely composed of white muscle fibres, give high values for ΔpH whereas in the slow-twitch *Masseter*, composed of red fibres, there is neither a distinct ΔpH nor an acceleration of $d\text{pH}/dt$. It is reported by Swatland (1981) that within the *Sternomandibularis*, fast fibres with strong ATPase and weak succinic dehydrogenase (SHD) reactions showed most glycogenolysis in response to electrical stimulation, while slow fibres with weak ATPase and strong SDH showed the least. Different muscle types have different responses to frequencies because the rate at which the Ca^{2+} is reabsorbed differs. Fast-twitch muscles have a better developed sarcoplasmic reticulum, so they can reabsorb Ca^{2+} faster and produce full fusion at a higher frequency. The ΔpH (drop in pH during ES) should include the variable of the duration of the stimulation when different frequencies are compared: at low frequencies, the ΔpH will be the same as standard 15 Hz but require more time than is usually allowed for during the stimulation period, whether experimental or commercial. What isn't usually discussed or understood is why the ΔpH decreases at the higher frequencies. Simmons *et al.* (2008) have proposed that this is due to membrane fatigue rather than metabolic fatigue; accordingly, responsiveness is lost due to K^+ accumulation at the membrane level. Muscles also differ in their optimum response frequency, where *Longissimus dorsi* is more responsive to 14

pulses per second than to 40 pulses per second (Chrystal & Devine, 1978), and the opposite has been shown for beef *Semimembranosus* (Bouton *et al.*, 1980).

It is well known that the stimulation increases the rate of post-mortem glycolysis; however, other biochemical and biophysical effects have also been indicated with the use of electrical stimulation (Hwang *et al.*, 2003). Electrical stimulation generally lowers shear-force values compared to non stimulated carcasses (Strydom *et al.*, 2005). However, the response should be controlled because too rapid a pH decline leads to protein denaturation (Thompson *et al.*, 2005). Denaturation can have detrimental effects on shear force and water-binding capacity of meat.

Takahashi *et al.* (1987) showed that stimulation with a 60 Hz waveform produced areas of super-contractions and myofibrillar stretching. Their observations found that 60 Hz causes only a small pH decline following stimulation with a greater tenderising effect than a 2 Hz waveform with a larger pH decline. They concluded that since the 2 Hz does not cause any physical disruption, tenderisation occurs due to structural damages rather than a faster pH decline.

Another theory is that increased tenderness is possibly due the acceleration of the proteolysis of the myofibrillar and cytoskeletal proteins (Hollung *et al.*, 2007; Salm *et al.*, 1983). There is a high correlation between the degradation of these proteins and an increase in the tenderisation of meat (Penny & Dransfield, 1979). The acceleration of post-mortem proteolysis in electrically stimulated meat is due to the increased proteolytic activity of μ - and m-calpain (Hwang & Thompson, 2001). Electrical stimulation increases the concentration of intracellular Ca^{2+} (Westerblad & Allen, 1991) and calpains require Ca^{2+} for proteolytic activity, thus initiating the proteolytic activity of μ -calpain. Electrical stimulation also accelerates pH decline which can increase calpain proteolytic activity (Dransfield, 1993; Geesink & Koohmaraie, 1999).

The third theory of the effects of electrical stimulation on tenderness is based on accelerated pH decline (Chrystal & Hagyard, 1975; Chrystal & Devine, 1985). This mechanism is based on the principle that the tenderisation process begins at or near rigor mortis, when the ultimate pH is attained. Since electrical stimulation accelerates the onset of rigor mortis, tenderisation begins sooner, and, more importantly, this earlier onset of rigor mortis is associated with a higher carcass temperature so that the rate of tenderisation is faster than would be the case if rigor mortis is attained later and the carcass temperature is lower. Of course, the carcass temperature would eventually decline to normal chill temperatures, but by then, the stimulated carcass will have had a head start.

Due to the many different layouts, sizes and cooling regimes of chillers adopted by the various abattoirs, it is important that electrical stimulation can be slightly modified to change the interaction between the temperature and pH decline in accordance with the specific product being produced. This study examines an easy method to change the pH decline based on a variation in responses to electrical frequency during electrical stimulation. The study intends to illustrate that frequency is an important factor in electrical stimulation that could be manipulated readily due to its effect on pH decline and will affect pH decline and subsequent tenderness of beef muscles.

4.2 Materials and methods

4.2.1 Treatments and pH measurement

Thirty-six Holstein steers were electrically stunned (head only, 4 Amps, 50 Hz, 3 seconds), followed by a throat cut and thoracic stick. Carcasses were electrically immobilised (EI) using high frequency (HF) (800 Hz, 200 V for 20 seconds) EI within 2 minutes of slaughter. After carcass dressing, electrical stimulation (ES) was applied approximately 30 minutes post mortem using (MVES) (300 V) with either a frequency of 5 Hz, 15 Hz or 50 Hz (12 carcasses per treatment).

pH and temperature measurements were taken at 0.5, 0.75, 3, 4.5 and 24 hours post-mortem in the *Longissimus dorsi* (LD) in the region of the 12th rib on the left side of each carcass. The Δ pH was calculated as the difference between the pH (30 minutes post-mortem) just before electrical stimulation, and the following measurement was taken about 15 minutes later in the chiller. Ten carcasses were randomly chosen per treatment group for meat quality assessment. All carcasses were subjected to a two-stage cooling regime comprising of 8 °C for the first 8 hours followed by 0 °C until 24 hours post-mortem, when the LD were cold boned (from between the 12th rib and the last lumbar vertebrae) and used for meat-quality assessment at day 1.

4.2.2 Shear force and Cooking loss

Shear-force evaluation of tenderness was measured using a Digital Tenderometer™. The samples of the LD and SM, approximately 400 g to 500 g, were cooked in a water bath at 100 °C in weighted plastic bags until the internal meat temperature reached 75 °C as measured by an Ebro TFX 392 temperature meter. The portions were then removed and immediately chilled on ice. The basic principle in assessing meat tenderness is to determine the force required to shear through a 10 x 10 mm square cross-section sample at right angles to the fibre axis. Ten bites per sample were sheared using a triangular-shaped tooth mounted on a Tenderometer (G2 Digital) and used to give an average shear force value in kilogram force was calculated, which was converted to Newton (1 kgf = 9.8 N). The temperature of the samples was ± 1 °C.

Before cooking, each muscle sample was weighed. The following day, after the samples were cooled, the samples were weighed again. The cooking loss was measured as the difference between the sample mass before and after and was expressed as a percentage of the starting mass.

Equation 4.1 Determination of cooking loss percentage

$$\text{Cooking loss \%} = (\text{weight before cooking} - \text{weight after cooking}) / \text{weight before cooking} \times 100.$$

4.2.3 Statistical analysis

Statistical analysis was conducted using MINITAB® Release 14 statistical software. Basic statistics were used to calculate the mean and standard error of the mean. A one-way analysis of variance (ANOVA) were done on the pH, shear force measurements and cooking loss using a mixed model with the main effect being the ES frequency (HFI MVS with 5 Hz, 15 Hz and 50 Hz). Differences between the stimulation treatments were determined with Tukey's 95% test for difference.

4.3 Results

4.3.1 pH

The amount of variation (coefficient of determination; R^2) indicated by the model in the ANOVA was 94.65%. There was an interaction ($p = 0.0001$) between stimulation treatment and the time of pH measurement. The mean values for pH at various times post-mortem are shown in Table 1. There were no significant differences ($p > 0.05$) between treatments before ($pH_{0.5}$) and after ($pH_{0.75}$) stimulation, although there was a significant ($p < 0.05$) decline during stimulation. At 3 hours post-mortem stimulation with 50 Hz had a higher pH ($p < 0.05$) compared to the other treatments. While at 4.5 hours there were ($p < 0.05$) differences between all the treatments, with 50 Hz stimulation having the highest and 15 the lowest pH. When the ultimate pH was measured at 24 hours, 50 Hz stimulation still had higher ($p < 0.05$) ultimate pH compared to the other treatments. ΔpH was the largest with the 15 Hz stimulation which differed ($p < 0.05$) from stimulation with 50 Hz, but not with 5 Hz.

Table 4 Mean pH and ΔpH values with corresponding temperature readings of beef Longissimus dorsi muscle after electrical stimulation with different frequencies at various hours post-mortem.

pH	Temperature ($^{\circ}C$)	Treatment			SEM
		15 Hz	5 Hz	50 Hz	
$pH_{0.5}$	35	6.49 ^a	6.48 ^a	6.40 ^a	0.021
$pH_{0.75}$	35	6.03 ^a	6.07 ^a	6.11 ^a	0.012
pH_3	20	5.69 ^a	5.88 ^b	5.9 ^b	0.02
$pH_{4.5}$	18	5.64 ^a	5.76 ^b	5.86 ^c	0.021
pH_u	2	5.47 ^a	5.50 ^a	5.55 ^b	0.009
* ΔpH		0.47 ^a	0.41 ^a	0.29 ^b	0.024

^{ab}Values with different letters within a row differ significantly ($p < 0.05$)

* ΔpH taken before stimulation at 30 mins and after stimulation at 45 mins.

4.3.2 Shear force and cooking loss

The mean values for cooking-loss percentage and shear force are displayed in Figures 1 and 2 respectively. Stimulation with 15 Hz caused a higher ($p < 0.05$) cooking loss than 50 Hz. However, stimulation with the evaluated frequencies had no effect ($p > 0.05$) on shear-force values.

