Beef tenderness evaluation using early post-mortem muscle nanostructure

Zimkhitha Soji^{1,*} and Amanda Y. Chulayo²

* Corresponding Author: Zimkhitha Soji
Tel: +27-406022123, Fax: +27-866282967,
E-mail: zsoji@ufh.ac.za

¹ Department of Livestock and Pasture
Science, University of Fort Hare, Alice 5700,
South Africa

² Dohne ADI, Private Bag X15, Stutterheim
4930, South Africa

ORCID

Zimkhitha Soji
<https://orcid.org/0000-0001-8510-0366>
Amanda Y. Chulayo
<https://orcid.org/0000-0003-0505-9012>

Submitted Mar 9, 2020; Revised Jun 8, 2020;
Accepted Jul 7, 2020

Objective: Tenderness is believed to start immediately after slaughter, but there is little work that directly links tenderness to the muscle nanostructure early post-mortem. This study attempted early diagnosis of meat tenderness using muscle nanostructure at approximately 45 minutes and 24 hours post-slaughter.

Methods: Carcass intrinsic factors (carcass mass, muscle pH, and temperature) were measured at 45 minutes and 24 hours post-slaughter on 52 A2-class beef carcasses from Bonsmara, Beefmaster, Hereford and Simbra steers. The muscle nanostructure (myofibril diameter [MYD], myofibril spacing [MYS], muscle fibre diameter [MFD], muscle fibre spacing [MFS], and sarcomere length [SL]) was also analysed at 45_{mins} and 24_{hrs} post-slaughter on 20 representative *longissimus* samples using a scanning electron microscope, while tenderness was measured using Warner Bratzler shear force (WBSF).

Results: At 45 mins post-slaughter breed affected MYD and MYS while it also affected MFD and MFS at 24 hours. While pH₂₄ affected MYD and MYS; muscle Temp_{45mins} affected MYD, MFD, and MFS; and Temp_{24hrs} affected MYD; WBSF and SL were not affected by these factors. WBSF negatively correlated with pH₀, MFD, and SL at 45 mins post-slaughter, while it positively correlated with MYD, MYS, MFS, warm carcass mass, and Temp_{45mins}. At 24 hours post-slaughter, WBSF had linear negative correlations with MYS, MFS, MFD, and SL, with WBSF decreasing as these muscle nanostructure components increased, thus enhancing tenderness. Meat tenderness was further enhanced by the muscle fibre bundle characteristics which include longer, visible, and finer muscle grain.

Conclusion: Muscle tenderness is enhanced by muscle fibre bundle characteristics at approximately 24 hours post-slaughter. However, the myofibrillar structure at 45 mins post-slaughter can be a good predictor of the required ageing period for individual breeds to further enhance tenderness.

Keywords: Myofibril Spacing; Myofibril Diameter; Muscle Fibre Spacing; Muscle Fibre Diameter; Sarcomere Length

INTRODUCTION

The intrinsic structure of the muscle including sarcomere length (SL), myofilament diameter and fibre types are important beef meat characteristics [1]. Beef tenderness can be partly explained by understanding these muscle nanostructure components. For instance the genetic correlation of SL and tenderness suggest the potential use of tenderness influencing factors as indirect selection criteria to improve palatability attributes [2]. Moreover the microscopically observed differences in the muscle fibres has been anecdotally reported to be due to the finer muscle grain that some genotypes possess, thus enhancing tenderness [3,4].

Muscle structure and composition on the other hand is influenced by breed, and is a factor which must be considered during the selection of meat animals to ensure beef quality

as well as quantity [5,6]. Although the aforementioned intrinsic structure of the muscle as well as extrinsic factors (which include but not limited to breed) are known to be related to tenderness and have been extensively researched, Modika et al [7] suggested that the tenderisation process is expected to begin immediately after slaughter depending on each carcass. Nonetheless there seems to have been little to no work that directly demonstrates that the muscle nanostructural changes early post-mortem from as soon as 45 minutes post-slaughter may be linked to tenderness.