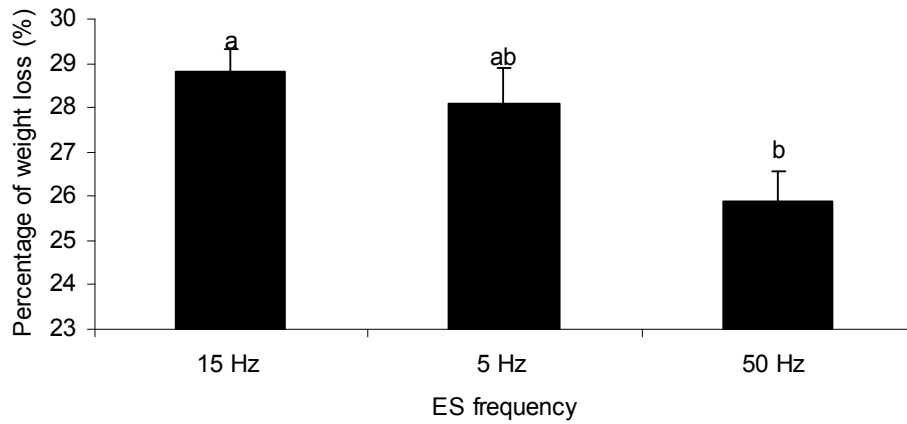


Figure 30 Effect of electrical stimulation frequency on the cooking loss (%) of beef *Longissimus dorsi*

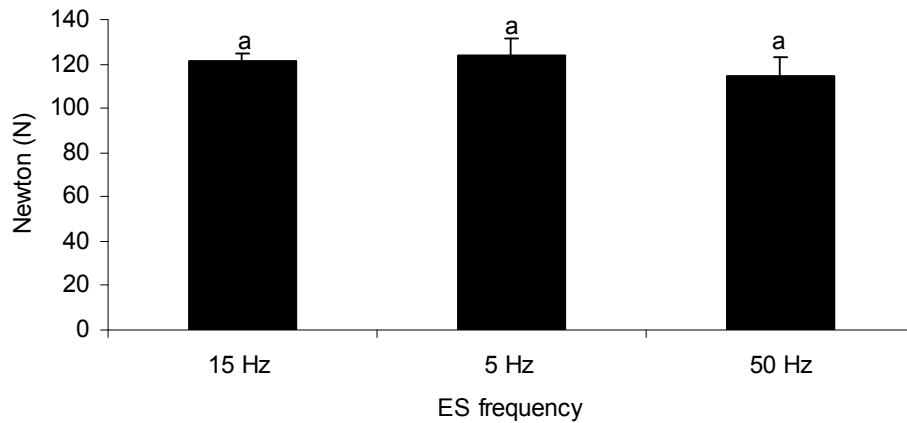


Figure 31 Effect of electrical stimulation frequency on shear force of beef *Longissimus dorsi*

4.3 Discussion

4.3.1 pH

Stimulation with 15 Hz led to the most rapid pH decline, which coincides with the work of Crystall & Devine (1978) which indicated that stimulation with 9 to 16 pulses per second causes the maximum drop in pH. Δ pH for stimulation with 50 Hz was the lowest, while there was no significant difference between 15 Hz and 5 Hz. A possible explanation is the way that muscles react to stimulation frequency. Frequency has a large effect on force because of the summation effect; if the Ca^{2+} is not fully reabsorbed between each pulse, it gradually accumulates. Also, as a result, increasing frequency also fuses the twitches, so a full tetanus produces a much higher peak force than an individual maximal pulse. The hypothetical muscle-contraction response is illustrated in Figure 3.

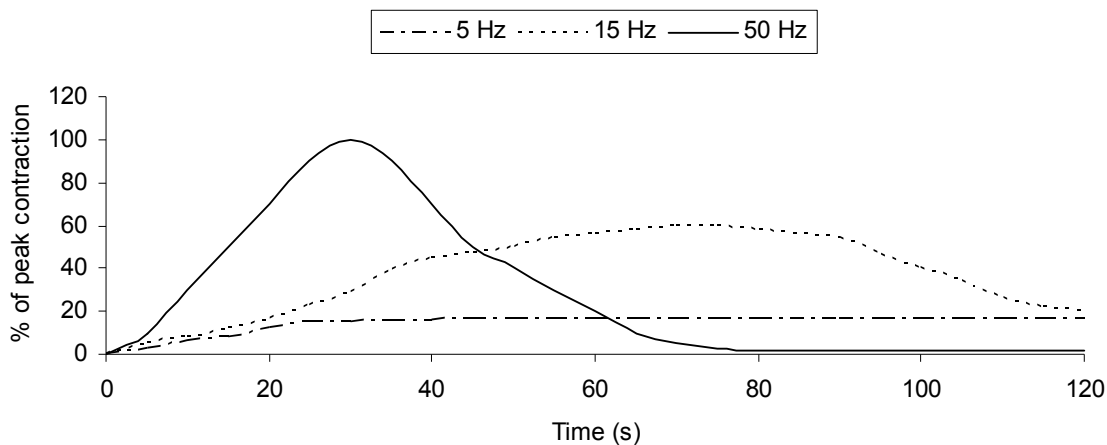


Figure 32 Hypothetical muscle contraction response for electrical stimulation at 5, 15 and 50 Hz (Adapted from Simmons *et al.*, 2008)

The measure of the work done by each muscle is the area under the graph which is therefore related to the pH decline. Simmons *et al.* (2008) reported that the area under the curve is 35% less in the 50 Hz stimulation than in the 15 Hz. A possible explanation of this phenomenon can be attributed to accumulation of extracellular potassium in response to the rapid rate of stimulation that renders it refractory to further stimulation (Juel, 1988; Cairns *et al.*, 1995). With the use of intermittent stimulation waveforms (2s on/2s off) the short rest period would allow the membrane to recover, and an effective decline in pH is then possible with high frequencies. Ultimately, the muscle becomes unresponsive due to metabolic fatigue and extreme pH decline (Simmons *et al.*, 2008).

Muscle contraction that occurs with stimulation with 5 Hz has a lower peak force of contraction. Due to a longer period between pulses, Ca^{2+} has enough time to be reabsorbed, and less summation with the contractile process takes place. Thus, a low frequency leads to a lower force generation, but can continue to produce this force for a longer period of time, since there is no occurrence of membrane fatigue. If stimulation occurs for long enough, the effect on ΔpH would be the same as with 15 Hz, because the muscles become unresponsive due to muscle fatigue (Simmons *et al.*, 2008).

4.3.2 Cooking loss

Cooking losses were the highest for stimulation with 15 Hz, coinciding with the most rapid pH decline, while, the lowest cooking losses coincided with the slowest pH decline. There was a linear correlation of 0.43 ($p = 0.01$) between the cooking loss % and ΔpH . The pH value before stimulation ($pH_{0.5}$) also had a linear correlation ($r = 0.44$; $p = 0.007$) with the cooking-loss percentage. Conditions of high temperatures and low pH denature muscle structural proteins (Offer, 1991), which leads to a reduced water-binding capacity.

4.3.3 Shear force

Shear-force values (tenderness) were unaffected by the stimulation frequency. Three possible mechanisms that may alter the rate of tenderisation have been postulated: reduced cold-induced shortening, enhancement of the rate of proteolysis and alteration of protein structure (Hwang *et al.*, 2003). Cold shortening did not occur because the temperature at pH 6 was higher than 10 °C. There is a potentially great

association between the physical disruption of the myofibrillar complex and the increase in tenderness in meat (Ho *et al.*, 1996). Takahashi *et al.* (1987) showed that stimulation with a 60 Hz waveform produced areas of super-contractions and myofibrillar disruption. They showed that a 60 Hz waveform causes only a small pH decline following stimulation with lower shear-force values than a 2 Hz waveform with a larger pH decline. They concluded that, since the 2 Hz did not cause any physical disruption, tenderisation occurred due to structural damages rather than a faster pH decline. Simmons *et al.* (2008) stated that it is unclear whether this is directly as a result of the physical disruption, as such, or whether the disruption facilitates ageing in other ways. Contracture bands are not a direct consequence of electrical current passing through the muscle, but rather due to the super-contraction caused through localised excessive calcium ion release from the sarcoplasmic reticulum. It is possible that it is the presence of this extra calcium which facilitates the increased tenderisation (Hwang *et al.*, 2003). However, Simmons *et al.* (2008) suggested that electrical stimulation needs to produce highly exaggerated levels of free intercellular Ca^{2+} compared to those produced during normal physiological muscle activity *in vivo*; otherwise, exercising would have limited health benefits.

The effects of electrical stimulation on tenderness can also be based on accelerated pH decline (Chrystall & Hagyard, 1975; Chrystall & Devine, 1985). This mechanism is based on the principle that the tenderisation process begins at or near rigor mortis, when the ultimate pH is attained. Since electrical stimulation accelerates the onset of rigor mortis, tenderisation begins sooner; this earlier onset of rigor mortis is associated with a higher carcass temperature, so that the rate of tenderisation is faster than would be in the case if rigor mortis were attained later and the carcass temperature was lower. Of course, the carcass temperature would eventually decline to normal chill temperatures, but, by then, the stimulated carcass will have had a head start. The stimulated and non-stimulated carcasses will reach the same level of tenderness, but with the stimulated carcasses reaching it sooner (Chrystall & Daly, 1996). Sometimes, the slow ageing rate caused by a slow pH decline and rapid chilling can be misinterpreted for cold shortening, as shown by Simmons *et al.* (1999) where non-stimulated *Longissimus dorsi* took 30 days to reach acceptable tenderness scores, but the persistent toughness normally associated with cold shortening did not occur.