Consistency in meat tenderness and earlier classification of meat according to its quality, specifically the degree of tenderness with high precision have always been and is still the primary concern in the beef industry [8,9]. Tenderness is a very complex feature and the process of its formation is very complicated and not fully understood. Its diversification is one of the most important problems of beef production. Thus this article attempted an early diagnosis of meat tenderness based on muscle nanostructure.

MATERIALS AND METHODS

Ethical consideration

Consent to carry out the study was granted by the University of Fort Hare Research Ethics Committee (UFH/UREC) with reference number: MUC411SSOJ01.

Description of the study site

The study was conducted at a typical high throughput abattoir in East London at the Buffalo City Metropolitan Municipality of the Eastern Cape Province, Republic of South Africa. The abattoir operates under the standard commercial abattoir procedures and slaughters up to 1,000 livestock units per day. It complies with the stipulations of the Meat Safety Act [10] which created an official system of meat inspection to provide measures in promoting meat safety and the safety of animal products; as well as regulations set by Act No 119 of 1990 for classification of meat intended for sale in the republic of South Africa [11].

Study animals and slaughter procedure: Fifty two A-class steers ($n = 52$) of four breed types (Bonsmara [19], Beef master [7], Hereford [9], and Simbra [17]) typically processed in different South African feedlots were studied. The animals were humanely slaughtered following the commercial standard procedures. For the purpose of this study, all animals used were of the same age (A class), fat class (fat class 2), conformation (medium score 3), sex (steers) and had no traces of bruises. These classification characteristics were selected since they are considered as ideal classes in the South African meat market. The classification was in accordance with Act no 119 of 1990 (Agricultural Product Standards Act).

Meat sample collection and quality measurements

After slaughter, warm carcass mass (WCM) and cold carcass mass (CCM) were recorded and then carcasses were dressed and split into half at the slaughter line. Immediately after dressing, a portable digital fibre-optic pH meter (Model HQ11d United States of America) was used to measure pH0 directly on carcasses at 45 minutes post-mortem on the *Longissimus thoracis et lumborum* (LTL) muscle. The pH meter gave convenient temperature compensated pH measurements, thus muscle temperature was recorded simultaneously. Thereafter, a small incision was made on the carcasses to harvest approximately a 20 g subsection of the LTL muscle on the left side of each carcass between the 10th rib and the third lumbar vertebra which was immediately immersed into a 3% formalin for muscle nanostructure analysis. Carcasses were then stored in cold rooms ($\pm 4^{\circ}\text{C}$). At approximately 24 hours post-slaughter ultimate pH (pH_{24}) was also measured directly from carcasses. The 24 hour smaller subsections of the LTL muscle (20 g) were harvested and immediately immersed into 3% formalin for further muscle nanostructure analysis.

Determination of muscle nanostructures: The samples were immediately put in small bottles containing 3% formalin for fixation after collection. They were then transported to the Botany lab (University of Fort Hare) for approximately 2 hours in cooler box filled with ice. During the analysis, the samples were dehydrated to remove formalin and kept in ethanol for 20 minutes in an ascending order of 10% up to 100%, respectively. In order to improve electrical conductivity of the sample surface in the scanning electron microscope (SEM), a thin film of gold palladium was used for sputter coating to enhance the analysis.

Critical point drying was performed using the Hitachi critical point dryer HCP-2 (Hitachi Koki Co Ltd, Tokyo, Japan) to prevent the samples from alteration and to boost good structural preservation. This was done by mounting the samples on aluminium stubs with double-sided carbon tape then the sputter coating with gold-palladium (Au-Pb) using the Eiko IB.3 Ion Coater (EIKO Engineering Co TD, Tokyo, Japan). The samples were then observed under the JEOL JSM-6390LV SEM for the determination of the skeletal surface area of beef muscles. The nanostructure of the skeletal surface area for the samples was then viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification.