It was expected that there would be a degree of difference in the tenderness in the present investigation. However, the fact that the pH after stimulation was relatively low for all the treatments, combined with the slow cooling rate, may have resulted in the treatment effect on tenderness being lost.

4.4 Conclusion

This paper confirms that different electrical stimulation frequencies can have a profound effect on pH decline, which can be explained by basic muscle physiology. Lower frequencies produce less summation and thus a lower force, but the same number of recruited fibres. Whereas higher frequencies lead to a higher peak contraction, it tails off more quickly due to membrane fatigue. Frequencies between 9 Hz and 16 Hz are regarded by Chrystall & Devine (1978) as the optimal number of pulses per second needed to induce the biggest ΔpH . This situation gives rise to many possibilities. Each abattoir has a unique layout, which can have an effect on meat quality. The changing frequencies of the stimulation unit can thus alter the rate of pH decline. The interaction between pH and temperature decline during the rigor development phase has a profound effect on the meat quality outcomes, where PSE and DFD are at the very end of the spectrum. The pH decline can be manipulated independently of temperature by means of electrical stimulation. Thus, by

changing the frequency, the effect on the pH decline can be manipulated according to the market requirement (fresh, frozen or chilled export products) of the specific abattoir.

4.4 References

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THE EFFECT OF SMALL PULSE WIDTHS DURING ELECTRICAL STIMULATION ON MUSCLE pH DECLINE AND DENATURATION OF BEEF PROTEINS

Abstract

Electrical stimulation (ES) is used on carcasses to induce rapid tenderisation due to its effect on pH decline rates and plays an integral role of the slaughter process in modern abattoirs. Optimisation of the temperature and pH decline is important, as it affects the meat quality. The aim of the trial was to evaluate the effects of small pulse widths during ES on the meat quality of beef. On the first day of the kill, 124 steers were electrically stunned, and 98 steers were stunned on the second day (head only, 2 A, 50 Hz, 2 s), and slaughtered and then electrically immobilised (EI) for 20 seconds using high frequency (800 Hz, 110 V peak, 0.2 ms pulse duration; HFI) within 2 minutes of being killed. After carcass dressing, ES was applied, using medium voltage (300 V peak, 15 Hz; MVS) with either a 10 ms, 1 ms, 0.5 ms or 0.1 ms pulse width. The *Longissimus dorsi* (LD) and *Semimembranosus* (SM) of 40 carcasses per kill were cold boned 24 hours later and their physical attributes measured. Ten (0.46±0.020), one (0.43±0.020) and 0.5 ms (0.44±0.019) pulse widths had no effect on the pH drop (Δ pH) while 0.1 ms (0.33±0.020) had a lower drop. The 1 ms (94.6±5.6 N) pulse width had the lowest shear force, which differed from 10 ms (111.3±3.8 N) to 0.1 ms (111.3±5.8 N), while cooking loss and water-holding capacity was not affected in the LD. Colour measurements on the SM indicated that a 0.1 ms (a^* = 19.38±0.50; chroma = 22.70±0.51), 0.5 ms (a^* = 20.89±0.49; chroma = 24.34±0.56) and 10 ms (a^* = 19.69±0.46; chroma = 22.98±0.58) pulse widths had a deeper red and a more vivid colour than 1 ms (a^* = 6.66±0.37; chroma = 19.99±0.32) at day 9 of retail display. The reduction of pulse widths from 10 ms to 0.1 ms had no detrimental effects on meat quality in this study, thus smaller pulse widths can be used in MVS systems to improve worker safety.

5.1 Introduction

The application of electrical stimulation has been widely used in commercial practices. The most variable aspects around stimulation have been either high- or low voltage, hide on/off stimulation or the placement of the electrodes. By increasing or decreasing the voltage, frequency or pulse width, the pH decline can be manipulated. Voltage, the amplitude of the waveform and pulse width has a direct interaction on the amount of muscle force generated. There is a distinct interaction between pulse width and amplitude. If the pulse amplitude is held constant while reducing the pulse width, the response will weaken and eventually disappear altogether (Aston, 1991). Pulse amplitude modulation is, in effect, equivalent to pulse-width modulation, since an increase in either results in greater muscle force generation (Crago *et al.*, 1980) with an increase in pH decline. Thus, with an increase in amplitude, pulse width can be decreased without any effect on contraction and vice versa. The relationship can be described by the classical pulse strength-duration curve, which defines the threshold for producing an electrically-induced stimulus (Figure. 1) (Simmons *et al.*, 2008).

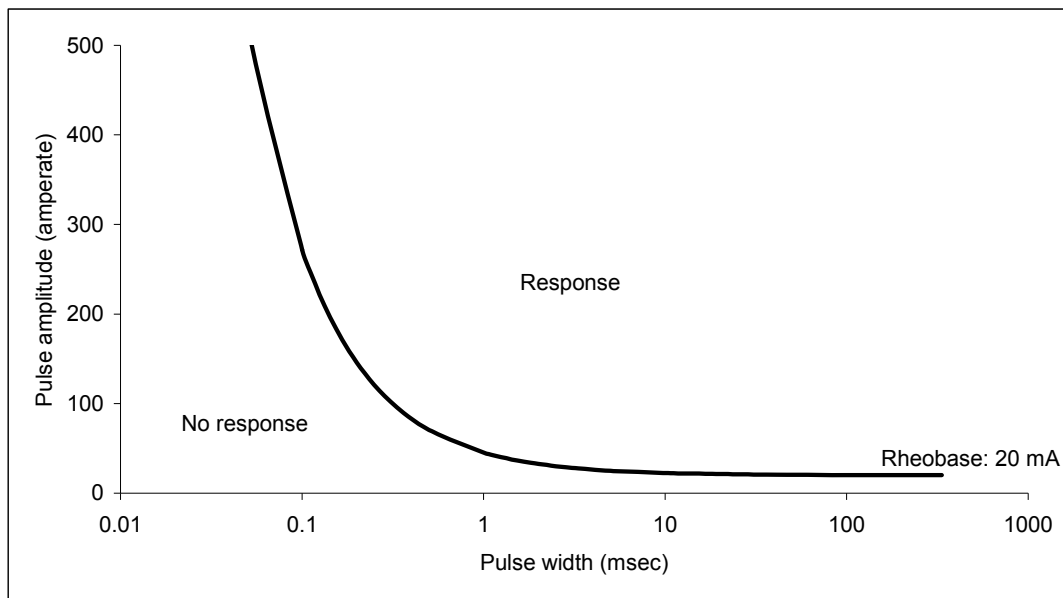


Figure 33 The pulse strength-duration curve of the response of muscle (adapted from Simmons *et al.*, 2008)

Simmons *et al.* (2008) used measurements of muscle pressure to acquire values for this relationship. They found that in carcasses stimulated immediately after slaughter, the muscle response is insensitive to pulse width and that the threshold current amplitude needed for a response is very low (< 50 mA). However, there are steep contrasts when the response is measured in the carcass after dressing (20-30 minutes post-mortem). The importance of pulse width becomes more evident as the carcass becomes more fatigued and the pH falls. The threshold for responses increases as the muscle becomes more fatigued and wider pulse widths are needed to maintain an effective response (Simmons *et al.*, 2008).

The pre-rigor temperature and pH environment determines the quality attributes of tenderness, colour and water-binding capacity. Within the extremes lie a range of processing options that can produce a spectrum of distinctive quality attributes which can be obtained by the manipulation of the pH decline by electrical stimulation with regard to the cooling rate. However, in commercial conditions, this is difficult to achieve. Due to the differences in muscle locations and size, there will always be differences in the rate of cooling of different muscles. The rate of pH fall between animals and the response to electrical stimulation is also variable, as shown by Simmons *et al.* (1997). The temperature at which the muscle enters rigor has a profound effect on the quality of meat produced (Hertzman *et al.*, 1993; Chrystall & Devine, 1985). When a muscle enters rigor at a high temperature, the proteolytic enzymes are stimulated (Dutson *et al.*, 1977); however, at high temperature and low pH the calpain activity and stability is compromised (Simmons *et al.*, 1996). Meat quality will be negatively affected under the abovementioned conditions because of the tightly-packed structure resulting from heat shortening. This could prevent the proteases access to their substrates (Rees *et al.*, 2003). The most notable adverse effect on meat quality is a result of the denaturation of muscle proteins which gives rise to the pale colour and low water-binding capacity (Geesink *et al.*, 2001; Offer & Knight, 1988). Loss of water-binding capacity is attributable to shrinkage of the myofibrillar lattice, triggered by the denaturation of myosin (Offer & Knight, 1988), which expels water from the intracellular to the extracellular space where it is poorly bound. Loss of water binding through the denaturation of soluble proteins also contributes to loss of water-binding capacity.

Rapid and early pH decline post-mortem with a simultaneous high muscle temperature promotes severe muscle protein denaturation (Briskey, 1964; Offer & Knight, 1988). Moreover, the combination of low pH and high temperature accelerates the inactivation of the oxygen-consuming mitochondrial enzymes and promotes the oxygenation of the muscle pigment to the bright red oxymyoglobin (Govindarajan, 1973). Together with the partial denaturation of the myosin and sarcoplasmic proteins, this increases light scattering of the surface characteristic for paler meat with high exudative drip loss (Bendall & Swatland, 1988).