Meat tenderness measurement: Warner Bratzler shear force (WBSF) measurements were done at 45 minutes and 24 hours post slaughter. The samples were labelled and placed in watertight PVC plastic bags for cooking in a water bath (Model TRH) for 45 minutes to a final internal temperature of 71°C according to standards by American Meat Science Association. After cooking the samples were cooled down at 25°C room temperature (measured using an analogue thermome-

ter) for approximately ± 20 minutes. Following cooking, from each sample three sub samples of approximately 12, 7 mm core diameter were cored parallel to the grain of the meat. The samples were sheared perpendicular to the fibre direction using a Warner Bratzler shear device mounted on an Instron 3344 Universal Testing Apparatus. Cross head speed at 400 mm/min, one shear in the centre of each core. WBSF was measured as the peak force (Newtons) average for three cores per sample.

Statistical analysis

Data was analysed using general linear model procedure (PROC GLM) of JMP 9.0 [12] to test the effect of breed, carcass mass, muscle pH and temperature on the muscle nanostructure characteristics (myofibril diameter [MYD], myofibril spacing [MYS], muscle fibre diameter [MFD], muscle fibre spacing [MFS], SL) and tenderness. Significant means ($p < 0.05$) were separated using the Duncan's multiple range test. The following model was used:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = response variable (MYD, MYS, MFD, MFS, SL, and tenderness); μ = overall mean; α_i = effect of breed; β_j = effect of carcass mass; γ_k = effect muscle pH; δ_l = effect of muscle temperature; ε_{ijkl} = random error.

Interactions between the variables were initially tested but were not significant. The strengths of relationships between muscle nanostructure properties, tenderness and carcass intrinsic factors (WCM, CCM, muscle pH, and temperature) were tested using Pearson correlation coefficient of XLSTAT version 2018.5 statistical software for excel.

RESULTS

At 45 minutes post-slaughter there were significant effects of breed ($p < 0.05$) (Table 1) on MYD and MYS, while at 24 hours in addition to MYD and MYS, breed had an effect on MFD and MFS. The MYD and MYS decreased in size at 24 hours post-mortem across all breeds. Furthermore, while the MFD and MFS also decreased in size for Beefmaster at 24 hours post-mortem, those of the Bonsmara, Hereford and Simbra increased in size. On the other hand the SL and WBSF were both not affected by breed at early post-mortem and 24 hours post-slaughter, while there was also no uniformity on the muscle nanostructure changes across all the breeds.

The pH of the muscles of the tested breeds was at a normal level, with overall pH_0 of 6.6 early post-mortem and the average pH_{24} of 5.5. The average pH values for individual breeds differed less or not at all with pH_0 values (6.63; 6.64; 6.51; 6.61) and pH_{24} (5.55; 5.51; 5.55; 5.56) for Bonsmara, Hereford, Simbra and Beefmaster, respectively. Early post-mortem pH (pH_0) had no effect on all muscle nanostructure properties (Table 2). Although ultimate pH (pH_{24}) had an effect on MYD and MYS, there was no uniformity on how they changed with either early post-mortem or ultimate pH. The WBSF and SL were also not affected by neither pH_0 nor pH_{24} .

Early post-mortem muscle temperature ($Temp_{45}$) had an effect ($p < 0.05$) on MYD, MFD, and MFS while muscle temperature at 24 hours post-mortem had an effect ($p < 0.05$) on MYD only (Table 3). Both muscle $Temp_{45}$ and $Temp_{24hrs}$ had no effect on MYS, SL, and WBSF. It was also noted that there was no uniformity on how the muscle nanostructure characteristics changed in size between 11°C to 40°C muscle temperatures. However, most nanostructure properties re-

Table 1. Changes in the muscle nanostructure properties and tenderness of each breed at 45 minutes and 24 hours post-slaughter