If smaller pulse widths can be used to minimise denaturation of the deeper lying muscles due to a rapid pH decline and a slow cooling curve while lowering the pH of more superficial muscles like the *Longissimus dorsi* (LD), it can have a beneficial effect for the overall meat quality of a carcass. Thus, the purpose of this study was to evaluate the effects of short pulse widths during electrical stimulation on the pH decline in the (LD) and denaturation of the *Semimembranosus* (SM) of beef muscles.

5.2 Materials and methods

5.2.1 Animals and treatments

The work consisted of two trials. The first and second kill used 124 and 98 Holstein steers respectively to determine pH decline. All animals were electrically stunned (head only, 4 Amps, 50 Hz, 3 seconds), followed by a throat cut and thoracic stick. Carcasses were electrically immobilised (EI) using high frequency (HF) (800 Hz, 200 V for 20 seconds) within 2 minutes of slaughter. After carcass dressing, electrical stimulation (ES) was applied using MVS (300 V, 14.3 Hz) with either a 10 ms, 1 ms, 0.5 ms or 0.1 ms pulse width.

Shear force, cooking loss and water-binding capacity were measured on the LD for the first kill. During the second kill, shear force, cooking loss and water-binding capacity were measured in the LD and water-binding capacity and colour during retail display were measured in the SM as an indication of the denaturation of muscle structural proteins.

5.2.2 pH measurement

pH measurements were taken at regular intervals pre-rigor and 24 hours post-mortem in the LD. The pH drop (ΔpH) was taken as the difference between the pH before stimulation (pH_{30}) and the pH as the carcasses entered the chiller (pH_{45}) after 45 minutes. Ten carcasses were randomly chosen per treatment group for meat-quality assessment. All carcasses were subjected to a two-stage cooling regime comprising of 8 °C air temperature for the first 8 hours followed by 0 °C air temperature until 24 hours post-mortem, when the LD (kill 1) or the LD and SM (kill 2) were cold boned and used for meat-quality assessment at 24 hours post-mortem.

5.2.3 Shear force and cooking loss

Shear-force evaluation of tenderness was measured using a Digital Tenderometer™. The samples of the LD and SM, approximately 400 g to 500 g, were cooked in a water bath at 100 °C in weighted plastic bags until the internal meat temperature reached 75 °C as measured by an Ebro TFX 392 temperature meter. The portions were then removed and immediately chilled on ice. The basic principle in assessing meat tenderness is to determine the force required to shear through a 10 x 10 mm square cross-section sample at right angles to the fibre axis. Ten bites per sample were sheared with a triangular-shaped tooth mounted on

a Tenderometer (G2 Digital) and used to give an average shear force value in kilogram force which was converted to Newton (1 kgf = 9.8 N). The temperature of the samples was ± 1 °C (Simmons *et al.*, 2006).

Before cooking, each muscle sample was weighed. The following day, after the samples were cooled, the samples were weighed again. The cooking loss was measured as the difference between the sample mass before and after cooking and was expressed as a percentage of the starting mass.

Equation 5.1 Determination of cooking loss percentage

$$\text{Cooking loss \%} = (\text{weight before cooking} - \text{weight after cooking}) / \text{weight before cooking} \times 100.$$

5.2.4 Water-binding capacity

The water-binding capacity (WBC) of the muscles was determined by the press method where a 500 mg fresh meat sample (24 hours) was placed on a filter paper (Whatman #2) sandwiched between two Perspex plates and pressed at a standard pressure (588 N) for 1 minute. The WBC was calculated by determining the ratio of meat area to the liquid area after compression. The measurement was taken in duplicate. Photos were taken of the two areas which were then measured by means of Image J computer package (Trout, 1988).

5.2.5 Colour stability

Steaks, roughly 20 mm, were cut from the SM and placed on a tray (vertex plix 810) and wrapped with an oxygen-permeable covering film (Progressive Enterprises, W/O ID 838485, Roll 404). The trays were placed in a chiller (Skope, B550PH2CX:V48JF) set at 5 °C to mimic retail storage. Three colour measurements were carried out across the individual sample surfaces on top of the covering film, and mean values were used for statistical analysis. Measurements were taken on the non-bloomed meat surface, and for each subsequent day until meat colour was visually brown with poor overall appearance (day 11). Non-bloomed colour was measured after cutting and bloomed colour (day 0) was taken 3 hours later.

Colour measurements followed the CIE colour convention, where the three fundamental outputs are L^* , a^* and b^* . L^* is lightness on a scale of 0 (all light absorbed) to 100 (all light reflected); a^* spans from +60 (red) to -60 (green) and b^* spans from +60 (yellow) to -60 (blue). Chroma (saturation) was calculated as $(a^{*2} + b^{*2})^{1/2}$, and hue angle as $\tan^{-1} b^*/a^*$. Colour was measured using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing Inc.) calibrated against a white tile ($L^* = 93.30$, $a^* = 0.3158$ and $b^* = 0.3322$). The aperture was 8 mm, and illuminant D65 and a 10° observer were used.

5.2.6 Statistical analysis

Statistical analysis was done using MINITAB® Release 14 statistical software. Basic statistics were used to calculate the mean and standard error of the mean. A general linear model (GLM) ANOVA was done with the main effects for kill (1 and 2), stimulation pulse width (0.1 ms, 0.5 ms, 1 ms and 10 ms) and in the case with WBC muscle (LD and SM). During colour assessment of the SM the main effects were stimulation pulse width and day of retail display (non-bloomed to day 11). Differences between main effects were determined by Tukey's 95% test for difference.

5.3 Results

5.3.1 pH

The results for mean pH values obtained in the beef LD muscles after ES with various pulse widths and measured at various times post-mortem are given in Table 1. The amount of variation (coefficient of determination; R^2) explained by the model in the ANOVA was 89.66%. The day of kill ($p = 0.807$) had no effect on the pH decline; however, there was an interaction ($p = 0.0001$) between stimulation treatment and the time of pH measurement.

It is evident that before electrical stimulation had taken place, there were no differences ($p > 0.05$) between the groups of treatments. However, after ES, the 10 ms treatment had the lowest pH at 45 minutes which differed ($p < 0.05$) from the other treatments. The 0.1 ms treatment had the highest pH_{45} which differed ($p < 0.05$) from the 10, 1 ms and 0.5 ms treatments. There were no difference between the 0.5 ms and 1 ms treatment at pH_{45} . Ultimate pH values were within the desired range, and there were no differences ($p > 0.05$) between stimulation pulse widths for pH_u . The pH drop during stimulation (ΔpH) was less ($p < 0.05$) in the 0.1 ms pulse treatment compared to the other treatments. There were no differences in ΔpH between 0.5 ,ms 1 ms and 10 ms pulse treatments.

Table 5 Mean pH values of beef *Longissimus dorsi* exposed to electrical stimulation using variable pulse widths at various times post mortem

pH	pulse widths (ms)				SEM
	0.1	0.5	1	10	
pH ₃₀	6.50 ^a	6.54 ^a	6.51 ^a	6.49 ^a	0.009
pH ₄₅	6.18 ^a	6.10 ^b	6.09 ^b	6.04 ^c	0.006
pH _{2.5}	5.97 ^a	5.89 ^{ab}	5.91 ^{ab}	5.83 ^b	0.013
pH _{3.5}	5.85 ^a	5.78 ^b	5.75 ^{bc}	5.72 ^c	0.01
pH _u	5.47 ^a	5.48 ^a	5.48 ^a	5.44 ^a	0.005
ΔpH	0.33 ^b	0.44 ^a	0.43 ^a	0.46 ^a	0.01

^{a,b} Values with a different subscript within a row differ significantly ($p < 0.05$)

*ΔpH taken before stimulation at 30 mins and after stimulation at 45 mins

5.3.2 Cooking loss

Mean cooking loss-percentage for the LD is depicted in Figure 2. There were no difference between the day of kill ($p = 0.153$) and the stimulation pulse width ($p = 0.255$) on the cooking loss percentage.

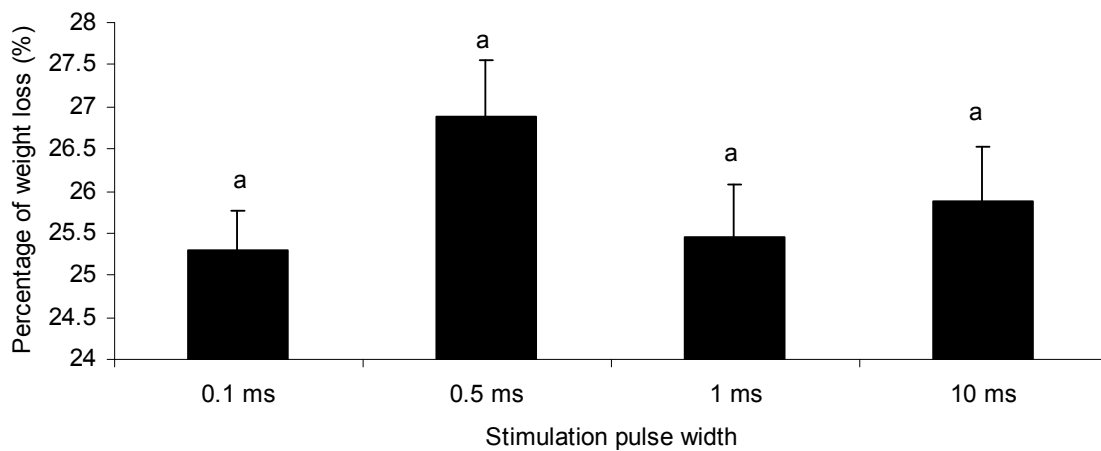


Figure 34 Cooking loss % of *Longissimus dorsi* muscle of cattle electrical stimulation using different pulses (0.1, 0.5, 1 and 10 ms)

5.3.3 Shear force

A mean shear-force value for the two days of kill is depicted in Figure 3. There were no difference between the day of kill ($p = 0.834$), however, stimulation pulse width ($p = 0.045$) had an effect on the shear-force values of the LD. Stimulation with a 1 ms pulse width had the lowest mean shear-force value which differed ($p < 0.05$) from that stimulated with 0.1 ms and 10 ms. Pulse widths 0.1 ms , 0.5 ms and 10 ms had the highest mean shear-force values that did not differ ($p > 0.05$) from each other.