Muscle nanostructure properties and tenderness	Breed				
	Beef master	Bonsmara	Hereford	Simbra	SE
			45 minutes post-slaughter		
Myofibril diameter	0.66 ^a	1.33 ^c	1.08 ^{b,c}	0.86 ^{a,b}	0.100
Myofibril spacing	0.36 ^a	0.57 ^{ab}	0.46 ^a	0.79 ^b	0.092
Muscle fibre diameter	37.26	41.32	40.62	39.59	3.192
Muscle fibre spacing	8.00	10.65	7.05	8.84	1.574
Sarcomere length	1.49	1.50	1.28	1.52	0.158
WBSF (Tenderness)	25.00	26.58	24.28	25.38	1.682
			24 hours post slaughter		
Myofibril diameter	0.55 ^a	0.90 ^b	0.79 ^{a,b}	0.88 ^b	0.085
Myofibril spacing	0.22 ^a	0.47 ^b	0.42 ^{a,b}	0.41 ^{a,b}	0.077
Muscle fibre diameter	33.93 ^a	62.93 ^b	53.74 ^{a,b}	44.32 ^{a,b}	7.901
Muscle fibre spacing	5.54a	14.36 ^b	7.11 ^a	8.8 ^{a,b}	2.174a
Sarcomere length	1.05	1.23	1.10	1.07	0.101
WBSF (Tenderness)	23.38	21.47	21.90	20.12	1.328

SE, standard error; WBSF, Warner Bratzler shear force (measured in newtons); μm , micrometres (unit measurement for muscle nanostructure).

^{a-c} Means within a row are different while those with no superscripts are similar at $p < 0.05$.

Table 2. Effect of pH on the muscle nanostructure properties and tenderness at 45 minutes and 24 hours post-slaughter

Muscle nanostructure properties	Muscle pH			
	45 minutes post-slaughter		24 hours post-slaughter	
	6.5 ≥ (n = 36)	6 to 6.4 (n = 16)	5.5 to 5.9 (n = 37)	5 to 5.4 (n = 15)
Myofibril diameter	1.02±0.100	0.92±0.111	0.79 ^a ±0.057	0.99 ^b ±0.090
Myofibril spacing	0.47±0.870	0.66±0.100	0.30 ^a ±0.058	0.50 ^b ±0.094
Muscle fibre diameter	36.43±1.743	36.64±2.000	42.28±6.175	42.66±9.931
Muscle fibre spacing	8.37±1.128	8.19±1.300	7.81±1.748	12.12±2.812
Sarcomere length	1.44±1.128	1.42±0.147	1.11±0.085	1.10±0.136
WBSF (Tenderness)	25.97±1.321	25.80±1.516	22.66±1.084	22.076±1.745

WBSF, Warner Bratzler shear force (measured in newtons); µm, micrometres (unit measurement for muscle nanostructure).

^{a,b} Means within a row are different while those with no superscripts are similar at p<0.05.

Table 3. Effect of muscle temperature at 45 minutes and 24 hours post-slaughter on the muscle nanostructure properties and tenderness

Muscle nanostructure properties	Muscle temperature					
	45 minutes post-slaughter			24 hours post-slaughter		
	36°C to 40°C (n = 18)	31°C to 35°C (n = 26)	26°C to 30°C (n = 8)	16°C to 20°C (n = 10)	11°C to 15°C (n = 36)	5°C to 10°C (n = 6)
Myofibril diameter	1.28 ^b ±0.122	0.88 ^a ±0.100	0.75 ^a ±0.169	1.22 ^b ±0.125	0.81 ^a ±0.445	0.64 ^a ±0.108
Myofibril spacing	0.51±0.110	0.58±0.900	0.61±0.152	0.453±0.129	0.44±0.046	0.31±0.111
Muscle fibre diameter	39.96 ^b ±2.195	43.76 ^b ±1.804	30.39 ^a ±3.030	41.58±13.670	54.69±4.892	31.14±11.770
Muscle fibre spacing	11.06 ^b ±1.420	7.66 ^{a,b} ±1.168	6.12 ^a ±1.961	11.84±3.870	10.41±1.385	7.66±3.333
Sarcomere length	1.35±0.161	1.42±0.133	1.52±0.223	1.12±0.182	1.13±0.067	1.07±0.161
WBSF (Tenderness)	27.04±1.664	24.38±1.368	26.24±2.297	24.49±2.402	21.13±0.859	21.48±2.068

WBSF, Warner Bratzler shear force (measured in newtons); µm, micrometres (unit measurement for muscle nanostructure).

^{a,b} Means within a row are different while those with no superscripts are similar at p<0.05.

corded a decrease in size at 5°C to 10°C muscle temperatures.