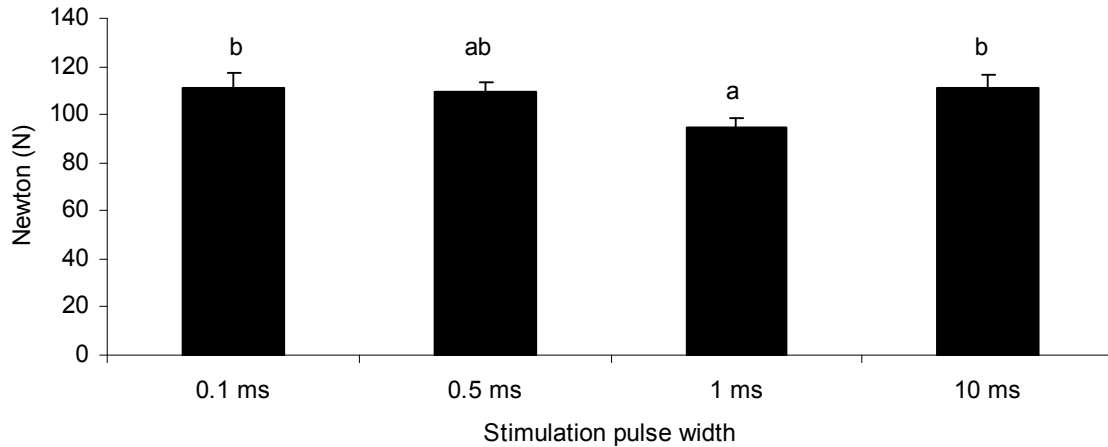


Figure 35 Shear-force values of the *Longissimus dorsi* muscle of cattle electrical stimulation using different pulses widths (0.1 ms, 0.5 ms, 1 ms and 10 ms)

5.3.4 WBC

The mean values for percentage of expressible water, or WBC, are depicted in Figure 4. There were no difference between the different days of kill ($p = 0.203$) but there was an interaction between stimulation pulse width and muscle ($p = 0.003$). Stimulation pulse width had no effect ($p > 0.05$) on the amount of expressible water of the LD. However, stimulation with a 10 ms pulse width in the SM led to less ($p < 0.05$) expressible water than the stimulation with 0.1 ms pulse width. There were no differences ($p > 0.05$) between the other treatments. There was a difference ($p < 0.05$) between the LD and SM, with the latter muscle having the higher level of expressible water, irrespective of the pulse width used during the ES.

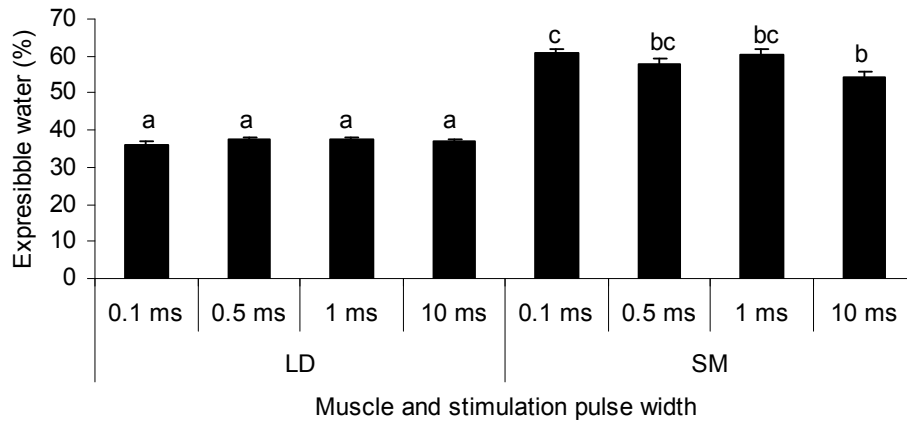


Figure 36 Illustrates the interaction between muscle and stimulation pulse width on the amount of expressible water of the *Longissimus dorsi* and *Semimembranosus*

5.3.5 Retail Colour Display

The mean L^* values during retail display of the SM over an 11-day period is depicted in Figure 6. There were no interactions (0.984) between the day of retail display and stimulation pulse width; however, stimulation pulse width ($p = 0.0001$) and retail day ($p = 0.0001$) had an effect on the L^* value of the SM. Stimulation with a 0.1 ms (45.60 ± 0.14) pulse width had a lower ($p < 0.05$) mean L^* value compared to the other treatments; 0.5 ms (47.12 ± 0.15) was higher ($p < 0.05$) than 10 ms (46.23 ± 0.13) but not 1 ms (46.76 ± 0.18). There was no difference ($p > 0.05$) between 1 ms and 10 ms.

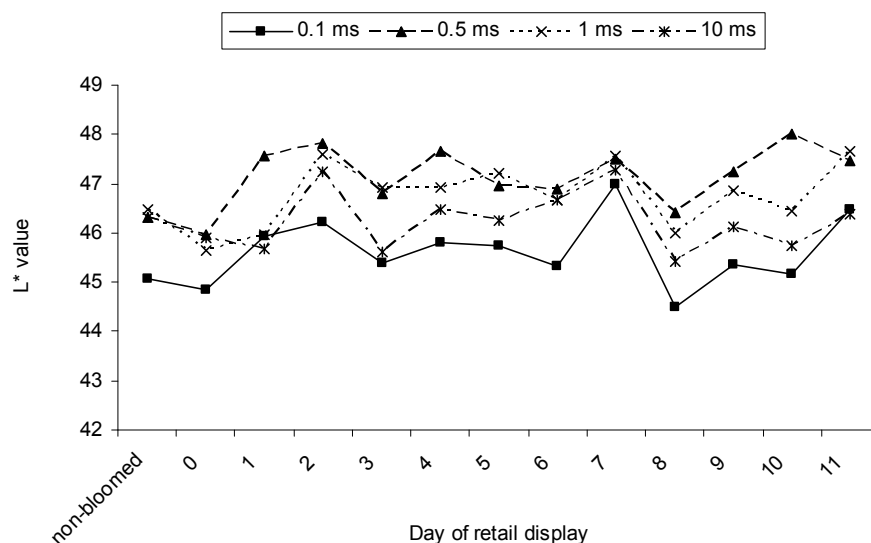


Figure 37 The change in mean values of L^* (lightness) of the *Semimembranosus* muscle of cattle subjected to varying pulse widths during electrical stimulation whilst under retail display

The mean a^* values for retail display of the SM over an 11-day period is shown in Table 2. There was an interaction ($p = 0.0001$) between day of retail display and stimulation pulse width on the a^* values of the SM during the mock retail display. For all the treatments, there was a distinct pattern. The redness of the meat increased from non-bloomed state to the bloomed state at day 1. Then there were a gradual decrease in a^* values with an increase in retail display time up to day 8, when the colour decreased more rapidly.

A more detailed analyses of the daily changes indicated no significant differences for non-bloomed meat colour between the different treatments. The bloomed colour for the SM on day 0, stimulated with a pulse width of 0.1 ms had a significantly higher a^* value compared to stimulation with a pulse width of 0.5 ms. However, there were no differences between the 0.1 ms stimulation and the other treatments. During retail display from days 1 to 7, there were no significant differences in the a^* values between the different treatments. At day 8, stimulation with pulse widths of 0.1 ms or 0.5 ms had significantly higher a^* values than stimulation with a 1 ms pulse width. Values of a^* for a pulse width of 10 ms was intermediate and did not differ significantly to any of the treatments.

At day 9, stimulation with pulse widths of 0.1 ms and 0.5 ms resulted in the SM having significantly higher a^* values compared to the treatments with pulse widths of 1 ms and 10 ms. However, on day 10 of retail display, the 0.5 ms and 10 ms treatments had significantly higher a^* values compared to 1 ms, whilst a pulse width of 0.1 ms did not differ significantly from any of the treatments. At day 11 of retail display, treatments of 0.5 ms and 10 ms had the highest a^* values, which were significantly higher than stimulation with a pulse width of 1 ms. Stimulation with a pulse width of 0.1 ms did not differ significantly with treatments of 0.5 ms and 1 ms; however, it was significantly lower than stimulation with a pulse width of 10 ms.