The WCM ranged between 171 to 368.20 kg with an average mass of 255.75 kg while the CCM ranged between 165.90 to 357.20 kg with an average mass of 252.72 kg (Table 4). The WCM had no effect (p>0.05) on all muscle nanostructure characteristics while CCM had an effect on MFS only. Notably, there was also no uniformity on how the nanostructure characteristics changed in size prior and after chilling. However, most nanostructure properties recorded a decrease in size between 200 to 250 kg post-chilling.

Furthermore, at 45 minutes post-slaughter moderate pos-

itive correlations (p<0.05; r = 0.614) were found between muscle temperature and MYD (Table 5); while weak linear correlations were found between MFS and temperature (r = 0.484) as well as between MFS and MFD (r = 0.493). WBSF negatively correlated with pH₀, MFD, and SL, while it positively correlated with MYD, MYS, MFS, WCM, and Temp_{45mins}.

At 24 hours post-slaughter (Table 6) moderate positive correlations (p<0.05) were found between muscle temperature and MYD (r = 0.614), between MFS and MYS (r = 0.501), between MFS and MFD (r = 0.543) and between CCM and MFD (r = 0.468). WBSF had linear negative correlations

Table 4. Effect of carcass mass on the muscle nanostructure and tenderness during chilling and post-chilling

Muscle nanostructure properties	Carcass mass					
	Warm carcass mass (kg) 45 minutes before chilling			Cold carcass mass (kg) 24 hours post-chilling		
	200 to 250 (n = 12)	251 to 300 (n = 16)	≤301 (n = 24)	200 to 250 (n = 8)	251 to 300 (n = 28)	≤301 (n = 16)
Myofibril diameter	1.01±0.162	0.98±0.101	0.92±0.135	0.81±0.078	0.93±0.078	0.94±0.094
Myofibril spacing	0.48±0.146	0.56±0.091	0.66±0.123	0.36±0.080	0.52±0.800	0.32±0.097
Muscle fibre diameter	34.02±2.905	40.43±1.818	39.67±2.433	32.06±8.494	46.23±8.493	49.13±10.26
Muscle fibre spacing	7.10±1.880	9.23±1.178	8.48±1.575	6.70 ^a ±2.405	13.78 ^b ±2.400	9.43 ^{a,b} ±2.905
Sarcomere length	1.39±0.213	1.51±0.134	1.39±0.179	1.03±0.116	1.17±0.116	1.12±0.141
WBSF (Tenderness)	26.77±2.202	24.54±1.378	26.35±1.844	23.17±1.492	21.58±1.492	22.35±1.802

WBSF, Warner Bratzler shear force (measured in newtons); µm, micrometres (unit measurement for muscle nanostructure).

^{a,b} Means within a row are different while those with no superscripts are similar at p<0.05.

Table 5. Correlations among nanostructure properties, warm carcass mass, muscle pH, and temperature on the day of slaughter

Variables	pH ₀	WCM	Temp	MYD	MYS	MFD	MFS	SL	WBSF
pH ₀	1	0.105	0.050	0.159	-0.354	-0.126	0.085	0.046	-0.018
WCM	-	1	0.087	-0.039	0.221	0.294	0.157	-0.032	0.004
Temp	-	-	1	0.614	-0.140	0.422	0.484	-0.237	0.200
MYD	-	-	-	1	0.053	-0.009	0.316	-0.182	0.266
MYS	-	-	-	-	1	0.092	-0.020	0.021	0.119
MFD	-	-	-	-	-	1	0.493	0.307	-0.194
MFS	-	-	-	-	-	-	1	0.298	0.276
SL	-	-	-	-	-	-	-	1	-0.239
WBSF	-	-	-	-	-	-	-	-	1

Figures highlighted bold are significant at $p < 0.05$.

WCM, warm carcass mass; Temp, temperature; MYD, myofibril diameter; MYS, myofibril spacing; MFD, muscle fibre diameter; MFS, muscle fibre spacing; SL, sarcomere length; WBSF, Warner Bratzler shear force (measured in newtons); μm - micrometres (unit measurement for muscle nanostructure).

Table 6. Correlations among nanostructure properties, cold carcass mass, muscle pH, and temperature 24 hours post slaughter

Variables	pH ₂₄	Temp	CCM	MYD	MYS	MFD	MFS	SL	WBSF
pH ₂₄	1	-0.066	-0.210	-0.382	-0.453	-0.227	-0.345	0.012	0.169
Temp	-	1	-0.012	0.614	0.177	0.183	0.147	0.031	0.235
CCM	-	-	1	0.277	0.083	0.468	0.329	0.200	-0.213
MYD	-	-	-	1	0.369	0.279	0.413	0.097	0.025
MYS	-	-	-	-	1	0.150	0.501	0.182	-0.231
MFD	-	-	-	-	-	1	0.543	0.267	-0.269
MFS	-	-	-	-	-	-	1	0.243	-0.301
SL	-	-	-	-	-	-	-	1	-0.369
WBSF	-	-	-	-	-	-	-	-	1

Figures highlighted bold are significant at $p < 0.05$.

CCM, cold carcass mass; Temp, temperature; MYD, myofibril diameter; MYS, myofibril spacing; MFD, muscle fibre diameter; MFS, muscle fibre spacing; SL, sarcomere length; WBSF, Warner Bratzler shear force (measured in newtons); μm , micrometres (unit measurement for muscle nanostructure).

with MYS, MFS, MFD, and SL, with WBSF decreasing as these muscle nanostructure components increased.

Figures 1 to 8 depict the microscopic overview of the muscle structure in each breed at 45 minutes and 24 hours post-slaughter. At 45 minutes post slaughter, Bonsmara had long and thin muscle fibres with a very smooth surface structure i.e. can hardly distinguish between muscle fibres (Figure 1). While at 24 hours the muscles fibres were short and thick with moderately coarse surface structure and slight separation between fibre bundles (Figure 2). Similarly Beef master had long and thin muscle fibres at 45 minutes post-slaughter but with a slightly coarse surface structure and slight separation between muscle fibres (Figure 3); while at 24 hours post slaughter, muscle fibres were short and thick with a slightly abundant separation between muscle fibres i.e. fibres starting to pull apart and an extremely coarse surface structure (Figure 4).

Simbra had short and thick muscle fibres with a slightly coarse surface structure and slight separation between muscle fibres at 45 minutes (Figure 5); while at 24 hours the muscle fibres were thin and long with a moderate coarse surface structure and slight separation of fibres and bundles (Figure 6). At 45 minutes Hereford had very short and thick muscle

fibres with a very coarse surface structure and slightly abundant separation between muscle fibres (Figure 7); while at 24 hours the muscles fibres were long and thin with a very coarse surface structure and muscle fibres starting to pull apart (Figure 8).

DISCUSSION

It was noted in the present study that while breed affected some nanostructure components (MYD and MYS) at 45 minutes post-slaughter and some (MFD and MFS) at 24 hours post slaughter, these breed effects did not have an influence on meat tenderness as both WBSF values and SL were not affected by breed. Furthermore, at approximately 45 minutes post-slaughter, there were evident changes in the myofibril structure, while at 24 hours there were evident muscle fibre bundle characteristics changes. Although meat tenderness is known to be directly associated with the myofibrillar structure [13] these results suggest that meat tenderness may be enhanced by the muscle fibre bundle characteristics after 24 hours post-slaughter, while the structural integrity of myofibrils may further change during maturation [13] and thus can be good predictors of ageing period for individual

Figure 1. Lateral muscle nanostructure of Bonsmara; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Length of muscle fibres at 45 minutes post-slaughter (long and thin muscle fibres). (B) Length of muscle fibres at 24 hours post-slaughter (short and thick). μm , micrometre.

breeds to further enhance meat tenderness.

Moreover the microscopic view of the muscle structure

showed that Bonsmara and Beef master muscles contracted immediately after slaughter and maintained the contraction

Figure 2. Transverse muscle nanostructure of Bonsmara; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Texture of muscle fibres/ surface structure at 45 minutes post slaughter (very smooth surface structure i.e. can hardly distinguish between muscle fibres). (B) Texture of muscle fibres/surface structure at 24 hours post-slaughter (moderately coarse surface structure and slight separation between fibre bundles). μm , micrometre.