Table 6 Mean values of a^* (redness) of the *Semimembranosus* muscle of cattle subjected to different pulse widths during electrical stimulation over 11 days of retail display

Day of retail display (d)	Pulse widths (ms)				SEM
	0.1	0.5	1	10	
Non-bloomed	19.18 ^a	18.57 ^a	19.47 ^a	18.28 ^a	0.209
0	29.40 ^a	26.55 ^b	28.23 ^{ab}	26.86 ^{ab}	0.301
1	27.84 ^a	27.15 ^a	27.55 ^a	28.30 ^a	0.314
2	24.73 ^a	25.38 ^a	24.13 ^a	24.28 ^a	0.249
3	22.45 ^a	22.93 ^a	21.85 ^a	22.56 ^a	0.193
4	22.23 ^a	22.43 ^a	21.67 ^a	22.27 ^a	0.214

Table 7 (continue) Mean values of a* (redness) of the *Semimembranosus* muscle of cattle subjected to different pulse widths during electrical stimulation over 11 days of retail display

5	20.41 ^a	21.15 ^a	19.70 ^a	20.51 ^a	0.182
6	20.14 ^a	20.63 ^a	19.26 ^a	19.60 ^a	0.202
7	19.38 ^a	20.77 ^a	19.11 ^a	19.56 ^a	0.223
8	20.50 ^a	21.00 ^a	19.06 ^b	19.87 ^{ab}	0.184
9	19.38 ^a	20.89 ^a	16.66 ^b	19.69 ^a	0.283
10	17.54 ^{ab}	18.96 ^a	15.86 ^b	19.68 ^a	0.347
11	13.68 ^{ab}	15.32 ^{ac}	12.20 ^b	15.86 ^c	0.328

^{a,b} Values with a different subscript within a row differ significantly ($p < 0.05$)

The mean b* values during retail display of the SM over an 11-day period are depicted in Figure 7. There were no interactions ($p = 0.549$) between the day of retail display and stimulation pulse width. The stimulation pulse width caused no differences ($p = 0.114$) on the b* values during retail display. However, there was a difference ($p = 0.0001$) in b* values between day of retail display, with an increase ($p < 0.05$) in the b* value from the non-bloomed to the bloomed state. Thereafter, there was a gradual decrease in b* values as the days of retail display progressed.

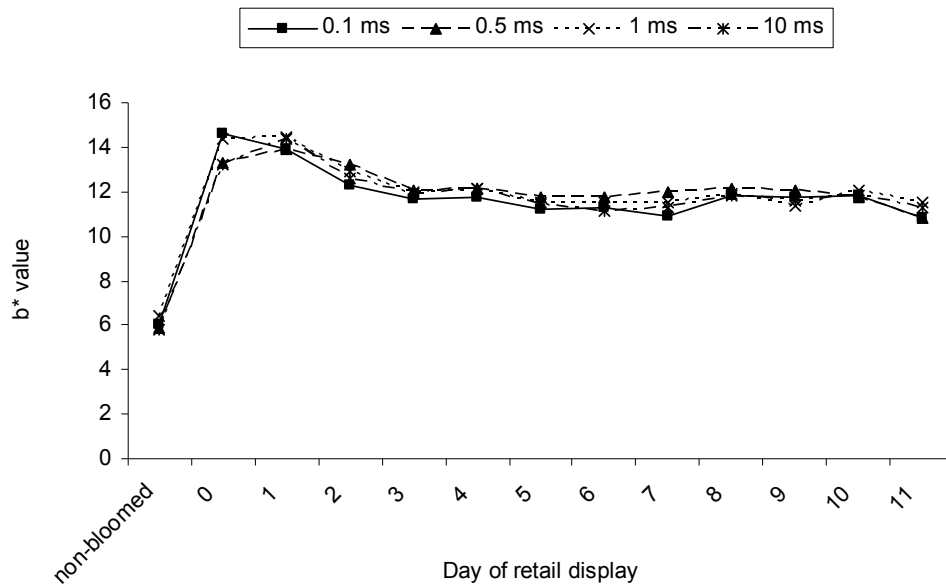


Figure 38 The change in mean values of b* (yellow-blue spectrum) of the *Semimembranosus* muscle of cattle subjected to varying pulse widths during electrical stimulation whilst under retail display

The mean hue angle values during retail display of the SM over an 11-day period are shown in Table 3. Hue angle, defined as $\tan^{-1}(b^*/a^*)$, describes the fundamental colour of a substance. There was an interaction ($p = 0.0001$) between day of retail display and stimulation pulse width on the hue angle of the SM. The hue angle increased rapidly (within 3 hours) from a non-bloomed colour to a bloomed colour. From day 8, there was an increase in hue angle. There were no differences ($p > 0.05$) between treatments for the non-bloomed and bloomed colour for days 0 and 1. Display from days 2 to 7, with the exception of day 6, had a distinct pattern, with stimulation with a 1 ms pulse width having a higher ($p < 0.05$) hue angle compared with a pulse width of 0.1 ms. Stimulation with pulse widths of 0.5 ms and 10 ms did not differ ($p > 0.05$) from the other treatments.

At day 8 of retail display, stimulation with a pulse width of 1 ms had a higher ($p < 0.05$) hue angle in comparison to stimulation with treatments 0.1 ms and 0.5 ms, whilst on day 9 of retail display, the hue angle was higher ($p < 0.05$) at this pulse width than with all the other treatments. At days 10 and 11 of retail display, the treatment of 1 ms pulse width still had higher ($p < 0.05$) hue values compared to those with treatments of 0.5 ms and 10 ms pulse widths, but there was no difference with the 0.1 ms pulse width treatment.

Table 8 Mean hue angle (fundamental colour) of the *Semimembranosus* muscle of cattle subjected to different pulse widths during electrical stimulation over 11 days of retail display

Day of retail display (d)	Pulse width (ms)				SEM
	0.1	0.5	1	10	
Non-bloomed	17.33 ^a	17.33 ^a	18.25 ^a	17.36 ^a	0.197
0	26.33 ^a	26.46 ^a	26.90 ^a	25.98 ^a	0.155
1	26.53 ^a	26.99 ^a	27.60 ^a	26.84 ^a	0.161
2	26.47 ^a	27.39 ^{ab}	28.08 ^b	27.24 ^{ab}	0.155
3	27.43 ^a	27.72 ^{ab}	28.645 ^{ab}	27.80 ^{ab}	0.144
4	27.85 ^a	28.39 ^{ab}	29.27 ^b	28.49 ^{ab}	0.148
5	28.81 ^a	28.96 ^{ab}	30.27 ^b	29.13 ^{ab}	0.164
6	29.30 ^a	29.52 ^a	30.77 ^a	29.53 ^a	0.191
7	29.41 ^a	29.91 ^{ab}	31.05 ^b	29.90 ^{ab}	0.174

Table 9 (continue) Mean hue angle (fundamental colour) of the Semimembranosus muscle of cattle subjected to different pulse widths during electrical stimulation over 11 days of retail display

8	29.89 ^a	30.04 ^a	31.99 ^b	30.63 ^{ab}	0.211
9	31.42 ^a	30.05 ^a	34.32 ^b	30.34 ^a	0.356
10	34.48 ^{ab}	32.01 ^b	37.50 ^a	30.92 ^c	0.646
11	38.76 ^{ab}	35.76 ^a	43.46 ^b	35.60 ^a	0.674

^{a,b} Values with a different subscript within a row differ significantly ($p < 0.05$)

The mean chroma values during retail display of the SM are shown in Table 4. Chroma, the square root of $a^{*2} + b^{*2}$, is an indication of the vividness of the meat colour. There was an interaction ($p = 0.0001$) between day of retail display and stimulation pulse width on the chroma values of the SM. Chroma increased ($p < 0.05$) from non-bloomed to bloomed colour 3 hours later at day 0. Chroma values decreased at a steady rate during retail display. There were no differences ($p > 0.05$) between treatments for chroma values in the non-bloomed meat samples, and for retail display from day 1 to day 7. However, for bloomed colour at day 0, stimulation with a pulse width of 0.1 ms had a higher ($p < 0.05$) chroma value compared to treatments with pulse widths of 0.5 ms and 10 ms. From day 3 onwards, an ES with an 1 ms pulse width consistently gave the lowest chroma values – these lower values were significantly lower from days 8 to 11 of retail display compared to 10 ms, while on day 9, it was lower ($p < 0.05$) than in all the other treatments.

Table 10 Mean chroma values (vividness) of the *Semimembranosus* muscle of cattle subjected to different pulse widths during electrical stimulation over 11 days of retail display

Day of retail display (d)	Pulse width (ms)				SEM
	0.1	0.5	1	10	
Non-bloomed	20.11 ^a	19.49 ^a	20.50 ^a	19.17 ^a	0.23
day 0	32.82 ^a	29.70 ^b	31.67 ^{ab}	29.94 ^b	0.361
day 1	31.13 ^a	30.50 ^a	31.10 ^a	31.77 ^a	0.373
day 2	27.64 ^a	28.61 ^a	27.36 ^a	27.35 ^a	0.295
day 3	25.29 ^a	25.93 ^a	24.91 ^a	25.53 ^a	0.23
day 4	25.16 ^a	25.52 ^a	24.85 ^a	25.35 ^a	0.255

Table 11 (continue) Mean chroma values (vividness) of the Semimembranosus muscle of cattle subjected to different pulse widths during electrical stimulation over 11 days of retail display

day 5	23.30 ^a	24.19 ^a	22.82 ^a	23.50 ^a	0.218
day 6	23.09 ^a	23.73 ^a	22.42 ^a	22.56 ^a	0.242
day 7	22.25 ^a	23.97 ^a	22.30 ^a	22.60 ^a	0.262
day 8	23.65 ^{ab}	24.28 ^a	22.48 ^b	23.12 ^{ab}	0.212
day 9	22.70 ^a	24.15 ^a	20.19 ^b	22.89 ^a	0.304
day 10	21.21 ^{ab}	22.34 ^{ab}	19.99 ^a	22.98 ^b	0.295
day 11	17.52 ^a	18.86 ^{ab}	16.83 ^a	19.52 ^b	0.346