Figure 3. Lateral muscle nanostructure of Beef master; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Length of muscle fibres at 45 minutes post-slaughter (long and thin muscle fibres). (B) Length of muscle fibres at 24 hours post-slaughter (Short and thick muscle fibres). μm , micrometre.

Figure 4. Transverse muscle nanostructure of Beef master; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Texture of muscle fibres/ surface structure at 45 minutes post slaughter (slightly coarse surface structure and slight separation between muscle fibres). (B) Texture of muscle fibres/surface structure at 24 hours post-slaughter (slightly abundant separation between muscle fibres i.e. fibres starting to pull apart and an extremely coarse surface structure). μm , micrometre.

Figure 5. Lateral muscle nanostructure of Simbra; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Length of muscle fibres at 45 minutes post-slaughter (short and thick muscle fibres). (B) Length of muscle fibres at 24 hours post-slaughter (thin and long muscle fibres). μm , micrometre.

throughout the 45 minutes to 24 hours post-slaughter testing. This resulted in a coarser surface structure, suggesting less

tender meat for both breeds. Contradictory, after 45 minutes of muscle contraction, Simbra and Hereford muscles were

Figure 6. Transverse muscle nanostructure of Simbra; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Texture of muscle fibres/ surface structure at 45 minutes post slaughter (slightly coarse surface structure and slight separation between muscle fibres). (B) Texture of muscle fibres/ surface structure at 24 hours post-slaughter (moderate coarse surface structure and separation of fibres and bundles). μm , micrometre.

Figure 7. Lateral muscle nanostructure of Hereford; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Length of muscle fibres at 45 minutes post-slaughter (long and thin muscle fibres). (B) Length of muscle fibres at 24 hours post-slaughter (contracted muscle fibres). μm , micrometre.

relaxed although the surface structure was still a bit coarser at 24 hours. This suggests that although all muscles were rea-

sonably tender in the study, Simbra and Hereford tenderness could further be improved by less ageing as compared to the

Figure 8. Transverse muscle nanostructure of Hereford; observed under the JEOL JSM-6390LV scanning electron microscope (SEM) and further viewed using JEOL JM-5600 scanning electron microscope at $\times 5,000$ magnification. (A) Texture of muscle fibres/ surface structure at 45 minutes post slaughter (very coarse surface structure and slightly abundant separation between muscle fibres). (B) Texture of muscle fibres/surface structure at 24 hours post-slaughter (very coarse surface structure and muscle fibres starting to pull apart). μm , micrometre.

Bonsmara and Beef master which could need longer ageing periods. This further suggests that breed influences the muscle structure and composition [14] thus indeed breed is a factor which must be considered during the selection of beef animals to ensure meat quantity and to predict ageing period to enhance tenderness.

Furthermore, although Guzek et al [1] indicated that the most variable factors in the meat production, even in similar conditions, that may also influence meat quality, are the weight of animal and pH of meat, this was not the case in respect with meat tenderness in the present study. The WBSF and SL were not affected by pH₀, ultimate pH₂₄, Temp₄₅, Temp_{24 hrs} and carcass mass although some muscle nanostructure properties were affected by these. This means that at early post-mortem, breed and some chemical muscle reactions may affect the muscle nanostructure but these effects may be indirectly linked to tenderness early post-mortem. This was further explained by the WBSF weak negative correlations SL, MFD, and pH at 45 minutes post slaughter; while most nanostructure components (MYS, MFS, MFD, and SL) negatively correlated with WBSF at 24 hours post slaughter with a slightly higher negative linear correlation than at 45 minutes.