^{a,b} Values with a different subscript within a row differ significantly ($p < 0.05$)

5.4 Discussion

5.4.1 pH

The pH₄₅ was at its the lowest in the 10 ms treatment; however, Δ pH were unaffected by pulse widths of 0.5, 1 and 10 ms. When the pulse width reach 0.1 ms, however, there was a significant difference. According to Aston (1991), there is a distinct interaction between pulse width and amplitude. If the pulse amplitude is held constant while the pulse width is reduced, the response will weaken and eventually disappear altogether (Aston, 1991). If direct stimulation of the muscle takes place, as in this case, there is a more defined force increase in response to increased stimulus strength. The wide distribution of the voltage threshold reflects the range of fibre diameters, the main determinant of electrically-induced depolarisation thresholds for both nerve and muscle tissue (Daly, 2005). Crystall & Devine (1978) reported that Δ pH is dependent on a few factors; post-mortem delay before stimulation can have a huge effect; muscle temperature can fall, greatly reducing the drop in pH (Δ pH); glycolysis may progress to such a level that the pH falls, reducing the Δ pH that can be achieved; and the nervous system can decay and become unresponsive, so that its stimulation cannot elicit any muscle response. It has been shown that the Δ pH produced by any given stimulation decreases as the pre-stimulation pH decreases (Chrystal & Devine, 1985; Hwang *et al.*, 2003)

5.4.2 Shear-force values

The shear-force values for stimulation with a pulse width of 1 ms were the lowest; however, these did not differ significantly with a pulse width of 0.5 ms. Stimulation with 0.1 ms and 10 ms pulse widths had the highest shear-force values.

There are three possible mechanisms that may alter the rate of tenderisation: reduced cold-induced shortening, enhancement of the rate of proteolysis and alteration of protein structure (Hwang *et al.*, 2003). In

this trial, no cold-induced shortening took place, as the pH was below 6 when carcass temperatures of 10 °C were reached. The theory that stimulation causes tenderisation by means of structural changes has been widely debated, and, is considered to be inapplicable in this experiment, since structural damage was not determined. The third theory on the effects of electrical stimulation on tenderness is based on accelerated pH decline (Chrystall & Hagyard, 1975; Chrystall & Devine, 1985). This mechanism is based on the principle that the tenderisation process begins at or near rigor mortis, when the ultimate pH is attained. Since electrical stimulation accelerates the onset of rigor mortis, tenderisation begins sooner, and, more importantly, this earlier onset of rigor mortis is associated with a higher carcass temperature so the rate of tenderisation is faster than would be the case if rigor mortis is attained later and the carcass temperature is lower. Of course, the carcass temperature would eventually decrease to normal chill temperatures, but, by then, the stimulated carcass will have had a head start. The effects of rigor temperature on the shear-force value was first thought to be optimum at 15 °C due to the minimal shortening that occurs at this temperature. However, Devine *et al.* (1999) showed that when shortening is prevented in beef by tight wrapping, mimicking skeletal restraint, accompanied by an increase in temperature from 15 °C to 35 °C at the attainment of rigor, shear force was greater at high rigor mortis temperatures. These differences continued with ageing. Hwang & Thompson (2001) measured calpain activity for muscle entering rigor at different temperatures. Their results indicate that μ -calpain activation occurs earlier in muscle entering rigor at higher temperatures (38 °C). However, with slow rigor (15 °C) muscles had higher μ -calpain levels at 24h hours post-mortem. At a constant rigor temperature of 35 °C almost 80% of the μ -calpain activity was lost during rigor development, while only about 20% of activity was lost during rigor development when exposed to a constant temperature of 15 °C (Wahlgren *et al.*, 1997). When a muscle enters rigor at a higher temperature, the proteolytic enzymes are stimulated (Dutson *et al.*, 1977); however, at high temperatures and low pH, the calpain activity and stability are compromised (Simmons *et al.*, 1996). Simmons *et al.* (1996) measured calpain activity throughout the rigor process, and they showed that calpain activity remained constant at all temperatures until a pH of approximately 6.2 is reached; then the activity decreased. Conditions of low pH and high temperatures are known to denature the contractile proteins, which are more stable at rigor mortis (Offer, 1991). Such conditions, in conjunction with greater autolysis of calpains at high temperatures (Dransfield *et al.*, 1992) would explain how ageing enzymes are reduced in effectiveness so that both shear force increases and the ageing potential is reduced when rigor is attained at a high temperature. If the tenderising mechanism of electrical stimulation is due to the pH temperature interaction, it would be expected that the treatment with the most rapid muscle pH decline would result in the samples with the lowest shear-force values and the treatment with the slowest pH decline would lead to the highest shear force. Stimulation with a pulse width of 10 ms led to the most rapid decline; however, it did not significantly cause the lowest shear-force values, whereas stimulation with 0.1 ms had the highest, as expected. Rigor shortening could have occurred when a 10 ms pulse width was used or, due to the high temperature at low pH the autolysis of the calpains (Dransfield *et al.*, 1992), could have lowered the capacity of the meat samples to tenderise.

5.4.3 WBC

WBC contributes to the tenderness, colour, flavour and overall quality of meat. Although it influences the juiciness of meat, its technological and economic significance are more important. Loss of fluid during storage and processing is a concern to meat producers, processors and consumers. Therefore, accurate

prediction of the potential water-retention ability through the measurement of WBC is important. Most of the water (95%) in lean muscle is immobilised within the structure by surface tension. This water is generally trapped within the muscles by capillary suction, generated by the small pores in between the thin and thick filaments (Trout, 1988). Therefore, the ability of post-mortem muscle to hold its moisture is very important to meat processors and the consumers.

There were no significant differences between the stimulation pulse widths on the WBC of the LD. This could be explained by the cooling rate of the LD, due to its location, being insufficient to bring about any differences between treatments. The SM, which has a slower temperature decline due to its deeper lying location, showed that stimulation with a pulse width of 10 ms had a significant lower amount of expressible water (higher WBC) compared to the other treatments. This is contradictory to what was expected. The pH_u did not differ; thus, it could not have had an effect on the WBC. Conditions of low pH and high temperature are known to denature the contractile proteins, which are more stable at rigor mortis (Offer, 1991). This would lead to higher purge losses and thus a lower WBC. Hence, it was expected that this stimulation pulse width would have the lowest WBC since it had the most rapid pH decline. A reason for this discrepancy could be that, due to the slow cooling rate of the SM, temperature overshadows the effects of ES on WBC of the SM. The SM is also known to be an unstable muscle and, although the samples were taken from around the same region in the muscle, significant temperature gradients within this large muscle could make accurate selection of the muscle region difficult.

5.4.4 Colour stability

To the naked eye, blooming is perceived as an increase in bright redness (Ledward, 1992). This represents the oxygenation of myoglobin and is influenced by the oxygen-consumption rate of the meat: higher oxygen consumption limits oxygen penetration into the meat and results in less redness (darker meat). The oxygen-consuming machinery of the mitochondria is affected by pH/temperature conditions during processing, as well as by the time elapsed during ageing (Young *et al.*, 1999). The L^* values did not change during blooming. Certainly, a^* values increase during blooming, but so do b^* values. Therefore, blooming is probably well represented by chroma and L^* , the lightness. The bloomed chroma values were the highest in the 0.1 ms treatment and lowest in 0.5 ms and 10 ms treatments due to the redness of the meat being higher in 0.1 ms. Although pH decline was the fastest with a 10 ms pulse width, it had the lowest bloomed chroma after 3 hours of blooming, which is unexpected. However, at day 1 of retail display, it had the highest chroma value, although not significantly. Due to the slow cooling rate of the SM, bloomed colour might be a temperature effect (Ledward, 1985), rather than a muscle stimulation effect as such.

Colour stability, when defined as the rate of metmyoglobin accumulation on the surface layer, is generally reduced by electrical stimulation (Wiklund *et al.*, 2001; Moore & Young, 1991). The redness (a^* value) of the meat increased rapidly until fully bloomed at day 1 of retail display, but declined steadily as days of retail display increased. There are several factors that contributed to this phenomenon. Oxygen-consumption rates decrease with increasing days of retail display for all beef muscles (McKenna *et al.*, 2005). Another factor that has an influence is the amount of electrical stimulation applied. Meat tends to be redder after high- (Eikelenboom *et al.*, 1985) and low voltage (Unrah *et al.*, 1986; Sleper *et al.*, 1983; Eikelenboom *et al.*, 1985) electrical stimulation compared to non-stimulated carcass muscles. This effect is due to damage to the enzyme systems of the mitochondria responsible for oxygen consumption, reduced oxygen consumption

rates and thus higher concentrations of oxymyoglobin in the surface meat layer (Ledward, 1992). This observation is confirmed by Lawrie (1998) who also claims that the brighter red colour can be attributed to a fast pH decline. This results in the carcasses reaching their iso-electric point much sooner, thereby “opening up” the structure and easing the oxygenation of myoglobin. A more open muscle structure and the enzymes important to muscle colour are degraded because of the temperature and pH conditions that result due to ES, and may account for the increased rate of colour deterioration during display (Sleper *et al.*, 1983). The result by Wiklund *et al.* (2001) suggests that electrical stimulation accelerates the loss of metmyoglobin reductase activity so that, after a week, metmyoglobin accumulation is accelerated. This action equilibrates after 3 weeks with non-stimulated meat and the effect is then lost. Unrah *et al.* (1986) found that at day 1 steaks were brighter red, but at day 5 the control (non-stimulated) was redder. Van Laack & Smulders (1990), however, found the opposite – that electrical stimulation increased the display life. They postulated that the more open structure of the muscle may allow more oxygenation and thus increased redness. Similarly, when stimulation is omitted, the tighter structure would limit oxygen diffusion and thus reduce the layer of the red oxymyoglobin. Consequently, the metmyoglobin layer beneath the oxymyoglobin will be nearer to the surface.

The literature gives conflicting results on the effects of ES on colour stability. Colour stability in electrical stimulated meat is dependent on two factors: the depth of the oxymyoglobin layer on the surface and the stability of the metmyoglobin reducing enzymes. With an increase in stimulation, a more open structure leads to a deeper oxymyoglobin layer; however, at the same time, the temperature and pH interaction leads to less stable metmyoglobin reductase. There are thus two conflicting mechanisms at work, which might explain the conflicting results of the literature. It is important to optimise the two conflicting mechanisms affecting colour stability to improve the retail display.

Hue angle is a better measure of browning than chroma. Ledward (1985) found that the temperature at which a muscle went into rigor strongly affected metmyoglobin formation on display. The results from this investigation indicate that from day 8 of retail display, there are significant differences and that stimulation with a pulse width of 1 ms had the lowest a^* value (redness) and chroma values (vividness) and highest hue angle (fundamental colour), thus having a browner appearance. Stimulation with 0.1 ms and 0.5 ms pulse widths had the highest a^* (redness) and chroma (vividness) values and lowest hue angle (fundamental colour) at day 9, but these declined rapidly during the next 2 days. It could be postulated that these treatments had more stable metmyoglobin reductase activity, which kept the formation of metmyoglobin to a minimum up until day 9, and, for the last two days, the activity decreased rapidly. For the stimulation with either 1 ms or 10 ms pulse width, the faster pH decline led to less stable metmyoglobin reducing enzymes; however, the layer of oxymyoglobin formed with a 1 ms pulse width could have been less in comparison to the 10 ms pulse width, resulting in the most rapid loss in a^* values during the retail display period. The fine balance between too little or too much stimulation is evident here.

5.5 Conclusion

The results show that decreasing the pulse width from 10 ms to 0.5 ms had no effect on ΔpH in the LD. There were also no detrimental effects on shear force or WBC in the LD for a pulse width of 0.1–1 ms compared to 10 ms. Thus, for this experiment, it was evident that the treatments induced a similar amount of

contraction for the LD. It was only with the 0.1 ms pulse width that there were reductions in the pH decline. This shows that the LD becomes less responsive only when stimulated with very small pulse widths.

Current is known to follow the shortest route with the least resistance. It could be postulated that sufficient current can flow through the LD with decreasing pulse widths, with the cut-off being between 0.5 ms and 0.1 ms, depending on the fatigue state of the muscle. In the SM, due to the less direct route of stimulation, the resistance might be higher, which would elicit a weaker response of muscle contraction. This could have beneficial effects on the meat quality of the SM. The slower cooling curve and lower stimulation inputs will lead to a less rapid pH decline. Thus, conditions known to denature muscle structural proteins can be eliminated. However, the SM is a very unstable muscle, and it is suggested that the same electrical parameters should be tested on other muscles with different cooling curves.

The use of smaller pulse widths can be beneficial when used in combination with high- or medium-voltage electrical stimulation, not only for their effects on the meat quality but also because of the opportunity to provide a safer working environment for operators. By decreasing the pulse width, although at a high peak voltage, the root mean square (RMS) of the voltage is decreased, allowing it to be classified in the same safety category as low voltage stimulation. This has the benefit that the stimulation unit need less protective barriers and that operators can safely work close to the unit.

5.6 References

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Chapter 6

Conclusion

Electrical stimulation is widely used in Australasian meat plants to maintain high throughputs and to induce the rapid tenderisation of their meat products. However, in South Africa, this application is scarcely used in plants, and when it is used, the principle is usually misunderstood. Since the deregulation of the South African red meat industry in 1993, a rapid growth in the number of registered abattoirs was experienced, namely from 330 in 1993 to 504 in 2001 (Anon, 2008). About 30% of these abattoirs make use of electrical stimulation in their conversion of muscle to meat; therefore, this study has great commercial application in terms of the different parameters of electrical stimulation and the implication of each of these on the meat quality of beef. Although this study was done on beef, the principles can also be implemented on the stimulation of lamb and mutton carcasses.

The variability in tenderness of these carcasses has been of great concern for producers, since consumers consistently rank tenderness high among the quality attributes that define an enjoyable eating experience. Since not all beef is created equally tender, meat scientists have sought ways to remedy this inconsistency. For centuries, the ageing of beef and other meats has been applied to improve tenderness. These days, carcasses are held in a chiller for a period of time (usually between 14 and 21 days) while natural enzyme activity enhances tenderness. However, aging beef properly has economic consequences. Many processors complain that holding beef in refrigeration facilities with controlled humidity and airflow for long periods of time is too costly. In addition, longer aging periods may result in increased product shrinkage and weight loss. Electrical stimulation reduces not only the variation between carcasses, but also ensures rapid tenderisation so that final meat tenderness is reached at an earlier period in the cold storage process. Processors can deliver tender meat within a very short ageing period to ensure that the consumer has an enjoyable eating experience. Shrinkage of the carcass is also minimised ensuring that the processor also benefits economically from electrical stimulation. However, caution should be applied when making use of this process because the application of electrical stimulation could have adverse effects on meat quality if applied incorrectly. This is a big problem in South Africa where the processors lack the knowledge and expertise necessary to ensure consistent improvement in meat quality. This thesis provides information on how electrical inputs interact and how electrical parameters can be used to influence pH decline in carcasses and thus optimise a processing plant's production.

pH-temperature interaction gives rise to the possibility of producing meat products for diverse markets, whether frozen, chilled local or export markets. The pre-rigor temperature-pH environment determines the quality attributes of tenderness, colour and water-binding capacity in meat products. Between the extremes lies a range of processing options that can produce a spectrum of distinctive quality attributes which can, with regard to the cooling rate, be obtained by the manipulation of the pH decline through electrical stimulation. In the studies done for this thesis, it was evident that HFI is an effective method to control post-stun convulsions and to manage the pH decline by subsequent HVS or MVS after dressing. HFI suppresses post-stun convulsion by causing the depolarisation of the nerves and not by means of muscle fatigue (rapid

decline in pH), as in the case of LFI. It is evident that keeping the pH high during the dressing of the carcass improves meat quality and offers the opportunity to optimise processing specifications to produce meat for diverse markets. In addition, it indicates that excessive electrical stimulation, for example using the standard LFI followed by HVS as is used in some New Zealand plants, can reduce meat quality by reducing tenderness and increasing purge losses.

The effects of electrical stimulation and immobilisation on meat colour and retail display life are complex, with complicated mechanisms at work. Electrical stimulation can have adverse effects on the display life (Moore & Young, 1991) although this might be a temperature effect (Ledward, 1985) rather than a muscle-stimulation effect as such. It is evident that stimulation increases the red bloom colour of meat after cold storage for various days. However, colour stability is affected adversely with an increase in the rate of pH decline. LFI HVS has a deeper red colour in the initial display life of meat but tends to discolour quicker as retail days progress. Thus, increasing storage time, which is beneficial for tenderness, also has the effect of reducing retail display life. ES would be useful when there is a limited cold storage time, and where extended periods of storage are used, it would be advisable not to stimulate, thus increasing the stability of the meat colour. The results also indicated that a MTT assay is a good method for predicting oxygen-consumption rates and the stability of the reductase enzyme is useful for predicting subsequent retail display life.

There are many different ES-stimulation systems available commercially, each with different electrical parameters. Each of these parameters has an effect on the way muscles contract and hence on their pH decline. The data from these trials confirmed that frequency and pulse width have significant effects on pH decline, with an optimum pH drop from stimulation obtained with 15 Hz and a 10 ms pulse width. By changing the frequency or pulse width of the stimulation unit, the rate of pH decline can be altered, with the various effects on meat quality.

The use of smaller pulse widths can be beneficial when used in combination with HVS and MVS, not only on the meat quality but also by creating a safer working environment for operators. Decreasing the pulse width, even at a high peak voltage, decreases the RMS of the voltage allowing it to be classified in the same safety category as low voltage stimulation. This has the benefit that the stimulation unit needs less protective barriers and that operators can safely work within close range of the unit.

It can be concluded that HFI improves meat quality when combined with either HVS or MVS and that MVS either improves (colour stability) or has no adverse effects on meat quality (tenderness and WBC) in relation to HVS when combined with HFI. In addition, it is clear that there are alternative electrical parameters to voltage that can be used to change the pH decline, and, by changing frequency and pulse width, subtle changes can be made to an electrical-stimulation system. Since every abattoir is different due to factors such as layout, chiller space and cooling regime, these electrical parameters can be modulated to optimise an electrical-stimulation system without expensive modification to the whole system. The previous chapters can be used as a guideline on how to optimise the electrical inputs applied within a specific abattoir.

The packager, retailer and consumer could therefore benefit if electrical stimulation is used as an integral part of the process of converting muscle into meat. Electrical stimulation is an important application in modern abattoirs, and, with correct application and the use of rapid chilling, it is beneficial to both the supplier and consumer.

6.1 References

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