The WBSF values decreased as these muscle nanostructure components increased at 24 hours post-slaughter, thus enhancing tenderness. So these results agree with Modika et al [7] who suggested that the tenderness process begins immediately after slaughter. This is because in the present study tenderness decreased as MYD and MYS increased at approximately 45 minutes, linking tenderness with the myofibril structure soon after slaughter. On the other hand at approximately 24 hours post-slaughter tenderness increased as MYS, MFS, MFD, and SL increased, directly linking the muscle fibre bundle characteristics to tenderness while the myofibril structure is maturing to further improve tenderness at 24 hours post-slaughter.

CONCLUSION

Instead of the myofibrillar structure, muscle tenderness is enhanced by muscle fibre bundle characteristics at approximately 24 hours post-slaughter. However, the myofibrillar structure at 45 mins post-slaughter can be a good predictor of the required ageing period for individual breeds to further enhance tenderness. Thus there is a need to extend the research in order to assess the effect of early diagnosis of the muscle nanostructure components on meat ageing for at least a 14 day ageing period.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manu-

script.

ACKNOWLEDGMENTS

This work was funded by the National Research Foundation (NRF) from two projects with Grant number(s): 101171-2016 and 116636 as well as the Meat Industry Trust (MIT) authorization number IT8114/98.

REFERENCES

1. Guzek D, Głaska D, Pogorzelski G, et al. Variation of meat quality parameters due to conformation and fat class in Limousin bulls slaughtered at 25 to 27 months of age. *Asian-Australas J Anim Sci* 2013;26:716-22. <https://doi.org/10.5713/ajas.2012.12525>
2. Kim JJ, Taylor J. Evaluation of beef carcass and palatability traits and prediction of tenderness in a cross of *Bos indicus* *Bos taurus* cattle. *Asian-Australas J Anim Sci* 2001;14:1621-7. <https://doi.org/10.5713/ajas.2001.1621>
3. Damez JL, Clerjon S. Meat quality assessment using biophysical methods related to meat structure. *Meat Sci* 2008;80:132-49. <https://doi.org/10.1016/j.meatsci.2008.05.039>
4. Christensen M, Ertbjerg P, Failla S, et al. Relationship between collagen characteristics, lipid content and raw and cooked texture of meat from young bulls of fifteen European breeds. *Meat Sci* 2011;87:61-5. <https://doi.org/10.1016/j.meatsci.2010.09.003>
5. Chulayo AY, Muchenje V. Effects of animal class and genotype on beef muscle nanostructure, pHu, colour and tenderness. *S Afr J Sci* 2016;112:1-9. <https://doi.org/10.17159/sajs.2016/20150056>
6. Sisson S, Grossman JD. The anatomy of domestic animals. 4th ed. Philadelphia, PA, USA: WB Saunders; 1953.
7. Modika KY, Frylinck L, Moloto KW, Strydom PE, Heinze PH, Webb EC. Visual evaluation of beef tenderness by using surface structural observations and its relationship to meat colour. *S Afr J Anim Sci* 2015;45:255-62.
8. Koohmaraie M, Wheeler TL, Shackelford SD. Beef tenderness: regulation and prediction. *Proc Meat* 1995;95:1-25.
9. Li P, Wang T, Mao Y, et al. Effect of ultimate pH on postmortem myofibrillar protein degradation and meat quality characteristics of Chinese yellow crossbreed cattle. *Sci World J* 2014; 2014:174253. <https://doi.org/10.1155/2014/174253>
10. Staatskoerant. Republic of South Africa government gazette. Meat Safety Act No. 40, 2000.
11. Staatskoerant. Regulations regarding the classification and marking of meat intended for sale in the Republic of South Africa. Agricultural Products Standards Act, Act No. 119 of 1990.
12. SAS Institute Inc. SAS/STAT software 9.2 release TS1M0. Cary, NC, USA: SAS Institute Inc; 2009.

13. Wyrwisz J, Półtorak A, Poławska E, et al. The impact of heat treatment methods on the physical properties and cooking yield of selected muscles from Limousine breed cattle. Anim Sci Pap Rep 2012;30:339-51.
14. Khasrad, Sarbaini, Arfa`i, Rusdimansyah. Effect of cattle breeds on the meat quality of *longissimus dorsi* muscles. Pak J Nutr 2017;16:164-7. <https://doi.org/10.3923/pjn.2017.164.167>